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CYTOPROTECTIVE TREATMENT OF FLAVONOID FRACTIONS OF *PHYLLANTUS AMARUS* LEAF ON THE HIPPOCAMPUS OF ADULT WISTAR RATS FOLLOWING PENTYLENETETRAZOL INDUCED CONVULSION

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Abstract

Phyllanthus amarus Schum and Thonn herb is in traditional medicine for more than 3000 years. It belongs to a family of Euphorbiaceae and commonly known by the name of carry me seed, stone breaker, gala of wind, etc. It is a branching annual herb of 30-60 cm height widely spread throughout tropics and sub-tropics as a weed. Phyllanthus amarus is gaining momentum for its hepatoprotective, anti-carcinogenic, anti-bacterial, anti-viral, anti-inflammatory and more activities as it contains different combinations of secondary metabolites, which render them medicinal properties. The major class of bioactive compounds like alkaloids, flavonoids, lignans, sterols, tannins, triterpenes and volatile oils has been isolated. Flavonoids are secondary metabolites that are very abundant in plants, fruits, and seeds, responsible for the color, fragrance, and flavor characteristics. In plants, flavonoids perform many functions like regulating cell growth, attracting pollinators insects, and protecting against biotic and abiotic stresses. The study grouped animals used into seven (7) groups with six (6) animals per group. Group 1 were given standard animal feed and water only, group 2 were given 33mg/kg of PTZ only, group 3 were given 100mg/kg of tannin fractions and 33mg/kg of PTZ, group 4 were given 200mg/kg of tannin fractions and 33mg/kg of PTZ, group 5 were given 400mg/kg of tannin fractions 33mg/kg of PTZ, group 6 were given 400mg/kg of tannin fractions while group 7 were given 6.4ml of carbamezepine drug and 41.2mg/kg of PTZ. Administration of Flavonoid was oral while PTZ was administered intraperitoneally all for a period of 20 days. Data generated was analyzed using SPSS ver. 23. Thresh-hold responses to PTZ as recorded with a digital timer and histological observations were done. Findings from this study showed that PTZ caused considerable weight loss, animals in group 2 which were given 33mg/kg of PTZ only showed that highest latency response and those in group 3 which were pretreated with 100mg/kg of flavonoid and given 33mg/kg of PTZ exerted the highest thresh-hold response. Histological observations showed that 33mg/kg of PTZ caused degranulation of neurons in the dentate granular layer of the hippocampus and mild tissue traumatic encephalopathy of the dentate gyrus. Flavonoid fractions also exhibited protective traits for neurons in the dentate gyrus.

Keywords: Flavonoid; Convulsion; Phyllanthus amarus; encephalopathy

Introduction

It has been well-known that natural compounds have an important role in the management and treatment of depression (Nabavi *et al.,* 2015; Pathak *et al.,* 2013*).* Phyllanthus amarus (P-amarus) belongs to the Euphorbiaceae family and is traditionally used for treatment of kidney ailments, diabetes, pain, jaundice, gonorrhea, chronic dysentery, skin ulcer, and hepatitis B (srividiya *et al*, 1995).

Recently, the plant has received increasing attention and has been studied for various pharmacological properties such as immunomodulatory, antinociceptive, anti-inflammatory, antioxidant, antibacterial, anticancer, antiulcer, gastroprotective, antifungal, antiplasmodic, antiviral, aphrodisiac, contraceptive, hepatoprotective, antihyperglycemic, antilipidemic, nephroprotective, and anti-amnesic activities (Joshi and Parle, 2007; Patel *et al*., 2011). Although it demonstrates a wide spectrum of pharmacological actions, the unifying features of all these actions are directed towards the anti-inflammatory and antioxidant properties of the plant. PA contains various phytoconstituents such as lignans, alkaloids, phenolics, terpenes, tannins, flavonoids, sterols, and volatile oils (Patel *et al.,* 2011). Of all these, phytochemicals, phyllanthin, hypophyllanthin, corilagin, and geraniin are found in abundance and potentially responsible for the reported anti-inflammatory actions of PA (Patel *et al.,* 2011; Jantan *et al.,* 2014). Most of the anti-inflammatory studies carried out with PA were performed in models of inflammation either *in vitro* or *in vivo*. However, limited available data to substantiate the effects of PA in neuroinflammation have warranted a study to explore the anti-inflammatory actions of PA in the central nervous system (CNS).

Anxiety has been conceptualized as a frequent and serious disorder affecting the world's population, independent of ethnicity, and is considered a cardinal symptom of many psychiatric disorders (Cassano *et al*., 1999). Psychopharmacological research has aided in the identification and treatment of anxiety disorders, such as generalized anxiety disorder, panic disorder, phobias, obsessive compulsive disorder, acute stress, and posttraumatic stress disorder. Many patients with these anxiety disorders are subjected to the adverse effects of drug treatments and present comorbid difficulties in memory and cognitive tasks (Eysenck & Calvo, 1992).

Pentylenetetrazol also known as pentylenetetrazole, metrazol, pentetrazol (INN), pentamethylenetetrazol, Corazol, Cardiazol, Deumacard, or PTZ, is a drug formerly used as a circulatory and respiratory stimulant. High doses cause convulsions, as discovered by Hungarian-American neurologist and psychiatrist Ladislas J. Meduna in 1934. It has been used in convulsive therapy, and was found to be effective – primarily for depression – but side effects such as uncontrolled seizures were difficult to avoid (Read, 1940). In 1939, pentylenetetrazol was replaced by electroconvulsive therapy, which is easier to administer, as the preferred method for inducing seizures in England's mental hospitals. In the US, its approval by the Food and Drug Administration was revoked in 1982. It is used in Italy as a cardio-respiratory stimulant in combination with codeine in a cough suppressant drug (Minkel, 2007).

The hippocampus (via Latin from Greek "seahorse") is a major component of the brain of humans and other vertebrates. It is the part of the limbic system and plays important roles in the consolidation of information from short-term memory to long-term memory, and in spatial memory that

enables navigation. The hippocampus is located under the cerebral cortex in the allocortex (Martin, 2003; Amaral *et al.*, 2007; Anderson *et al.*, 2007) and in primates it is in the medial temporal lobe. It contains two main interlocking parts: the hippocampus proper (also called Ammon's horn (Pearce, 2001) and the dentate gyrus. In Alzheimer's disease (and other forms of dementia), the hippocampus is one of the first regions of the brain to suffer damage; short-term memory loss are included among the early symptoms. Damage to the hippocampus can also result from oxygen starvation (hypoxia), encephalitis, or medial temporal lobe epilepsy.

People with extensive, bilateral hippocampal damage may experience anterograde amnesia: the inability to form and retain new memories. Since different neuronal cell types are neatly organized into layers in the hippocampus, it has frequently been used as a model system for studying neurophysiology. The form of neural plasticity known as long-term potentiation (LTP) was initially discovered to occur in the hippocampus and has often been studied in this structure. LTP is widely believed to be one of the main neural mechanisms by which memories are stored in the brain. In rodents as model organisms, the hippocampus has been studied extensively as part of a brain system responsible for spatial memory and navigation. Many neurons in the rat and mouse hippocampus respond as place cells: that is, they fire bursts of action potentials when the animal passes through a specific part of its environment. Hippocampal place cells interact extensively with head direction cells, whose activity acts as an inertial compass, and conjecturally with grid cells in the neighboring entorhinal cortex (Hernández-Pérez, Cooper, &, Newman, 2020).

The convulsive disorders are among the more frequently occurring neurologic disorders of childhood and adult, affecting more than 4% of all children (Allen, 1995). Anxiety has been conceptualized as a frequent and serious disorder affecting the world's population, independent of ethnicity, and is considered a cardinal symptom of many psychiatric disorders (Cassano *et al*., 1999). The use of *P. amarus* is gaining momentum because of its novel antiviral activity against hepatitis B virus and for several other biological activities such as kidney and gallbladder stones, for cold, flu, tuberculosis, and other viral infections; liver diseases and disorders including hepatitis, jaundice and liver cancer (Unander *et al*., 1993). It also acts against liver cell toxicity and improves the immune system of patients and has been found effective against hepatitis A (Jayaram *et al*., 1997). *P. amarus* is often used in the traditional system of medicine for a variety of ailments including dropsy, diabetes, jaundice, asthma and bronchial infections (Foo and Wong, 1992). In the Ayurvedic system of medicine, it is used in problems of stomach, genitourinary system, liver, kidney and spleen. It is bitter, astringent, stomachic, diuretic, febrifuge and antiseptic. The whole plant is used in gonorrhea, menorrhagia and other genital affections. It is useful in gastropathy, diarrhea, dysentery, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds. It is also used as a good tonic (Foo and Wong, 1992). Therefore, this research assesses the anti-anxiolytic properties of phyllantusamara on hippocampus of adult Wistar Rat following the induction of PTZ.

This study will add to existing knowledge of health benefit of flavonoid fraction of *Phyllantus amarus* and would also validate the effect of flavonoids on the hippocampus. It will be of interest to both pharmaceutical companies and research institutes in generation of new therapeutic

strategy to effectively cure the pentylenetetrazol damage on the hippocampus and will also help improve patient care since plant drugs are considered to be less toxic and freer from side effects.

This study will use H & E, IBA 1 stain, and cresyl fast violet stain in the study of the histo-architecture of the Hippocampus, Elevated Plus Maze (EPM), and Y-maze test to determine neurobehavior. Statistical analysis will be carried out using SPSS software analytical tool (version 23) with $P \leq 0.05$ considered as level of significance.

Historical information about Phyllanthus Amarus

Medicinal plants are widely used and assumed to be safe, but they can also be potentially toxic especially in pregnancy (Nasri and Shirzad, 2013). However, it has been considered that if a drug is effective, it will have side effects. Therefore, herbal medicines as drug either have side effects or are ineffective (Nasri and Shirzad, 2013). Nevertheless, people every year turn to herbal medicine because they believe plant remedies are free from undesirable side effects (Philomena, 2011). These beliefs can be wrong sometimes and the side effects can be detrimental to the health also as there have been increasing reports on the adverse reactions associated with herbal consumption for many of these adverse reactions, the underlying biochemical mechanisms are unknown, but bioactivities of herbal compounds to generate reactive intermediates have been implicated (Chen *et al*., 2011).

Phyllanthus amarus belong to the family Euphorbiaceae. It is a medicinal plant that has been used in traditional Thai medicine for treatment of fever, jaundice, ascites, hemorrhoid and diabetes (Pongboonrod, 1976). Several pharmacological activities of *P. amarus* have been reported including antiamnesic, antibacterial, antifungal, antiviral, anticancer, anti-diarrheal, gastroprotective, antiulcer, analgesic, anti-inflammatory, antioxidant, diuretic, antiplasmodial, hypocholesterolemic, immunomodulatory, nephroprotective, radioprective and spasmolytic activities (Patel *et al*., 2011) – hepatoprotective against ethanol-paracetamol and carbon-terachloride-toxicity (Pramyothin *et al*., 2017; Wongnawa *et al*., 2006; Krithika and Verma, 2009).

The secondary metabolites present in P.amarus are alkaloids, flavonoids, hydrolysable tannis (ellagitannis), major lignaus, polyphenols, triterpines, sterols and volatile oil (Patel *et al.*, 2011). An alcoholic extract of P.amarus was found to inhibit some cytochrome P450 (CYPIA1, IA2, 2B1/2, 2E1) which are responsible for activation of various procarcinogens both in vivo as well as in vitro suggesting its inhibitory mechanisms of action on carcinogenesis (Kumar and kuttan, 2006). Moreover, Taesotikul *et al*. (2011) have reported the inhibitory potency of the ethanolic and aqueous extract of P. amarus on CYP3A4 activity in vitro in human liver microsome which was about 2-3 orders of magnitude stronger than the known CYP3A4 inhibitors such as erthromomycin and clarithromycin suggesting its potential to cause herb-interaction since CYP3A4 is an enzyme responsible for metabolism of various drugs. Previous studies have demonstrated that inhibition of CYP3A4 increased plasma concentration of drugs which are CYP3A4 substrate leading to serious side effects or toxicity (Lilja *et al.*, 2000). Scientific studies have shown that P. amarus has antihyperlipidermic effect (Danladi *et al.*, 2018).

Description of the Plant

Phyllantus amarus is an annual glabrous herb; grows up to 10-60 cm high. It has an erect, stem terete, younger parts rough, cataphylls 1.5-1.9mm long, deltoid acuminate. Leaves are green, 3.0-11.0 × 1.5-

6.0mm in size, elliptic oblong to obvate, obtuse or minutely apiculate at apex, obtuse or slightly inequilateral at base. Flowers axillary, proximal 2-3 axils with unisexual 1-3 male flowers and all succeeding axils with bisexual cymules. Male flowers – pedicel 1mm long, calyx – 5, sub equal 0.7 × 0.3 mm, oblong, elliptic, apex acute, hyaline with unbranched mid rib; disc segments – 5, rounded, stamens – 3, filaments connate. Female flowers-pedicel 0.8-1.0mm long, calyx lobes – 5, 0.6×0.25mm, ovate-oblong, acute at apex; disc flat deeply 5-lobed. Lobes often toothed at apex, styles – 3, free, shallowly bifid at apex. Capsule 1.8mm in diameter, oblate and rounded, seeds about 0.9mm long, triangular with 6-7 longitudinal ribs and many transerve striations on the back (Bagchi *et al*., 1992). It is widespread throughout the tropics and subtropics in sandy regions as a weed in cultivated and wasteland (Ross, 1999).

Table 1: Ethnomedicinal uses of Phyllanthus amarus Schum & Thonn

S/N	PLACE	LOCAL NAME	PLANT PART USED	DISEASES/ AILMENTS	METHOD OF USE OF P. A.	REFERENCE
1	Dharapuram Taluk, Tamil Nadu, India	Keelanelli	Whole plant	1. Migraine 2. Jaundice	• Whole plant is boiled in gingelly oil, filtered and applied on the head • The fresh root is used with water. Paste or fresh roots are given orally	Balakrishnan et al. (2009)
2	Paliyar tribals in Theni district of Tamil Nadu, Indian	Keelanelli	Leaves	Jaundice	Leaf paste is given internally	Ignacimuthu et al. (2008)
3	Eastern part of Rajasthan, India	Bhumiamla	Whole plant, leaves	Gonorrhea and syphilis Skin diseases, malaria	Decoction of leaves, sugar and cumin seeds are taken orally to treat gonorrhea and syphilis. Leaves are crushed with salt to make paste and applied locally against skin diseases. Plant is crushed into paste, mixed with seed powder of pepper, candy and water and taken as a refrigerant. Decoction of whole plant is taken as an antimalarial	Upadhyay et al. (2010)
4	Uttara kannada, Western Ghats, India	Nelli	Whole plant	Malaria	Not stated	Kuppusamy and Murugan (2010)

5	Eastern region of Shimoga district, Karnataka, India	Nelanelli	Root juice	Jaundice	Root juice is taken orally with cow's milk early in the morning for 1 week	Rajakumar and Shivanna (2009)
6	Dindigul District, Tamil Nadu, Indian	Kizhnelli	Leaves	Menstrual problem	Leaf extract with milk and onion is given during night, three times once in 3 days	Samuel and Andrews (2010)
7	Buldhana district; Maharashtra, Indian	Bhui-awala	Whole plant	Jaundice	Extract, one spoonful per day for 3 days	Ahirrao and Patil (2010)
8	North Andaman Island, India	Nallesari	Whole plant	Jaundice	Handful of leaves is crushed with a pinch of turmeric and one teaspoonful of extract is taken orally for 3–5 days	Prasad et al. (2008)
9	Sivagangai district, Tamil Nadu, India	Keelaanelli	Leaves	Diabetes Jaundice	Diabetes: A. Leaves of Piper betle, Cynodondactylon, Azadirachta indica and P. amarus are dried and powdered with the stem park of Syzygiumcumini. The powder is boiled in water and the extract is given orally B. Leaf extracts of A. indica and P. amarus are mixed and given orally Jaundice: • Leaves of C. dactylon and P. amarus are grounded with the fruits of Piper nigrum and extracted. The extract is given orally. • Leaves of Eclipta alba, P. amarus and Leucas aspera are grounded and extracted. The	Shanmugam et al. (2009)

					extract is given orally • Leaf extracts of C. dactylon and P. amarus are mixed and given orally	
10	Shimoga district of Karnataka, India	Nelanelli (Bhumyamalaki)	Leaves	1. Jaundice 2. Chronic dysentery	• Leaf paste with cardamom is taken internally, two tea spoons daily • Leaves are ground with Acacia Senegal leaves, add sugar and give orally, or tender leaves ground with cow's milk curd given orally, for 2–5 days, before food.	Mahishi et al. (2005)
11	Kattunaykas tribes of Mudumalai Wildlife Sanctuary, Nilgiris district Tamil Nadu, India	Kila nelli	Whole plant	Jaundice	15 mL whole plant juice is taken internally in empty stomach along with one tumbler goat's milk against jaundice	Udayan et al. (2007)
12	Kancheepuram district Tamil Nadu, India	Keezhanelli	Leaves	Jaundice	Fresh leaves are ground and mixed with a cup of cow or goat's milk and taken internally to cure jaundice	Muthu et al. (2006)
13	India	Bhumi amalaki	Whole plant	Liver disease, dyspepsia, anorexia, moderate constipation, chronic colitis, irritable bowel syndrome, urinary tract infection	20% whole plant and 10% Plumbago zeylanica (roots). 4 g of powdered mixer is given to the patient twice daily, half an hour before meals with water	Samy et al. (2008)
14	Northern India	Bhui amla	Whole	14 Jaundice, aphrodisiac, dysentery	Not stated	Kala et al. (2006)
15	Sitamata Wildlife Sanctuary of Chittorgarh	Not stated	Leaves	15 Syphilis, gonorrhea, jaundice	Leaf paste and decoction of leaves	Jain et al. (2005)

	and Udaipur district Rajasthan, India					
16	Esan North East local govt. area of Edo State, Nigeria	Abenaghe	Leaves	Stomachache	Ground leaves with pepper and salt. Half of cup is taken twice daily	Idu et al. (2008a)
17	17 Delta State Nigeria	Ibuko-oyeke	Leaves	Stomachache	The leaves are infused in alcohol and drunk as a remedy for stomachache	16 Idu et al. (2008b)
18	Akwa Ibom State in Nigeria	Oyomokiso, amankeeden	Leaves	Malaria	Boiled in water as decoction. Use internally 4 times a day for 5 days	18 Ajibesin et al. (2008)
19	South West Nigeria	Eyinolobe	Whole plant	Diabetes	Decoction	Abo et al. (2008)
20	Semi-arid Northeasten Brazil	Quebra-pedra	Leaves	Kidney problems	Decoction of leaves soaking drink	Cartaxo et al. (2010)
21	Dangme West district of Ghana	Ofobiokpabi	Whole plant	Malaria	Boil about 50 g of plant in 1 L of water and drink a cupful of decoction three times daily after meals until recovered. Children should take half of the dose. Sweeten with honey or sugar if desired. The decoction may cause dizziness	Asase et al. (2010)
23	Surinamese migrants in Netherland	Finibita	Whole plant	Stomach-ache, cleaning uterus, laxative, health promotion, disease prevention	Whole plant is boiled in water or soaked in alcohol and drunk to purify the blood. These so-called BITAS were said to promote one's health, purity of blood and prevent and cure diseases like malaria, skin sores, diabetes and pimples. Bitter tonics are also reported to be popular among HIV-positives to support their body function	Van Andel and Westers (2010)
24	Akha people in Thailand and China	Yu Jae	Leaves	Rashes, itches	Poultice	Inta et al. (2008)

Adapted from: Patel, Tripathi, Sharma, Chauhan, & Dixit (2011)

Medicinal uses of Phyllanthus amarus

Phyllanthus amarus has an antiviral, anti-diabetic, anti-ulcer and antiseptic property and is used in the treatment of jaundice, diarrhea, dysentery, wound, ulcer and urogenital diseases (Calixto *et al.,* 1998; Santos *et al.*, 1995).

Antibacterial activity of Phyllanthus Amarus

Hexane, methanol and water extracts of aerial parts of P. amarus were screened for antimicrobial activities against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Candida albicans using the agarcup diffusion protocol. The aqueous and methanolic extracts of P. amarus were active against all the test microorganisms. The methanolic extract of P. amarus also showed a broad spectrum of activity with a minimum inhibitory concentration of 1.56 mg/mL against all the test microorganisms. The extracts were also screened for secondary metabolites and the result indicated the presence of alkaloids, saponins, tannins and terpenoids (Alli *et al.*, 2011). The 80% methanolic extracts obtained from seven *Phyllanthus sp.* were evaluated for antibacterial activity using the broth microdilution assay. Best antibacterial activity was obtained by P. amarus against S. aureus (gram-positive) with a MIC value of 17.7 g/mL (Eldeen *et al*., 2011).

P. amarus was examined against ocular infections causing bacteria P. aeruginosa, Micrococcus lylae, Bacillus licheniformis, Staphylococcus hominis, S. aureus, Staphylococcus haemolyticus, Micrococcus luteus, Bacillus lentus, Bacillus firmus and Pseudomonas stutzeri using agarwell diffusion method. Results revealed that P. amarus exhibited remarkable bioactivity against M. lylae, S. haemolyticus, B. lentus, B. firmus, P. stutzeri, P. aeruginosa and S. aureus (Koday *et al*., 2009). 80% methanolic extract of whole plant of P. amarus showed the least MIC on the tested bacteria viz. B. stearothermophilus, S. aureus, B. subtilis, M. leuteus, S. typhi, Enterobacter aerogens, Proteus mirabilis, and Proteus vulgaris. The MIC and MBC were 30 and 40 g/mL respectively. Ampicillin was used as standard (Komuraiah *et al*., 2009).

Antiplasmodial activity of Phyllanthus Amarus

The aqueous and ethanolic extracts of the whole plant of P. amarus were administered to Swiss albino mice at doses of 200, 400, 800 and 1600 mg/kg/day to investigate the prophylactic and chemotherapeutic effect of the extract against Plasmodium yoelii infection and compared with those of standard antimalarial drugs pyrimethamine and chloroquine, artesunate/amodiaquine respectively. Results showed that extracts demonstrated a dose dependent prophylactic and chemotherapeutic activity. The aqueous extract showed slightly higher effect than the ethanolic extract.

The antiplasmodial effects of extracts were comparable to the standard prophylactic and chemotherapeutic drugs used in chloroquine resistant plasmodium infection. The extracts showed prophylactic effect by significant delay in the onset of infection with the suppression of 79% at a dose of 1600 mg/kg/day. The results indicate that the extracts of the whole plant of P. amarus possess repository and chemotherapeutic effects against resistant strains of P. yoelii in Swiss albino mice (Ajala *et al.,* 2011). The aqueous extract of leaves and stem of P. amarus at doses of 108.33, 165 and 325 mg/kg in Swiss albino mice was found to cause a significant dose-dependent suppression of P. berghei parasites [$p < 0.05$] sulfadoxine/pyrimethamine caused a similar

significant suppression of P. berghei parasites (Dapper *et al.,* 2007). Ethanolic, methanolic and methylene chloride extracts of entire plant of P. amarus showed significant activity against the chloroquinesensitive strain of P. falciparum 3D7. The IC50 of methanolic extract and methylene chloride extract was 5 and 14.53 g/mL respectively (Adjobimey *et al.,* 2004)

Contraceptive effect of Phyllanthus Amarus

Antifertility effect of an alcoholic extract of the whole plant of P. amarus at a dose of 100 mg/kg body weight for 30 days orally was investigated in cyclic adult female mice. The results revealed no significant change in absolute body and organ weights in extract fed animals indicated no alteration in general metabolic status. Cohabited females with normal male mice were unable to become pregnant as their cyclicity was affected. These factors are related to a change in the hormonal milieu that governs female reproductive function. Upon withdrawal of feeding for 45 days, these effects were reversible. Thus, this extract manifests a definite contraceptive effect in female mice (Rao and Alice, 2001).

Hypoglycemic and Hypocholesterolemic properties of Phyllanthus Amarus

The hypoglycemic potential of aqueous extract of whole plant of P. amarus was investigated in alloxan-induced diabetic Wistar albino rats. The extract at a dose of 260 mg/kg produced a significant ($p < 0.05$) reduction in blood glucose level by 112% at 24 h of oral administration. A significant reduction ($p < 0.01$) in blood glucose level of 81 and 61% (day 7) at doses of 130 and 260 mg/kg of extract were observed respectively. The extract also showed a highly significant ($p < 0.001$) decrease in blood glucose level of 38 and 30% (day 14) at doses of 130 and 260 mg/kg respectively. On the administration of 390 mg/kg dose of extract, significant reduction ($p < 0.001$) in blood glucose level of 41% on day 7 and 16% on day 14 was observed (Mbagwu *et al.,* 2011).

The antihyperglycemic and hypolipidemic activities of aqueous extract of whole plant of P. amarus were evaluated in streptozotocin (STZ)-induced diabetic male Wistar albino rats. Aqueous extract of P. amarus was administered at 200 mg/kg body weight/day to normal treated and STZ-induced diabetic treated rats by gavage for 8 weeks. During the experimental period, blood was collected from fasted rats at 10 days intervals and plasma glucose level was estimated. The plasma lipid profile was estimated at the end of experimental period. After the treatment period, kidney LPO, protein oxidation and GSH were estimated. The significant decrease in the body weight, hyperglycemia and hyperlipidemia was observed in STZ-induced diabetic rats with the treatment of aqueous extract of P. amarus in diabetic treated group. STZ-induced diabetic rats showed increased renal oxidative stress with increased LPO and protein oxidation (Karuna *et al.,* 2011).

The amylase inhibitory activity of ethanol, chloroform, and hexane extracts of P. amarus against porcine pancreatic amylase in vitro was evaluated. Different concentrations (10, 20, 40, 60, 80, and 100 g/mL) in DMSO were subjected to -amylase inhibitory assay using starch azure as a substrate. The ethanol and hexane extracts of P. amarus exhibited appreciable -amylase inhibitory activity with an IC50 values 36.05 ± 4.01 and 48.92 ± 3.43 g/mL, respectively, when compared with acarbose (IC50 value 83.33 ± 0.34 g/mL). However, the chloroform extract failed to inhibit -amylase activity (Tamil *et al.,* 2010). Hydro-alcoholic extract of leaves of P. amarus (HAEPA) was studied for its in vivo anti-hyperlipidemic potential using cholesterol diet induced hyperlipidemia model in rats. Results indicated that HAEPA possessed significant hypolipidemic activity at doses of 300 and

500 mg/kg (Umbare *et al.,* 2009). The aqueous extracts of leave and seed of P. amarus at oral dose of 150, 300 and 600 mg/kg produced a dose-dependent decrease in the fasting plasma glucose and cholesterol, and reduction in weights in treated male Swiss mice. The results suggested that the extracts could be enhancing the peripheral utilization of glucose (Adeneye *et al.,* 2006).

Anti-Diarrhoeal, Gastroprotective and Antiulcer activities of Phyllanthus Amarus

Methanolic extract of leaves and stems of P. amarus at the dose of 50, 200, and 1000 mg/kg body weight on adult male Wistar rats significantly inhibited gastric lesions induced by intragastric administration of absolute ethanol (8 mL/kg body weight). Mortality, increased stomach weight, ulcer index, and intraluminal bleeding were reduced significantly by P. amarus. Biochemical analysis indicated that reduced GSH of gastric mucosa produced by ethanol administration was significantly elevated by treatment with P. amarus (Raphael and Khuttan, 2003). Graded doses of the aqueous extract of P. amarus (100–800 mg/kg) administered orally produced a dose related inhibition of gut meal travel distance in normal mice. The highest intestinal transit inhibition of 31.65% was obtained with 400 mg/kg (Raphael and Khuttan, 2003).

In castor oil induced diarrhoea in mice, P. amarus extract (400 mg/kg) delayed the onset of diarrhoea, reduced frequency of defecation and reduced gut meal travel distance significantly resulting in intestinal transit inhibition of 79.94% compared to 86.92% produced by morphine (100 mg/kg). In addition, the activities of some intestinal mucosa enzymes (maltase, sucrase, lactase and alkaline phosphatase) in mice pretreated with extract before castor oil were not as severely depressed as those in castor oil treated mice (control) (Odetola and Akojenu, 2000).

Effect of Phyllanthus Amarus on reproductive organs

The effect of a carbamate insecticide, carbofuran was studied on estrous cycle and follicular growth in virgin female Wistar rats as well as recovering from the damaged estrous cycle with treatment of P. amarus lignans viz. phyllanthin and hypophyllanthin since, phyllanthin and hypophyllanthin at the dose of 100 mg/kg body weight have been found to be systemically transformed into enterolignan(s), which is known to be responsible for augmenting estrous cycle in rats (Islam *et al.,* 2008b). The aqueous crude extracts of P. amarus were administered to 38-week old sexually mature male albino to determine the effect of extract on the male reproductive organs of these animals. The results from the study revealed that the aqueous crude extracts of P. amarus caused varying degrees of testicular degeneration as well as reduction in the mean seminiferous tubular diameter (STD) in the treated rats (Adedapo *et al.,* 2003).

Hepatoprotective and Antioxidative Properties of Phyllanthus Amarus

In a research of the Anti-Inflammatory effects of Phyllanthus amarus through inhibition of NF-kB, MAPK and P13K-Akt signaling pathways in LPS-induced human macrophages; Phyllanthus amarus extract significantly inhibited the production of pro-inflammatory mediators (TNF-α, IL-1β, PGE2) and COX-2 protein expression in LPS-induced U937 human macrophages (Hemavathy *et al*, 2018). P. Amarus-pretreatment also significantly down regulated the increased mRNA transcription of pro-inflammatory markers (TNF-α, IL-1β, and COX-2) in respective LPS-induced U937 macrophages. It down regulated the phosphorylation of NF-κB (p65), IκBα, and IKKα/β and restored the degradation of IκBα, and attenuated the expression of Akt, JNK, ERK, and p38 MAPKs phosphorylation in a

dose-dependent manner (Hemavathy *et al.*, 2018)

P. amarus extract also down regulated the expression of upstream signaling molecules, TLR4 and MyD88, which play major role in activation of NF-κB, MAPK and PI3K-Akt signaling pathways. The quantitative amounts of lignans, phyllanthin, hypophyllahtin and niranthin, and polyphenols, gallic acid, geraniin, corilagin, and ellagic acid in the extract were determined by HPLC (High Performance Liquid Chromatography) analysis (Harikrishnan *et al.*, 2018)

In the Antioxidant potential of aqueous extract of Phyllanthus amarus in rats, PAAEt (Phyllanthus Amarus Aqueous Extract) treated rats showed a significant decrease in plasma LPO and a significant increase in plasma vitamin C, uric acid, GSH levels and GPx, CAT and SOD activities. SCGE (Single Cell Gel Electrophoresis) experiment reveals that PAAEt (Phyllanthus Amarus Aqueous Extract) was devoid of genotoxicity and had a significant protective effect against H(2)O(2), STZ(Streptozotocin) and nitric oxide (NO) induced lymphocyte DNA damage (Karuna *et al*., 2009).

In the hepatoprotective effects of ethanolic extracts of Phyllanthus Amarus on aflatoxin B1-induced liver damage in mice, it was discovered that Phyllanthus amarus possesses a potent protective effect against aflatoxin B (1)-induced hepatic damage, and the main mechanism involved in the protection could be associated with its strong capability to reduce the intracellular level of reactive oxygen species by enhancing the level of both enzymatic and non-enzymatic antioxidants. (Naaz *et al.*, 2007). In the hepatoprotective potentials of Phyllanthus amarus against ethanol-induced oxidative stress in rats, it was discovered that Phyllanthus amarus leaf extract could protect the liver against ethanol-induced oxidative damage by possibly reducing the rate of lipid peroxidation and increasing the antioxidant defence mechanism in rats (Faremi *et al.*, 2008).

In Hepatoprotective activity of Phyllanthus amarus extract in ethanol treated rats; In vitro and in vivo studies showed that the Primary cultures of rat hepatocyte, PA (14 mg/ml) increased %MTT and decreased the release of AST (Aspartate Aminotransferase) and/or ALT (Alanine Aminotransferase) as compared to the effect of ethanol alone (Pornpen et al, 2007). In rats, single toxic dose of ethanol showed significantly increased levels of ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) (Pornpen *et al.*, 2007). In the Hepatoprotective effect of aqueous extract of Phyllanthus niruri on nimesulide-induced oxidative stress in vivo, the results suggested that intraperitoneal administration of the extract could protect liver from NIM-induced hepatic damage more effectively than oral administration (Chatterjee *et al.*, 2006).

Antioxidant property of the aqueous extract of Phyllantus Niruri was also compared with that of a known potent antioxidant, vitamin E. The Phyllantus Niruri extract at a dose of 100mg/kg body weight along with NIM (nimesulide) was more effective in suppressing the oxidative damage than the Phyllantus Niruri extract at a dose of 50 mg/kg body weight. Results suggested that beneficial effect of the aqueous extract of Phyllantus Niruri, probably through its antioxidant property, might control the NIM-induced oxidative stress in the liver (Chatterjee *et al.*, 2006).

In the Water-extractable phytochemistry, Phyllanthus niruri exhibited distinct in vitro antioxidant and in vivo hepatoprotective activity against paracetamol-induced liver damage in mice. The results of the present study suggested the potential use of P. niruri in the treatment of various diseases, among which liver disease

is the most important, due to its ability to act as an antioxidant (Sabir and Rocha, 2008). Furthermore, since the treatment of human intoxications with paracetamol is always limited to the administration of N-acetyl-cysteine, additional studies would be important to determine whether aqueous extracts of P. niruri could increase the efficacy of N-acetyl-cysteine against paracetamol acute hepatotoxicity (Sabir and Rocha, 2008).

In the in-depth hepatoprotective mechanistic study of Phyllanthus niruri: in vitro and in vivo studies and its chemical characterization, the results indicated that CCl4 (Carbon Tetrachloride) induced toxicity in clone-9 cell line and Hepg2 cell line, caused significant elevation of AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), GSH (Glutathione), and SOD (Superoxide Dismutase) levels (Ezzat *et al.,* 2020). The toxicity induced by CCl4 (Carbon Tetrachloride) in the clone-9 and Hepg2 cells was significantly ($p< 0.001$) recovered by treatment with P. niruri extracts (Ezzat *et al.,* 2020). In the comparative study on hepatoprotective activity of Phyllanthus amarus and Ecliptaprostrata against alcohol induced in albino rats, the results clearly suggested that, the Phyllanthus amarus and Ecliptaprostrata have enormous hepatoprotective value (Arun *et al.,* 2011). Among the two plants, Phyllanthus amarus has slightly high activity as compared to Ecliptaprostrata. These herbal drugs have equivalent therapeutic value with the standards drug, Silymarin. Moreover, it is very important to study the specific phytochemical compounds responsible for this hepatoprotective effect (Arun *et al.*, 2011).

Nephroprotective effect of PhallanthusAmarus

Phyllantus amarus has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites. The Spanish name ‘chanca33hylla’ means “stone breaker or shatter stone.” In South America, ‘chanca33hylla’ has been used to eliminate gall bladder and kidney stones, and to treat gall bladder infections (Petal *et al.*, 2011; Calixto, 2000). Phyllantus amarus is gaining momentum because of its novel antiviral activity against hepatitis B virus and for several other biological activities such as kidney and gallbladder stones (Petal *et al.*, 2011). The traditional uses of Phyllantus amarus for kidney stones and gall bladder stones have been validated by clinical research, where Phyllantus amarus extract was found to exhibit a potent and effective non-concentration dependent inhibitory effect on calcium oxalate crystal formation, the building blocks of most kidney stones (Calixto, 2000).

Recent investigations of the traditional uses of the plant leaf and seed aqueous extract among some Ijebu herbalists (in Ogun State, South-West Nigeria) revealed its ethnomedicinal use in the management of suspected poison-related renal disease. The plant hot decoction is administered to suspected adult renal diseased patient at a dose of 30–50 mL three times daily for 2–3 weeks depending on the severity and type of poison-related renal disease. The decoction is believed to enhance renal excretion of the suspected ingested poison and restores deranged renal function to normal (personal communication) (Adeneye & Benebo, 2008). Its effectiveness in protecting against nephrotoxicity induced by gentamicin and acetaminophen was evaluated by Adeneye and Benebo (2008). Data from the study was in support of this plant attenuating renal dysfunction and tubulonephrosis induced by these two therapeutic agents, thus validating its folkloric use in traditional African medicine. The nephroprotective action also was reported to be due to the inherent antioxidant and free radical scavenging constituent of the plant extract (Ekor, 2014).

Phytochemical investigations revealed the presence of flavonoids, tannins, alkaloids, terpenoids, steroids, saponins and cardiac glycosides. The methanol extract of Phyllanthus amarus leaves (50-800mg/kg) caused a statistically significant ($P<0.05$ student's t-test) decrease in the levels of total cholesterol, Aspartate transaminase, Alanine transaminase, urea, uric acid, total protein, prostatic, alkaline, and acid phosphatases. The highest reduction effect was obtained with uric acid at 400mg/kg of Phyllantusamarus extract while the least effect was observed in total cholesterol. These effects were dose- and time-dependent. This shows that the leaves of Phyllantusamarus have hepatoprotective, nephroprotective and cardio protective properties (Obianime& Uche, 2008).

Among many other ailments, renal disease is considered as the 9th major cause of mortality across the world and is considered the sole clinical sign of this disease. Renal failure disease is mainly caused by environmental pollution, particularly by heavy metals. For instance, cadmium (Cd) enters the kidney where it deposits in the proximal tubules. Phyllantus amarus extracts have no prophylactic or amelioratic effects on Cd-mediated kidney damage and rather that continuous exposure to these extracts are deleterious to the kidney (Olubunmi *et al.*, 2017).

Cardioprotective properties of Phylanthus Amarus

A large number across the globe suffer from heart/stroke attacks, mainly due to poor life styles and increased sugar/carbohydrate intakes. In a recent study, the cardioprotective action of aqueous extracts of Phyllantus amarus was studied against high-sugar (fructose) diet-mediated cardiac damage in Wistar rats following 60-days of sugar diet. Heart and aorta tissue samples were collected for further histopathological and biochemical analyses. Coadministration of Phyllantusamarus aqueous extracts plus glucose-diet for a specified time period (60 days) inhibited cardiac and aortic lipids levels (total lipids, triglycerides, total cholesterol and free fatty acids) and reduced phospholipid formation (Putakala *et al.,* 2017).

Histopathological evaluations of the heart and aorta tissues highlighted that the plant aqueous extracts treatment lessened the deposition of fats and necrosis. This study showed the obvious cardioprotective potential of Phyllantusamarus aqueous extract for treatment of high sugar-diet mediated oxidative stress in rats is mainly due to its ameliorative antioxidant potential along with its anti-hyperglycemia and anti-hyperlipidemic properties. Moreover, the phytochemical profiling of the aqueous extracts of Phyllantusamarus indicated it may contain the flavonoids quercetin and rutin, the lignans phyllanthin and hypophyllanthin, saponins and the phenolic compound gallic acid, all of which are potent drivers of cardioprotective action (Putakala *et al.*, 2017).

Anti-Inflammatory properties of Phyllanthus Amarus

Phyllanthus Amarus have been reported to contain various phytoconstituents such as lignans, alkaloids, phenolics, terpenes, tannins, flavonoids, sterols, and volatile oils. Of all these phytochemicals, phyllanthin, hypophyllanthin, corilagin, and geraniin are found in abundance and potentially responsible for the reported anti-inflammatory actions of Phyllanthus amarus (Patel *et al.,* 2011; Jantan *et al.,* 2014).

Phyllanthus amarus have promising anti-inflammatory activity which acts through the suppression of Nuclear factor-kappa B (NF-κB), Mitogen activated protein kinase (MAPKs), and PI3K-Akt signaling pathways and may have beneficial therapeutic applications for treating inflammatory disorders (Harikrishnan *et al.,*

2018). A different group from laboratory observations has similarly demonstrated an anti-inflammatory action of Phyllanthus Amarus through inhibition of LPS-induced responses in human macrophages (Harikrishnan *et al.,* 2018). The aqueous extracts of Phyllanthus amarus was investigated for analgesic and anti-inflammatory activities using both thermal and chemical models of pain assessment in rats. The extract caused a significant ($p < 0.05$) dose related increased inhibition of the carrageenan-induced paw oedema in the rats. The inhibition produced by 200mg/kg aqueous extract of Phyllanthus amarus (70.20%) was significantly higher than that of the reference drug (acetylsalicylic acid). The extract produced a marked analgesic activity by inhibiting both early and late phases of pain stimulus in formalin induced paw licking rats and also a significant and dose related increase in inhibition of the mean tail immersion duration at varying water bath temperature (50, 55 and 60^0C) (Iranloye *et al.,* 2011).

Methanol extract of the leaves of Phyllanthus amarus has great anti-inflammatory and analgesic potential. These biological effects exhibited by the extract of this plant may be attributed to the presence of flavonoids and other phenols contained therein (Ofuegbe *et al.,* 2013).

In a related report, the effects of methanol extract of Phyllanthus amarus on different phases of inflammation were examined by Mahat & Patil in 2007 through investigations performed using different phlogistic agents-induced paw edema, carrageenan-induced air-pouch inflammation and cotton pellet granuloma in rats. The methanol extract of Phyllanthus amarus significantly inhibited carrageenan, bradykinin, serotonin and prostaglandin E1-induced paw edema, but failed to inhibit the histamine-induced paw edema. The extract significantly decreased the formation of granuloma tissue in chronic inflammation model (Mahat and Patil, 2007).

Anti-viral properties of Phyllanthus amarus

The aqueous extract of Phyllanthus amarus showed partial antiviral activity against white spot syndrome virus in shrimp at the concentration of 150 mg/kg of animal body weight for 30 days (Balasubramanian *et al.,* 2007). Effect on viral RNA replication was investigated using Taq Man Real time RT-PCR. Phyllanthus amarus root extract showed significant inhibition of HCV-NS3 protease enzyme; whereas Phyllanthus amarus leaves extract showed considerable inhibition of NS5B in the in- vitro assays. Results suggested the possible molecular basis of the inhibitory activity of Phyllanthus amarus extract against HCV which would help in optimization and subsequent development of specific antiviral agent using Phyllanthus amarus as a potent natural source (Ravikumar *et al.,* 2011). Again, n-hexane leaf extract from Phyllanthus amarus has significant antiviral potentials against Newcastle disease virus in broiler chickens and as submitted by Faeji et al. (2019), prophylactic administration of n-hexane leaf extract from P. amarus at 500 mg/l might be a safer approach in utilization of the leaf extract against Newcastle disease (Faeji *et al.,* 2019).

Pentylenetetrazol and its uses

History of Pentylenetetrazol

Pentylenetetrazol also known as Metrazol, penetrazol (INN), pentamethylenetetrazol, corazol, cardiazol, deumacard or PTZ is a drug formerly used as a circulatory and respiratory stimulant. High doses cause convulsions as discovered by Hungarian-American neurologist and psychiatrist Ladislasj.Meduna in 1934. It has been used in convulsive therapy and was found to be effective primarily for depression but side effects such as uncontrolled seizures were difficult to avoid (Charles, 1940). In

1939, pentylenetetrazol was replaced by electroconvulsive therapy, which is easier to administer as the preferred method for inducing seizure in England's mental hospital. In the US, it's approval by the food and drug administration was revoked in 1982 (Minkel, 2007). Pentylenetetrazol has been used as a respiratory stimulant for 30 years and as a convulsant for 20 years (Berman *et al.*, 1957).

Pentylenetetrazol has been used experimentally to study seizure phenomena and to identify pharmaceutical effects that may control seizure susceptibility. It is also a prototypical anxiogenic drug and has been extensively used in animal models of anxiety. Pentylenetetrazol produces a reliable discriminative stimulus, which is largely mediated by the $GABA_A$ receptor. Several classes of compounds can modulate the pentylenetetrazol discriminative stimulus including 5-HT (1A), 5-HT (3), NMDA, glycine and L-type calcium channel ligands (Jung *et al.*, 2002).

General uses of Pentylenetetrazol

Pentylenetetrazol is a potent convulsant commonly used to produce focal or generalized epileptiform discharges in the mammalian central nervous system (Ajmone, 1969) and has been used experimentally to study seizure phenomena and to identify pharmaceuticals that may control seizure susceptibility. Pentylenetetrazol is also a prototypical anxiogenic drug and has been extensively utilized in animal model of anxiety. Pentylenetetrazol produces a reliable discriminative stimulus which is largely mediated by the $GABA_A$ receptor. Several classes of compounds can modulate the Pentylenetetrazol discriminative stimulus including 5-HT1A, 5-HT3, NMDA, glycine and L-type calcium channel ligands (Jung *et al.*, 2002).

Recently, Stanford University Researchers have renewed interest in PTZ as a candidate for pharmacological treatment of Down syndrome published in the April 2007 issue of Nature Neuroscience. Their brief communication outlined an experiment designed to test the underlying theory proposed to explain the purported efficacy of $GABA_A$ antagonist in restoring the declarative memory deficits associated with the mouse model of human Down syndrome (Fernandez *et al.*, 2007). TS6DN mice injected with a 2-week regimen of either of two compounds picrotoxin or bicobalide (Both GABA antagonists) showed marked improvements in both exploration and recognition of novel objects over controls injected with only saline. These results were duplicated in a second experiment with mice fed either plain milk or a combination of milk and a non-epileptogenic dose of PTZ daily for 17 days (Fernandez *et al.*, 2007). PTZ-fed mice achieved novel object task scores comparable to wild-type (normal) mice. These improvements persisted at least 1-2 months after the treatment regimen. Not surprisingly, these compounds' efficacies were accompanied by the normalization of long-term potentiation in the dentate gyrus one month after the end of the treatment, further suggesting persistent drug-mediated improvements in learning and memory (Fernandez *et al.*, 2007).

The finding of Pentylenetetrazol effectiveness in treating a mouse model of Down syndrome has led to it being explored as a means of correcting other learning deficiencies. Specifically, hamsters denied their natural circadian rhythm (though not denied sleep) had their memory restored to near-normal levels when treated with Pentylenetetrazol (Ruby *et al*., 2008).

Pentylenetetrazol is a central and respiratory stimulant similar to doxapram hydrochloride. It is a $GABA_A$ receptor antagonist and is anxiogenic (Aronson *et al.*, 2016). It has been used in respiratory depression and has also been included in multi-ingredient formulations for respiratory

tract disorders, including cough and for the treatment of hypotension and pruritus (Aronson *et al.,* 2016). It has also been used to provoke seizures during the use of SPET scanning to localize the epileptic focus as a diagnostic procedure in patience with drug-resistant epilepsy (Aronson *et al.*, 2016).

Side effects of Pentylenetetrazol

Key-ringing or PTZ induced single and repeated seizures result in increased oxidative damage and lipid peroxidation and decreased antioxidant defence mechanisms (Dillioglugil *et al.,* 2010). An intraperitoneal injection of PTZ into an animal induces an acute, severe seizure at a high dose, whereas sequential injections of a subconvulsive dose have been used for the development of chemical kindling. A single low-dose injection of PTZ induces a mild seizure without convulsion. Repetitive low-dose injections of PTZ decreased the threshold to evoke a convulsive seizure. Finally, continuous low-dose administration of PTZ induces a severe tonic-clonic seizure (Shimada *et al*., 2018).

Mechanism of action of Pentylenetetrazol

The mechanism of Pentylenetetrazol is not well understood, it may have multiple mechanisms of action. A report was published and it analyzed Pentylenetetrazol and several structurally related convulsant drugs. They found that in vivo convulsant potency was strongly correlated to invitro affinity to picrotoxin binding site on the GABA-A receptor complex. Many GABA-A ligands are effective anticonvulsant such as sedatives diazepam and Phenobarbital but presumably Pentylenetetrazol has the opposite effect when it binds to the GABA-A receptor (Squires *et al.,* 1984).

Several studies have focused on the way Pentylenetetrazol influences neuronal ion channels. A 1987 study found that Pentylenetetrazol increases calcium influx and sodium influx both of which depolarizes the neuron. Because these effects were antagonized by calcium channel blockers, Pentylenetetrazol apparently acts as calcium channels and it causes them to lose selectivity and conducts sodium ions as well (Papp *et al.,* 1987). PTZ suppreses the function of inhibitory synapses leading to increased neuronal activity. This regulation causes generalized seizures in animals (Tourov *et al.,* 1996).

Physiological mechanism of action of PTZ

PTZ is a bicyclic tetrazole derivative. According to Kalinowsky (1986), Von Meduna for the first time used PTZ as a stimulant for the nervous system, in 1934. PTZ is characterized by high bioavailability due to easy penetration through biological membranes, rapid distribution to organs after intraperitoneal (I.P.) injection, a very short latency of action, uniform distribution in the brain, and ability to stimulate epileptogenic activity by blocking g-aminobutyric acid (GABA)-mediated transmission (Zienowicz *et al.*, 2005).

Kindling is a process in which repetitive sub-threshold applications of any convulsant agent may result in prolonged seizures, gradually increased in duration and degree of behavioral disorder (Vosu and Wise, 1975). The kindling model induced by PTZ was originally described by Mason and Cooper (1972). They found that an effect comparable to the electrical kindling appeared after chronic intraperitoneal applications of a subconvulsive dose of PTZ. It was shown that PTZ-induced kindling is a long-lasting phenomenon so once a behavioral manifestation on PTZ application was achieved; there is a grand seizure response on the renewed applications even after months without applying convulsive stimuli.

PTZ kindling can be realized by short or long protocols: the daily or each alternate day injections of sub-threshold dose during 15 or 38 days long, respectively (Atack *et al.,* 2000; Becker *et al.,* 2000). The critical PTZ

dose for development of tonic-clonic seizures after the I.P. injection in accordance with kindling protocol ranges from 20 to 50 mg per kg. Davoudi *et al.* (2013) and Dhir (2012) described detailed standard experimental protocol of PTZ kindling and modified win-PTZ kindling method. A significant decline in PTZ concentration in the brain by 6h after intraperitoneal application was found by using precise high-performance liquid chromatography (Sierra-Paredes *et al.,* 1989). Therefore, the complex evaluation of the effect of PTZ kindling model should be performed in view of time after the last injection of PTZ to avoid an acute effect. Depending on the aims of the experiments, different groups of researchers use different seizure scales. The major studies evaluate a PTZ-induced kindling seizure severity based on the Racine's scale of seizure classification. Racine established a positive correlation between the increase of epileptiform AD and the development of motor seizures in amygdala kindling model (Racine, 1972). He categorized progressive behavioural changes of rodents into five stages manifesting as: motor arrest, eye blink, chewing, and head nodding (stages 1 and 2); forelimb clonus when the skeletal motor response is activated and the convulsion itself is initiated (stage 3); rearing and bilateral clonus (stage 4) followed by loss of balance control (stage 5).

However, the development of motor seizures in PTZ-induced kindling has some particular behavioural patterns that have been described by Fischer and Kittner (1998): no seizure (stage 0); weak nodding (stage 0.5); ear, face and eyelid twitching (stage 1); mild forelimb clonus (stage 1.5); myoclonic body jerks, clonic convulsions of forelimb (no rearing; stage 2); partial rearing and rapid clonic seizures of forelimb (stage 2.5); powerful bilateral forelimb clonus with complete rearing (≥10 s; stage 3); rearing and falling with intense bilateral forelimb clonus (stage 3.5); generalized clonic seizures with rearing– falling down episodes, or jumping (stage 4); generalized clonic-tonic seizures with failure of righting reflex (stage 4.5); generalized clonic-tonic seizures and status epilepticus (≥2 min; stage 5). Such an extended scale may be important for subtle monitoring of changes in different parameters such as expression of different genes of binding sites or energy metabolism.

Involvement of Brain structures

First 1–3 injections of PTZ (20–45 mg/kg) cause absence seizures (Snead, 1992). Typically generalized 3–4 Hz spike-wave discharges (SWDs) with short duration, characteristic paroxysmal electrical activity of absence epilepsy syndrome, were observed in local field potentials and were followed by behavioral manifestations, namely, freezing of animals along with tremor of vibrisses, staring, and tilting of head (Sang and Lee, 1995). In accordance with the focal cortical theory of genetic origin of absence epilepsy, cortex and thalamus form a unified oscillatory network, responsible for SWDs generation (Meeren *et al.,* 2005).

To study the applicability of cortical theory to induced SWDs in PTZ kindling, the expression of c-Fos was traced, as c-Fos is an immediate-early gene that allows visualizing the brain structures involved in each specific stage of chemical kindling. Indeed, it was found that thalamus and hypothalamus were the most rapid structures involved in the early absence-like stage of kindling in adult animals. But the occurrence of clonic and tonic-clonic seizures after repetitive sub-convulsive applications of PTZ was characterized by the sequel involvement in epileptogenesis of the midbrain, brainstem regions and most of the hippocampal subfields. The latter study that used classified seizures severity revealed more exhaustive involvement of the brain structures in PTZ kindling (Szyndler *et al.,* 2009).

The earliest expression of c-Fos (at stages 1 and 2 of kindling according to Racine's acute seizure scale) was observed in piriform cortex prefrontal cortex, and striatum. It is certain that striatum, like piriform cortex and prefrontal cortex, is characterized by a low excitation threshold to PTZ in kindling model. These brain structures are important for the propagation of epileptic activity due to their dense projections to other regions of the brain: amygdala, hippocampus, entorhinal cortex, perirhinal cortex, and substantia nigra (Loscher *et al.,* 1991). At the third stage of kindling, central amygdala nuclei, entorhinal cortex, and lateral septal nuclei had enhanced concentrations of c-Fos. At the fourth stage of kindling, the increase in c-Fos expression was observed in the basolateral amygdala and CA1 region of the hippocampus. Finally, when tonic-clonic convulsions were fully developed, c-Fos labelling was found enhanced in the dentate gyrus.

Biochemical mechanism of the PTZ Action

Although the kindling seizures model based on applications of PTZ is widely used for the antiepileptic drug screening, the mechanism by which PTZ elicits its action is not well understood. In the last four decades, many studies have mapped the PTZ targets. PTZ was shown to interact with benzodiazepine (BZ) recognition sites of the GABAA receptor (Rocha et al., 1996). However, a slow cyclic nucleotides degradation and changes in ion movements have also been implicated (McCrohan and Gillette, 1988).

Alterations in Glutamate-mediated excitation

Upregulation of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) binding depending on the duration of the PTZ-treatment was shown by Ekonomou *et al.* (1999). Autoradiographic studies in mice demonstrated a gradual and long-lasting increase of N-methylD-aspartate (NMDA) binding by glutamate receptors in the dentate gyrus and CA3 area of the hippocampus together with a short-term increase in somatosensory cortex (Ekonomou *et al.,* 1999). Cremer demonstrated that upregulation of NMDA receptors in the CA1 area of the hippocampus and dentate gyrus, as well as in the somatosensory, piriform, and entorhinal cortices, occurs already after two weeks. The decrease in kainate binding sites in the cortex and hippocampal regions of PTZ-treated animals was shown (Cremer *et al.,* 2009). At the same time, increase in the density of specific [3H]-L-glutamate binding sites has been revealed (Meldrum *et al.,* 1999). An increase in the number of AMPA and NMDA binding sites along with the decrease in kainate receptors densities was demonstrated in several studies of human epileptic tissue from patients with focal and temporal lobe epilepsy (TLE) (Zilles *et al.,* 1999). Schunzel *et al*. (1992) reported elevation of the level of glutamate in the brain of PTZ-treated animals. Therefore, alterations in the glutamatergic transmitter system follow PTZ-induced reduction of GABA-mediated inhibition (Bradford, 1995).

Alterations in Gaba-mediated inhibition

The acute effect of PTZ on cultured mammalian neurons was originally described by MacDonald and Barker (Macdonald and Barker, 1977). It was characterized by blocking GABA-mediated transmission. Biochemical and electrophysiological studies indicated that the pharmacological effects of PTZ were induced by blockade of the binding sites of the GABAA receptor complex, namely as benzodiazepine recognition sites. The structure of benzodiazepine sites is formed as adjacent at the interface of the g2 subunit to either an a1, a2, a3 or a5 (but not a4 or a6) subunits of GABAA receptor (Atack, 2010). BZ sites together with the other subunits are arranged around a central waterfilled pore, which gates to conduct Cl

ions when GABA is bound. In accordance with experimental data, the action of PTZ is antagonistic for benzodiazepine and leads to the development of seizure activity. In contrast, agonists of BZ sites have a marked anticonvulsive effect on tonic clonic seizures in PTZ-kindled rats, with marked dose–effect dependence (Rosenberg, 1995).

Phyllanthus amarus and the nervous system

Phyllanthus Amarus and the Glial cells of the Central Nervous System

Glial cell also known as neroglia are cells that primarily support neurons. There are four (4) types of Glial cells in the Central Nervous System, namely;

- Microglia
- Astrocytes
- Oligodendrocyte
- Ependymal cells.

Neurodegenerative disease such as Parkinson's disease (PD), Alzheimer's disease (AD), Amyotrophic lateral sclerosis (ALS) and Multiple sclerosis (MS) has a common underlying feature: neuroinflammation, characterized by dysregulation in the activation of immune cells of the brain, particularly the microglia (Wang *et al.,* 2011). Microglia activation leads to morphological and functional changes, which result in the transformation from ramified phenotype to amoeboid phenotype, aimed at eliminating foreign bodices (Kettenmann *et al* 2011). Upon activation, microglia releases different chemokines and cytokines which propagate immune responses (Amor *et al.,* 2014). Interaction between microglia and injured neurons produces uncontrolled inflammation and, progresses to brain injury (Jeong *et al.,* 2013).

Studies on anti-inflammatory activity of Phyllanthus amarus within the field of neuroinflammation are currently limited. A recent study focusing on corilagin, an isolated compound from Phyllanthus amarus demonstrated attenuation of radiation-induced brain injury through microglia activation and the expression of inflammatory cytokines (Tong *et al.,* 2016). Other studies using isolated constituents from Phyllanthus amarus were found to partially reverse oxidative damage in stressed rats (Nathiya and Vanisree, 2010).There is consistent evidence that stress leads to microglial activity in the hippocampus, and neuroinflammation in particular relates to elevated microglial activity which imply mental illnesses (Calcia *et al.,* 2016). Preliminary studies has shown that three out of nine compounds of Phyllanthus amarus; namely phyllanthin, ellagic acid and gallic acid, have demonstrated neuroprotective activities against LPS-induced activation of BV2 microglial cells when pretreated with these compounds individually (Ismail *et al*., 2020).

A study designed to determine effect of ethanol extract from Phyllanthus amarus (EPA) in LPS-induced BV2 microglia cells showed that no production was inhibited and iNOS protein was suppressed. The secretion of TNFα in LPS-activated nurine microglial cells was also decreased (Ismail *et al*., 2020). Various research evidences indicated that upregulation of iNOS leads to higher NO production (Sierra *et al*., 2014) resulting in neurotoxic effects and is associated with several neurodegenerative disorders (Panthi *et al.,* 2018; Andreasson, 2010; Ryan *et al.,* 2011). Study conducted to investigate the protective effect of ethanolic extract on the nervous system using microglias cells to elucidate the underlying cellular mechanism of its protective actions revealed, very low expression of IL-IB were detected after pretreatment with ethanol extract from P. Amarus (EPA) in LSP- induced BV2 microglia cells (Ismail *et al.,* 2020).

How Astrocyte react with Phyllanthus amarus in the CNS

Prolific research in the last two decades has expanded the role of astrocytes from a mere structural function to critical functions in CNS development, modulation of synaptic activity and glutamate homeostasis, blood-brain barrier (BBB) formation and neurovascular coupling, and inflammatory response. The basis of this astrocyte functional specialization remains largely unraveled, but it is plausible that specific subpopulations of astrocytes with distinct morphology and molecular equipment carry out different functions in different neural circuits (Chai *et al*., 2017).

Astrocytic reactivity is functional and morphological change of astrocytes as a result of variety of brain insults and it is characterized by increased gene expression of a number of astrocytes structural proteins, such as glial fibrillary acid protein (GFAP) and vimentin; morphological changes, such as hypertrophy of the cell soma and processing; and proliferation, which is particularly important in the formation of an astrocytes scar around tissue lesions (Dossi *et al*., 2018). It is usually implicated in several neurological disorders such as AD, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, and multiple sclerosis. Sustained reactive responses might be driven by positive feedback loops between microglia and astrocytes under conditions of severe and prolonged brain insults, thus providing detrimental signals that can compromise astrocytic and neuronal functions and lead to chronic neuroinflammation (Colangelo *et al.*, 2014). Inwardly rectifying potassium (Kir) channel subunits. Kir4.1 are specifically expressed in the brain astrocytes and form Kir4.1-containing channels (Kir4.1 channels), homo-tetramers of Kir4.1 subunits and hetero-tetramers of Kir4.1 and Kir5.1 subunits (Ohno *et al.,* 2015; Ohno, 2018). Kir4.1 channels play a key role in mediating the spatial potassium buffering currents, which remove excessive extracellular potassium ions (K^+) at tripartite synapses (Ohno *et al*., 2015). Kir4.1 channels regulate the resting membrane potential of astrocytes, which serves as a driving force of astrocytic glutamate uptake through excitatory amino-acid transporter 2 (EAAT2) (Frizzo, 2017). Therefore, dysfunction of Kir4.1 channels elevates not only extracellular K^+, but also glutamate levels at tripartite synapses (Ohno *et al*., 2015; Ohno, 2018). Inhibition (channel blockade or expressional suppression) of Kir4.1 channels facilitated the expression of brain-derived neurotrophic factor (BDNF) in astrocytes, which may produce diverse effects including synaptic plasticity, neural sprouting, neurogenesis and reactive gliosis in the brain (Kinboshi *et al*., 2017; Ohno, 2018).

Kir4.1 plays an important role in inducing and developing epilepsy (epileptogenesis) (Mukai *et al*., 2018). Astrocytic Kir4.1 expression was reported to be reduced in the brain regions related to seizure foci in patients with epilepsy and animal models of epilepsy (Inyushin *et al.*, 2010; *Das et al*., 2012; Heuser *et al.*, 2012; Steinhäuser*et al*., 2012; Harada *et al*., 2013). Although the effect of Phyllanthus amarus on the astrocytes has not been documented, findings suggest that enhancement of Kir4.1 channel activities can prevent the development of epilepsy (epileptogenesis) by facilitating astrocytic spatial potassium buffering (Mukai *et al.*, 2018).

How ependymal cells react with Phyllanthus amarus in the CNS

Ependymal cells are ciliated glial cells that form an epithelial barrier, called the ependyma, lining the brain's ventricular system and the spinal cord's central canal. They develop from radial glia along the surface of the ventricles of the brain and spinal canal starting from the first postnatal days, thereby providing an interface between

the parenchyma and cerebrospinal fluid (CSF)-filled cavities throughout life. This interface allows ependymal cells to control the bidirectional passage of immune cells and solutes between the CSF and interstitial fluid (Mastorakos and McGavern, 2019); while also providing homeostatic regulation of molecules (Bedussi *et al.*, 2015; Ma *et al.*, 2017); in addition to playing a critical role in sensing and propelling CSF via primary and motile cilia, respectively (Bolborea *et al.*, 2015).

The ependymal cell is responsible for the production, circulation and absorbtion of CSF. Three subtypes of ependymal cells have been described: E1, E2, and E3 ependymal cells. The three distinct ependymal cell subtypes are defined by their cilia number and regional distribution (Mirzadeh *et al.*, 2017) where E1 ependymal cells possess multiple motile cilia and are the most abundant subtype in the adult brain, occupying the majority of the lateral (forebrain), third (midbrain) and fourth (hindbrain) ventricles. E2 cells possess both primary and motile cilia and are bi-ciliated (Mirzadeh *et al.*, 2008) and line the spinal canal (Alfaro-Cervello *et al.*, 2012) in addition to occupying a portion of the third and fourth ventricles of the brain (Mirzadeh *et al.*, 2017); while E3 ependymal cells have primary cilia, are uniciliated (Mirzadeh *et al.*, 2017), and exist primarily in a small portion of the third ventricle, inhabiting the preoptic and infundibular recesses (Mirzadeh *et al.*, 2017). The effect of P. Amarus on the ependymal cells is yet to be documented.

How Oligodendrocyte react with Phyllanthus Amarus in the CNS

Oligodendrocytes are the myelinating cells of the central nervous system (CNS). They are the end product of a cell lineage which has to undergo a complex and precisely timed program of proliferation, migration, differentiation, and myelination to finally produce the insulating sheath of axons. Due to this complex differentiation program, and due to their unique metabolism/physiology, oligodendrocytes count among the most vulnerable cells of the CNS (Bradl and lassmann, 2010).

Within the central nervous system (CNS) of jawed vertebrates, myelination is carried out by oligodendrocytes, a highly specialized glial cell (Zalc, 2016). The promotion of the speed and efficiency of action potentials has been the best understood purpose of oligodendrocytes and myelin over the past seven decades (Hartline and Colman, 2007). While this role is undeniably important, there is also an increasing appreciation that neurons require support by glia, including oligodendrocytes, for their long-term integrity. When oligodendroglial support is lost, axons become progressively compromised and vulnerable to loss. Consequently, remyelination strategies are being pursued in diseases such as multiple sclerosis (MS) with the hope of not only recovering nerve conduction in the short term but also protecting axons against degeneration in the long term (Franklin *et al.*, 2012; Franklin and Ffrench-Constant, 2017; Lubetzki *et al.*, 2020).

In the central nervous system (CNS), oligodendrocytes assemble myelin, a multilayered sheath of membrane, spirally wrapped around axonal segments and best known for its role in enabling fast saltatory impulse propagation (Nave & Werner, 2014; Cohen *et al.*, 2020)**.** An additional function of oligodendrocytes is the metabolic support of myelinated axons (Fünfschilling *et al.*, 2012; Lee *et al.*, 2012; Saab *et al.*, 2016) most important when axons spike at high frequencies (Trevisiol *et al.*, 2017).

Subtle defects of CNS myelin have been associated with various psychiatric diseases (Nave & Ehrenreich, 2014). Basic research has mostly focused on rodent models with white matter abnormalities and analyses largely restricted to the diagnosis

and assessment of reduced motor functions. These studies revealed, among other findings, that oligodendrocytes can myelinate axons in an activity-dependent manner and influence, as predicted, their conduction velocity properties (Gibson *et al.,* 2014; Hines *et al*., 2015).

The phytochemistry of Phyllanthus amarus

Chemical constituents of Phyllanthus amarus

Phytochemical analysis of *P. amarus* suggests that it contains tannins, flavonoids, lignans, triterpenes, alkaloids, sterols, and volatile oils (Kumar *et al.,* 2017; Wu *et al*., 2019). It is a rich source of lignans comprising of phyllanthin and gallic acid, a vital phytoconstituents responsible for its antioxidant, anti-inflammatory, antiulcer, colonoprotective potential (Chanrde *et al.*, 2015). A researcher (Kiemer, 2003) reported that *P. amarus* exerts its anti-inflammatory effect via modulation of endogenous biomarkers such as COX-2, iNOS (inducible NO synthase), and NF (Kiemer, 2003). Furthermore, Kandhare *et al.* (2013) reported that phyllanthin and hypophyllanthin from the standardized extract of *P. amarus* inhibited DNA damage and elevated levels of TNF-α, thus produced antiulcer potential in a murine model of ulcerative colitis (Kandhare *et al.*, 2013). Furthermore, a recent study demonstrated the antiasthmatic potential of phyllanthin from *P. amarus* through inhibition of IgE (Immunoglobulin E), iNOs, HO-1 (Heme oxygenase-1), TNF-α, IL's (interleukins) and TGF-β (Transforming growth factor beta) (Wu *et al.,* 2019). However, there is no significant data available to support antiepileptic efficacy of P. amarus in various available literature.

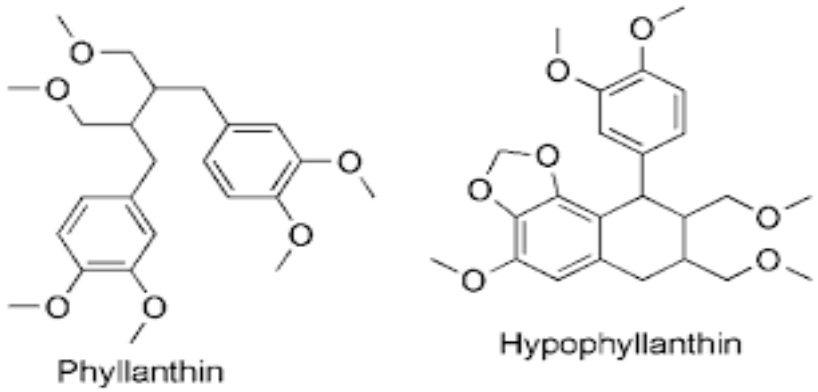


Fig. 1: Chemical Structures of major lignands in P. amarus+

Flavonoid

Flavonoids are a large family of over 5,000 hydroxylated polyphenolic compounds that carry out important functions in plants, including attracting pollinating insects, combating environmental stresses such as microbial infection and regulating cell growth (Kumar and Pandey, 2013). Flavonoids are an important class of natural products, particularly; they belong to a class of plant secondary metabolites having a polyphenolic structure widely found in fruits, vegetables and certain beverages. They have miscellaneous favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc. (Burak & Imen, 1999). Flavonoids are associated with a broad

spectrum of health promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is because of their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions. They are also known to be potent inhibitors for several enzymes such as xanthine oxidase (XO), cyclooxygenase (COX), lipoxygenase and phosphoinositide 3_kinase (Hayashi *et al.*, 1988). In nature, Flavonoid compounds are products extracted from plants and they are found in several parts of the plant. Flavonoids are used by vegetables for their growth and development against plaques (Havsteen, 2002). They belong to a class of low molecular weight phenolic compounds that are widely distributed in the plant kingdom. They constitute one of the most characteristic classes of compounds in higher plants. Many Flavonoids are easily recognised as flower pigments in most angiosperm families. However, their occurrence is not restricted to flowers but is found in all parts of plants (Dewick, 2001). Flavonoids are also abundantly found in foods and beverages of plant origin, such as fruits, vegetables, tea, cocoa and wine, hence they are termed as dietary Flavonoids. Flavonoids have several subgroups, which include chalcones, flavones, flavonols and isoflavones. These subgroups have unique major sources. For example, onions and tea are major dietary sources of flavonols and flavones (Dewick, 2001).

Functions of flavonoids in plants

Flavonoids play a variety of biological activities in plants, animals and bacteria. In plants, Flavonoids have long been known to be synthesized in particular sites and are responsible for the colour and aroma of flowers and in fruits to attract pollinators and consequently fruit dispersion to help in seed and spore germination and the growth and development of seedlings (Griesbach, 2005). Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV filters (Takahashi & Ohnishi, 2004), function as signal molecules, allopathic compounds, phytoauxins, detoxifying agents and antimicrobial defensive compounds(Samanta *et al*., 2011). Flavonoids have roles against frost hardness, drought resistance and may play a functional role in plant heat acclimatization and freezing tolerance (Samanta *et al*., 2011).

Classification of flavonoid

Flavonoids can be subdivided into different subgroups depending on the carbon of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring. Flavonoids in which the B ring is linked in position 3 of the C ring are called Isoflavones. Those in which the B ring is linked in position 4 are called neoflavonoids, while those in which the B ring is linked in position 2 can be further subdivided into several subgroups on the basis of the structural features of the C ring. These subgroups are:

- Flavones
- Flavonols
- Flavanones
- Flavanols or catechins, anthocyanins and chalcones

Flavones

Flavones are one of the most important subgroups of Flavonoids. They have a double bond between positions 2 and 3 and a ketone in position 4 of the C ring. Flavones are widely present in leaves, flowers and fruits as glucosides. Celery, parsley, red peppers, chamomile, mint and ginkgo biloba are among the major sources of flavones (Manach *et al.*, 2004).

- **Flavanols**

Flavanols are Flavonoids with a ketone group. They are building blocks of proanthocyanins. Flavanols occur abundantly in a variety of fruits and vegetables. The most studied flavonols are kaempferol, Quercetin, myricetin and fisetin. Onions, kale, lettuce, tomatoes, apples, grapes and berries are rich sources of flavonols. Apart from fruits and vegetables, tea and red wine are also sources of flavonols. They have a hydroxyl group in position 3 of the C ring compared to flavones, which may also be glycosylated like flavones. They are perhaps the most common and largest subgroup of Flavonoids in fruits and vegetables. For example, Quercetin is present in many plant foods (Iwashina, 2013).

Flavanones

Flavanones are another important class which is generally present in all citrus fruits such as oranges, lemons and grapes. Hesperitin, naringenin and eriodictyol are examples of this class of Flavonoids. They are associated with a number of health benefits because of their free radical scavenging properties. Flavanononones also called dihydroflavones, which have the C ring saturated; therefore, unlike flavones, the double bond between positions 2 and 3 is saturated and this is the only structural difference between the two subgroups of Flavonoids. Over the past 15 years, the number of flavanones has significantly increased (Iwashina, 2013).

Isoflavonoid

Isoflavonoids are a large and very distinctive subgroup of Flavonoids. Isoflavonoids enjoy only a limited distribution in the plant kingdom and are predominantly found in soyabeans and other leguminous plants. Some isoflavonoids have been reported to be present in microbes (Mathies *et al.,* 2008).

Neoflavonoid

Neoflavonoids are a class of polyphenolic compounds. While, flavonoids have a 2 phenylchromen 4 one backbone, neoflavonoids has a 4 phenylchromen backbone with no hydroxyl group substitution at position 2. The first neoflavone isolated from natural sources in 1951 was calophyllolide from calophyllolide inophyllum seeds. It is also found in the bark and timber of the sri Lankan endemic plant mesuathwaitessi (Linuma *et al.,* 1987).

Flavanols, Flavanols _3_Ols Or Catechins

Flavanols, flavanols _3_ols or catechins are derivatives of flavans that possess a 2_ phenyl _ 3, 4 _dihydro_2H_chromen_3_ol skeleton. They include a range of compounds such as catechin, epicatechin gallate, ihearubigins etc. They are found in most plants and have a role in plant defense (Ullah *et al.*, 2017).

Anthocyanins

Anthocyanins are pigments responsible for colours in plants, flowers and fruits. Cyanidin, delphinidin, malvidin, pelargonidin and peonidin are the most commonly studied anthocyanins. They occur predominantly in the outer cell layers of various fruits such as cranberries, black currants, red grapes, Merlot grapes, raspberries, strawberries, blue berries, bilberries and blackberries stability coupled with health benefits of these compounds facilitate them to be used in the food industry in a variety of application (Giusti & Wrolstad, 2003).

Chalcones

Chalcones are secondary metabolites belonging to the Flavonoid (C6_C3_C6 system) family. These metabolites are abundantly present in edible plants. A majority of naturally occurring chalcones is polyhydroxylated aromatic compounds and they are considered the bioprecursors of open chain Flavonoids and isoflavonoids (Ouyang *et al.,* 2021).

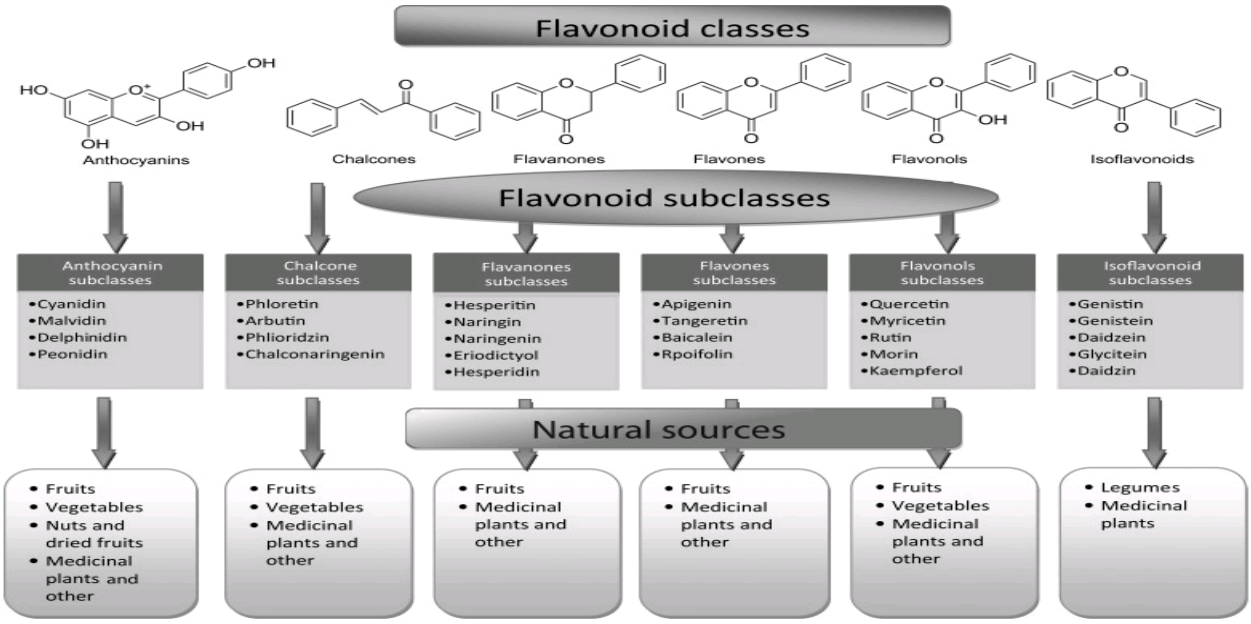


Fig.2: Flavonoid Classes, subclasses and natural sources (Adapted from Panche et al., 2016).

Pharmacological action of flavonoid

Flavonoid is an anti-Parkinson

Parkinson is a progressive degenerative disorder. Progressive degeneration of neurons occurs in substantia nigra, pars compacta and nigrostriatal tract (Tripathi, 2013). The etiology of Parkinson's disease is extremely complicated with various factors playing roles such as environmental, genetics and aging (Chu and Kordower, 2008). Neurodegeneration occurs as a result of several biological processes involving oxidative stress (Datla *et al.*, 2001), augmented iron deposition (Dexter *et al.*, 1987). External stimuli also trigger pro-apoptotic caspases by activating MAPK _induced inflammatory mediators which cause cellular apoptosis (Magalingam *et al.*, 2015). Commencement of pro-inflammatory cytokine genes (iNOS, TNF_ α and IL_IB) expressions induced by NF_KB is also caused by MAPK family (Magalingam *et al.*, 2015). Flavonoids for example, emodin (Jiaz *et al.*, 2013), kaempferol (Qureshi *et al.*, 2012) and morning (Zhu *et al.*, 2013) have been proved to suppress secretion of TNF _ α.

Flavonoid is an anti-Ulcer

Peptic ulcer occurs in that part of the gastrointestinal tract which is exposed to gastric acid and pepsin. It results probably due to an imbalance between the aggressive (acid, pepsin, bile and H.pylori) and defensive (gastric mucus, nitric oxide and bicarbonate secretion) factors (Tripathi, 2013). Quercetin has been found to have antiulcer activity in animals (Motilva *et al.*, 1992). It acts by inhibiting the enzyme histidine decarboxylase (Konturek *et al.*, 1985) and thus reduces the formation of histamine in the gastric mucosa, which stimulates the parietal cells and pepsinogen responsible for the secretion of hydrochloric acid and pepsin respectively (Izzo *et al.*, 1994). Manuka honey, which is rich in flavonoids, preserves the gastric mucosa GSH (Almasaudi *et al.*, 2016). GSH and gastric mucus both act as a barrier against gastric mucosal injury (Cnubben *et al.*, 2001).

Flavonoid is an anti-Depressant

In depression, monoaminergic transmission in the brain gets affected (5_HT and NE gets depleted) (Tripathi, 2013).

Several flavonoids including Quercetin have shown inhibitory actions against MAO_A (Chimenti *et al.,* 2006). Monoamine oxidase-A is responsible for oxidative deamination of 5 _HT and NA.

Flavonoid is an anti-Bacterial

An antibacterial agent is one which interrupts the propagation and growth of bacteria (kohanski *et al*., 2010). Apigenin _7_o_ triglycoside, apigenin, luteolin_7_ o_neohesperidoside, lucenin _ 2, saponarine and vitexin are some of flavonoids which are isolated from mosses and have been proved to possess inhibitory effect against various bacteria. They have been shown to have antibacterial effect against several bacteria including *Enterobacter cloaceae* and *Pseudomonas aeruginosa* (Basile *et al.*, 1999). Golnar extract has played a successful role in preventing food poisoning and exhibited antibacterial action against both gram positive and gram negative food poisoning causing bacteria (Mahboubi *et al.*, 2015).

Flavonoid is an anti-Hypertensive

Rutin and Quercetin are reported to regulate and restore elevated blood pressure and promote the antioxidant defense system. In one study, rats fed on 8 percent sodium chloride supplemented diet (i.e. a high salt diet) for 12 fort nights showed an increase in systolic, diastolic, pulse and mean arterial blood pressures. Liquid peroxidation was increased and the antioxidant enzymes were down regulated. Treatment with rutin and quercetin for nearly 2 weeks resulted in notable reversals of these indices compared to the animals fed only the high salt diet (the without treatment group) (Olaleye *et al.*, 2014).

Chemical structure of flavonoid

Flavonoids are naturally occurring compounds present in plants. They have variable phenolic structures. Stereo-chemically flavonoids are composed of a 15-Carbon skeleton comprising of two benzene rings (A and B as shown in Figure 1) which are linked through a heterocyclic pyrane ring (C). Flavonoids have been classified into a number of classes. Biosynthesis of flavonoids occurs via phenylpropanoid pathway. In this pathway phenylalanine which is an amino acid, gets transformed into 4-coumaroyl-CoA and this 4-coumaroyl-CoA conjugates with malonyl-CoA to give chalcones consisting of two phenyl rings. Conjugate ring-closure of chalcones produces a three-ringed similar form of flavonoids called as flavone. This pathway continues via a sequence
of various enzymatic modifications to form flavanones, dihydroflavonols and anthocyanins. Along with these compounds flavan-3-ols, proanthocyanidins (tannins), flavonols and several other poly-phenolics can also be formed (Kuete, 2013). Flavonoid's basic structure is aglycone (Figure 2) (Mendes *et al.,* 2012). In the structure of flavonoids, a six-member ring that is condensed with the benzene ring can either be a α-pyrone (flavanones and flavonols) or its dihydroderivative (flavanones and flavonols) (Mendes *et al*., 2012; Novza& Popova, 2016; Watson &Preedy, 2009). Flavanones differ from flavonols by lacking a hydroxyl group (OH) at the 3- position and a C2-C3 double bond (Grumwzecu, 2016; Narayana *et al.,* 2001). Different class of Flavonoids are frequently hydroxylated at different positions (2, 3, 3', 4', 5, 5' and 7). The carbohydrates (D-glucose, L-rhamnose, glucorhamnose, galactose or arabinose) are formed via glycosidic linkage generally positioned at positions 3 or 7 (Kumar & Pandey *et al.*, 2013; Ohadoma, Akah& Okolo, 2016).

General uses of flavonoid

Flavonoids are widely distributed in plants, fulfilling many functions (Delage, 2015). They are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals

designed to attract pollinator animals. In higher plants, they are involved in UV filtration, symbiotic nitrogen fixation, and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors. Flavonoids secreted by the root of their host plant help Rhizobia in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy. Rhizobia living in soil are able to sense the flavonoids and this triggers the secretion of Nod factors, which in turn are recognized by the host plant and can lead to root hair deformation and several cellular responses such as ion fluxes and the formation of a root nodule. In addition, some flavonoids have inhibitory activity against organisms that cause plant diseases, e.g. *Fusarium oxysporum* (Galeotti *et al.,* 2008).

In recent years, many polyphenols have been used as natural antioxidants in oils and fats to prevent lipid oxidation, protect food and beverages from light exposure, to prolong shelf-life of
food, supplement for animal feeds to improve their health, to protect animal products, like antimicrobial agent in foodstuffs and health functional ingredient in foods and dietary supplements; these applications can be attributed mainly to antioxidant and antimicrobial activities (Arct *et al*., 2008; Huntley *et al*., 2009; Huvaere *et al*., 2015). The use of synthetic additives in the food industry has been declining in recent years; this is mainly because man seeks to reduce risk in suffering from diseases which have been associated with synthetic products consumption. Flavonoids are phytochemicals that cannot be synthesized by humans; however, these compounds may be used as food additives to improve health-beneficial effects and increase their amount in humans (Rodríguez *et al*., 2015).

Flavonoids are present in significant amounts in many fruits and vegetables; natural antioxidants and flavonoids have been reported as two of the most important micronutrients, which can be used in industry to reduce the use of synthetic compounds on foods and improve health in humans due to their potential to decrease several diseases. These bioactive compounds can be used to prolong shelf-life and preserve many foods due to their antimicrobial and antioxidant properties (Pandey *et al*., 2009).

Flavonoids are suitable compounds that may be used as food preservative due to their beneficial effects in the prevention of fat and oils oxidation, supplement for animal feeds, protection of vitamins and enzymes, inhibition of microbial growth in foodstuffs and health functional ingredient in foods and dietary supplements (Huvaere *et al*., 2015). Flavonoids have been used in natural dyes (Villela *et al*., 2019; Paramita *et al*., 2018), in dermatology and cosmetic preparations for a long time due to several associated properties such as antioxidant, antimicrobial, anti-inflammatory and therapeutic properties (Malinowska *et al*., 2013; Kole *et al*., 2005), and anti-wrinkle skin agents (Chuarienthong *et al*., 2010). Recent dermatological studies have demonstrated that herbal extracts were added to cosmetic preparations due to antioxidant properties of flavonoids in addition to impart UV protection and inhibit metal chelating properties to protect skin (Kole *et al*., 2005). The protective action of flavonoids on the skin is manifested through their anti-inflammatory activity. This activity is given by the action of bioactive compounds on the enzymes and other factors that promote inflammation stages. The other mechanism of action is the inhibition of enzymes linked to cellular activation that promotes skin deterioration and the secretion of regulatory substances for their propagation. The affinity for protein structures and with estrogen receptors is another property of flavonoids, as well as anti-irradiating activities; because of these

features, they are used in the cosmetic industry to reduce congestion problems, with the aim of reducing inflammatory symptoms that damage humans (Arct *et al*., 2008).

The most pronounced applications of these polyphenols, however, are in the field of medicine. Flavonoids have been used extensively as anticancer (Zhao *et al*., 2019), antimicrobial, antiviral, antiangiogenic (Zhao *et al*., 2019; Camero *et al.*, 2019), antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative agents (Patel *et al*., 2018). It also prevents cardio-metabolic disorder (Mazidi *et al*., 2019) and displays better preservation of cognitive performance with aging (Aguiar *et al*., 2019).

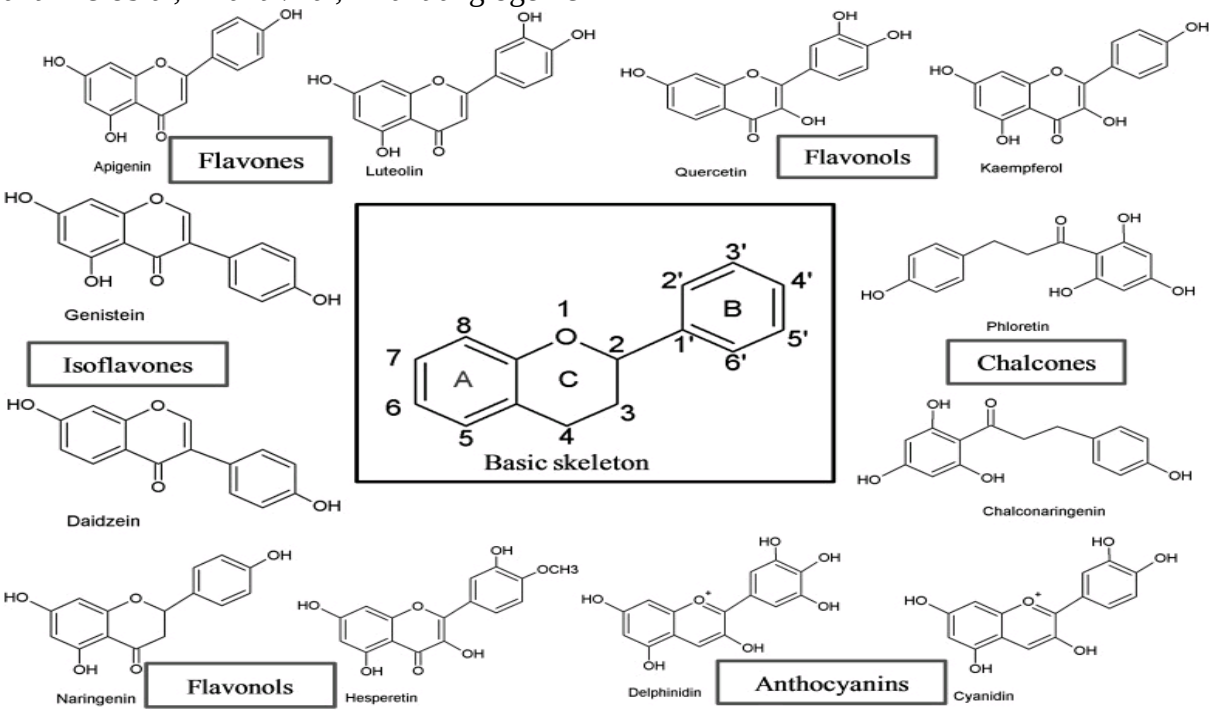


Fig.3: Flavonoid Classes, subclasses and natural sources (Adapted from Panche et al., 2016)

Side effect of flavonoid

Interestingly, there is no evidence of side effects associated with dietary intake of flavonoids. The reason could be attributed to their low bioavailability, that is, poor absorption and quick elimination. However, flavonoids in the supplement form do have side effects, and sometimes severe ones. For example, they can cause nausea, headache, or tingling of the extremities in some people when taken in doses of 1000mg per day. Similarly, a study showed that tea extracts supplement can cause liver toxicity in some cancer patients. It is best to avoid flavonoid supplements during pregnancy and lactation because their safety has not been established in these conditions (Mita, 2002).

At higher doses, flavonoids may act as mutagens, pro-oxidants that generate free radicals, and as inhibitors of key enzymes involved in hormone metabolism. Thus, in high doses, the adverse effects of flavonoids may outweigh their beneficial ones, and caution should be exercised in ingesting them at levels above that which would be obtained from a typical vegetarian diet (Skibola and Smith, 2000). However, the potential for flavonoid toxicity exists if they are consumed at extraordinary amounts in the form of high-potency supplements. In addition, flavonoids are potentially toxic in vulnerable populations such as the elderly who are

marginally iron deficient, because flavonoids may bind nonheme iron (Corcoran *et al.*, 2012).

Medicinal uses of flavonoid

Anti-Cancer action

Cancer is a major health problem caused by abnormal cell growth. There are various anticancer drugs available, and yet only a few display inhibitions against oncogenesis, and a majority of them are toxic and have adverse side effects (Wagner *et al.*, 2009). Natural biomolecules with secondary metabolites have phytomediated content, and display biological activities over a wide range of spectrum, laying the basis for cancer prevention and treatment. Flavonoids are known to inhibit cell growth and act as an anticancer agent (Wang *et al.*, 2016; Patil and Masand, 2019). Hesperedin (Hsp) is an important flavonoid which displays efficient anticancer activity (Devi *et al.*, 2015). Polylactic-co-glycolic acid (PLGA) nanoparticles were synthesized and loaded with Hsp to form hesperidin nanoparticles (HspNPs) to determine its potential application as an anticancer agent against C6 glioma cells. The encapsulated Hsp exhibited decreased in vitro cell viability against the C6 glioma cell line, and the controlled release of Hsp decreased the cytotoxicity of PLGA (Ersoz *et al.*, 2019).

Aurone, a benzo-furanone, is another flavonoid that has been extensively used as an anticancer agent (Alsayari *et al.*, 2019). Various analogues of aurone display different mechanisms against cancer cells because there are many possible targets. These targets include cyclin dependent kinase, histone deacetylase, the adenosine receptor, telomerase, sirtuins, and microtubules (Alsayari *et al.*, 2019). Quercetin is a natural flavonoid present in plants and in commonly consumed foods such as berries, green tea, and grains. It has been used most effectively for colorectal cancer. Cell cycle arrest, increase in apoptosis, antioxidant replication, modulation of estrogen receptors, regulation of signaling pathways, inhibition of metastasis and angiogenesis are among various mechanisms underlying the chemo-preventive effects of quercetin in colorectal cancer (Darband *et al.*, 2018). Luteolin, a natural flavonoid with pro-apoptotic activity in hepatocellular carcinoma (HCC) cells, arrests the cancer cell cycle at the G2/M stage (Yang *et al.*, 2019). Myricetin is an important flavonoid which has anti-inflammatory and anticancer activities, and in liver cancer it shows antimitotic effects, and it targets different metabolic pathways in mitochondria that result in cancer cell death (Devi *et al.*, 2015). Matricariarecutita L. (chamomile) flower has a flavonoid content of 157.9 ± 2.22 mg/g QE of dry extract. It displayed dose dependent enhanced mortality of HepG2 cells in HCC. Angiogenesis is an important process which is hijacked for progression of cancer. Vascular endothelial growth factor (VEGF) facilitates blood vessel formation via angiogenesis through VEGF receptors. This expression of VEGF was dose dependently reduced by synthesized extract, making it an efficient anticancer agent (Al-Dabbagh *et al.*, 2019). Flavonoids present in apple are reported to reduce the risk of colorectal cancer. Studies have shown that consumption of one apple per day reduces the chance of cancer up to 50% (Tu *et al.*, 2017). One of the most popular Chinese medicinal herbs is Scutellariabaicalensis. Wogonoside, bicalein, and baicalin are flavonoids extracted from Scutellariabaicalensis. These flavonoids are not only cytostatic, but also show cytotoxic effect on tumor cells both in vitro and in vivo (Li-Weber, 2009). Although majority of these studies on the anticancer activities of various flavonoids and their derivatives are in pre-clinical study phase, several flavonoids are under consideration for cancer treatment and are at various phases of clinical trials. For example, the icaritin (ICT) is in the third phase of clinical trial for

the cure of hepatocellular carcinoma. Further clinical trials should be conducted on flavonoids and their derivatives for anticancer and other diseases as they have lower toxicity and minor side effects.

Antioxidant Activity

Reactive oxygen species (ROS) are produced in the human body mainly as byproducts of the electron transport chain. They are essential for protein phosphorylation, initiation of numerous transcriptional factors, apoptosis, immunity, and differentiation processes. However, ROS also cause oxidative stress upon reacting with molecules such as lipids, proteins, or nucleic acids. Lipid peroxidation by ROS causes cellular membrane damage. This membrane has a potential, with positive charges on the outside of the cell, and negative charges inside the cell. The damage to membrane alters the cell membrane potential and the cell's osmotic pressure, eventually causing cell death. The human defense system use different mechanisms and enzymes to battle endogenous elevated ROS (Brunetti *et al.,* 2013). Flavonoids act as exogenous antioxidants and are directly oxidized by radicals to form less reactive species via four mechanisms, namely: (1) the inhibition of nitric-oxide synthase activity, (2) inhibition of xanthine oxidase activity, (3) modulation of channel pathways, or by (4) interacting with other enzyme systems (Nijveldt *et al.,* 2001; Kumar and Pandey, 2013). The antioxidant potential of flavonoids is associated with the molecular structure, and more precisely, with the location and total number of the –OH groups, the conjugation and resonance effects, the surrounding environment which modifies the thermodynamically favored antioxidant site, and the particular antioxidant mechanism for a compound (Zheng *et al.,* 2019). The most commonly used supplemented antioxidants are vitamins C and E. The antioxidant potential of flavonoids is more robust than vitamin C and vitamin E (Prochazkova *et al.*, 2011). It is therefore important to regularly include those fruits and vegetables that are rich in flavonoids in daily food intake. For example, due to enhanced and well-known antioxidant and anti-inflammatory properties, flavonoids improve bone health. The utilization of flavonoids in biomaterials has great prospects for bone tissue engineering (Preethi *et al.,* 2017). Quercetin, an antioxidant flavonoid, when present in the blood stream, improves vascular health and reduces risk of cardiovascular disease in its conjugated form. The quercetin and its derivatives prevent thrombosis or blood clotting and prevent chances of stroke (Teraol, 2017). Rutin, a flavonol, showed a number of biological activities that includes anticancer, antioxidant and cytoprotective etc (Ganeshpurkar and Saluja, 2017).

Bryonia Alba L. is a medicinal and homeopathic plant of Cucurbitaceae family with a wide range of biological activities. The methanolic extract of air-dried leaves of this plant was used to isolate four flavonoids (lutonarin, saponarin, isoorientin and isovitexin) using HPLC-DAD. Their antioxidant and antiradical activity were confirmed by their ability to suppress ROS produced by macrophages, the isolates being more effective in comparison to the crude extract (Ielciu *et al.,* 2019).

Effects on the Cardiovascular System

Dietary flavonoid shows a favorable relationship between their consumption and reduction of cardiovascular diseases (Feliciano *et al.,* 2015; Slavin and Lloyd, 2012). Several studies demonstrated that those who consume a large number of flavonoids have 18% lower mortality risk of cardiovascular diseases. Various studies have shown that flavonoids have cardioprotective and neuroprotective (Faggio *et al.,* 2017; Xie *et al.,* 2018) actions and chemoprotective abilities (Williams *et al.,* 2004). Tea is a rich source of flavonoids, and its intake reduces

the risk of cardiovascular diseases (Hodgson and Croft, 2010). Anthocyanidin and proanthocyanidin are flavonoids that have proven to be effective against cardiac diseases (Kruger *et al.,* 2014). Isoflavone, anthocyanins (Cassidy, 2017), and cocoa (Corti *et al.,* 2009) flavan-3-ols improve vascular health (Van Dam *et al.,* 2013). High consumption of these flavonoids decreases arterial stiffness which reduces the risk of cardiovascular diseases (Lilamand *et al.,* 2014). Oils from leaves and fruits of Hippophaerhamnoides (sea buckthorn) have many compounds including flavonoids that showed positive effects on the cardiovascular system (Olas, 2016). Morin hydrate showed high biological activity such as anti-inflammatory, anticancer, and protection against cardiovascular diseases (Venu Gopal, 2013). Brazil nuts are rich in flavonoids which help to prevent heart disease and cancer (Yang, 2009). Chrysin is a flavone and has beneficial effects on epilepsy and depression, also suppresses neuro-inflammation, and has neuro-protective effects (Nabavi *et al.,* 2009).

Morin, a bioflavonoid, has proven to be a cardio-protective agent via animal modeling. Rats were divided into groups, and morin was orally and dose dependently administered to the experimental group. Myocardial necrosis was induced, and the result suggested the improved antioxidant effects and apoptosis. The mechanism for this cardio protection was reported due to alteration of MAPK/NF-kappa B/TNF-alpha pathway (Verma *et al.,* 2019).

The high flavonoid content of dark chocolate cocoa has proven to be an effective cardioprotective agent (Gvozdjakova*et al.,* 2018) by acting as an effective anti-inflammatory agent, by inhibiting NF-kb, by acting as an antihypertensive agent by increasing the bioactivity of nitric oxide, displaying antiatherogenic activity by decreasing triglyceride concentration, and by decreasing insulin resistance and increasing platelet reactivity (Zięba *et al.,* 2019). Dihydro-quercetin (DHQ), a dihydroxyflavone used in animal models, proved to be effective against cardiac dysfunction by decreasing the generation of ROS and lipid peroxidation and increasing the biological function of antioxidant enzyme. The PI3K/Akt pathway activation is found to have protective effects (Shu *et al.,* 2019). Clinical trials on oxerutin showed that it is quite active in treatment of chronic venous hypertension (Petruzzellis *et al.,* 2002). Interestingly, there are no detected toxicities or side effects of oxerutin clinical trilas results (Petruzzellis *et al.,* 2002). Different studies and clinical trials showed that a mixture of flavonoids like diosmin, troxerutin, Rutin hesperidin, quercetin, etc., enhance the veins function, soothe the capillary permeability, and increase the lymphatic and hemorrhoidal drainage (Massimo *et al.,* 2020; Corsale *et al.,* 2018). Thus, flavonoids are useful in control of piles and hemorrhoid diseases. Diosmin that is the flavone glycoside of diosmetin effectively control various types of blood vessel disease like hemorrhoids, bleeding from gums and eyes, etc. (Visconte *et al.,* 2016). It enhances blood flow inside the body (Visconte *et al.,* 2016).

Effects on Nervous System

Flavonoids prevent age related neurodegenerative diseases, and in particular, dementia (Orhan *et al.,* 2015; Nakajima *et al.,* 2014), Parkinson's (Datla *et al.,* 2001; Gao *et al.,* 2012; Magalingam *et al.,* 2015), and Alzheimer's disease (Bakhtiari *et al.,* 2017). The ROS and nitrogen species (NOCs) have roles in many neurodegenerative diseases. Tangeretin, a flavonoid found in citrus fruits, acts as an antioxidant against ROS and NOCs species and provides protection in neurodegeneration disorders such as Parkinson's disease (Gao *et al.,* 2018).

Foods which have abundant amounts of flavonoids lower the hazard of neurodegenerative diseases and also counteract age related cognitive disorders (Spencer *et al.,* 2012). It is beneficial in two ways; first, it regulates neuronal signal cascade caused by cell apoptosis, and second, shows beneficial effects on the peripheral and central nervous system. Hesperidin (Hsd) and hesperetin (Hst) are two flavonoids known for their neuro-pharmacological effects, including neuroprotective, antidepressant, and effects on memory (Roohbakhsh *et al.*, 2014). Berries contain several natural flavonoids, such as polyphenolic compounds like stilbene, anthocyanins etc. These flavonoids are reported to be effective as anti-neurodegenerative agents, anti-mutagenic and antimicrobial agents (Nile and Park, 2014). Epicatechin, an antioxidant flavonoid abundantly found in wood plants, has an analog 3-O-methyl epicatechin which inhibits neurotoxicity in vitro (Lamuela-Raventós *et al.,* 2016). The polyphenolic luteolin flavonoid has neuroprotective effects and also as protective effect against age related neuro-disorders (Nabavi *et al.,* 2015). Forsythia suspensa is a dried fruit and a Chinese medicinal herb with activities determined against infectious diseases, showing antioxidant activity as well as acting as a neuroprotective agent (Wei *et al.,* 2019).

Excessive drinking of alcohol causes various health disorders and negatively affects the brain. The acetylpectolinarin (ACP) flavonoid obtained from the Linaria vulgaris Mill, has been reported to treat hangover by increasing the spontaneous network function of the cultured hippocampal neurons when treated with low concentration of ethanol. It does so by agonistic action on GABAergic synapses mediated by SK potassium channel (Botalova *et al.,* 2019). Hyperalgesia is an enhanced pain sensation due to peripheral nerve damage, and is associated with diabetic patients. Quercetin and sodium, when used together, can act as antinociceptives, decreasing diabetes complications (Narenjkar *et al.,* 2011).

Inhibition of Neuropathy

Nerve malfunction is called neuropathy. Peripheral neuropathy is one of the four types of neuropathy. It refers to the conditions that result when nerves that carry messages to and from the brain and spinal cord and to the rest of the body are damaged or diseased. High levels of glucose destroy the blood vessel that goes to the nerve, and thus, affects the nerves of hand and feet which progress with age. Natural compounds containing flavonoid have been used for to relieve neuropathic pain (Lim and Kim, 2016). A mixture of water and alcoholic solvent has been used for the preparation of root extract of *Cichorium intybus* (Blackburn and Warren, 2017). Pyridoxine or vitamin B6 is a coenzyme in biological reactions whose high dose intake causes damage to peripheral neurons (Blackburn and Warren, 20017). This process is known as pyridoxine-induced neuropathy. Cichorium intybus is a medicinal plant that contains a variety of beneficial biochemical constituents present, including flavonoid, saponin, and tannin. The presence of these compounds enables them to be used to suppress oxidative stress, and its possible interference of the two amino acid systems, namely GABAergic and glutamatergic systems in nerve injury and neuropathy (Hasannejad *et al.,* 2019). Diabetic neuropathy (DN) is a complication that is most commonly faced by 50% diabetes patients, with development of burning sensation to complete loss of sensation of heat and cold in legs and feet, and the loss of peripheral nerve fibers (Bayram *et al.,* 2016). Adult Sprague-Dawley rats have been used to evaluate the effect of flavanoglycone, hesperidin on DN pain induced by streptozotocin. The combination of insulin and hesperidin decreased the neuropathic

pain to a significant amount inducing neuroprotective effect (Visnagri*et al.,* 2014). Nobiletin is a non-polar methoxy flavone found in citrus fruits showing diverse biological activity including anticancer, reverse learning impairment by regulating ERK signal (Nakajima *et al.,* 2007), improves memory impairment by reducing AChE expression (Nakajima *et al.,* 2007). Nobiletin has been proven by animal models to dose dependently increase nerve conduction velocity in diabetic ulcer group (DU) (Parkar *et al.,* 2014). Oxaliplatin is a drug that is used in chemotherapy with an adverse side effect of causing painful neuropathy. The compound was introduced into the mice which induced peripheral neuropathy; the flavonoids rutin and quercetin reduced the production of ROS species by acting as an antioxidant agents and reduced the side effect induced by oxaliplatin (Azevedo *et al.,* 2013). The flavonoid quercetin reduced the neuropathic pain by inhibition of p-ERK induced in Sprague-Dawley rats by sciatic nerve injury (Ishii *et al.,* 2013).

Stroke Prevention

Chalcones are natural precursor compounds of flavonoids and iso-flavonoids. They are present in various plants and vegetables with a wide range of biological activities. Chalcone is an aromatic ketone and an enone with an ability to activate nuclear factor erythroid 2-related factor (2NRF2) pathway. Several novel dihydroxy chalcones were synthesized and evaluated for their ability to suppress ROS species and oxidative stress acting as an anti-ischemic stroke through KEAP1/NRF2/ARE pathway activation. Cerebral ischemia-reperfusion injury (CIRI) in stroke was studied using rat models. These compounds had a potent protection of H2O2-induced oxidative damage in the neuron-like PC12 cells, but also played a neuroprotective role against ischemia/reperfusion-related brain injury in animals (Wang *et al.,* 2019). Fisetin, a flavonoid inhibited LPS-induced TNFα production and suppressing nuclear factor B activation thus acting as a neuroprotective and anti-inflammatory agent after post ischemia injury in vitro (Gelderblom *et al.,* 2012). The cortical development, where neurons in brain failed to migrate in the proper formation in utero, results in malfunctioning known as Focal cortical dysplasia (FCD). The naturally occurring flavonoid rutin has been used on animal models to treat FCD. At a dose of 50 mg/kg, a pronounced recovery was observed in motor neurons. This may act as a clinical drug in the future (Rodrigues *et al.,* 2013).

Recovery of Injured Nerves and anti-inflammatory Properties

Injury to the spinal cord or central nervous system (CNS) causes paralysis of the body from the waist down, and this condition is known as Paraplegia. Experimentation of animal model using rats revealed that using isoflurane increased the number of motor neurons. The delayed preconditioning with a neuroprotective effect was observed, which may be associated with the expression of protein complex NF-κB (Kim *et al.,* 2008). The Hypericum perforatum L. is a medicinal plant with flavonoids as an active phytoconstituent. It has a flavonoid content of 6%. It protects the neuron of adrenal phaeochromocytoma from oxidative stress caused by ROS species such as H2O2. Sciatic nerve injury (SNI) was induced in order to determine the effect of plant extract on oxidative stress, cell signaling molecules, cytokine production, and caspase expression in brain muscle. Wistar albino female rats were used for the study. The result suggested a delay in the progression of SNI caused by the plant extract (Uslusoy *et al.,* 2019).

Angiogenesis is the development of new blood vessels from existing vessels which is important for normal development. Uncontrolled angiogenesis causes serious

diseases such as inflammatory disorders, obesity, multiple sclerosis, asthma, endometrioses, and cirrhosis. Plant polyphenols such as flavonoids and chalcones inhibit angiogenesis by regulating multiple signaling pathways (Mojzis *et al.,* 2008). Kaempferol is a flavonoid that possesses anti-inflammatory effects (Devi *et al.,* 2015). Rutin is a common dietary flavonoid, having various pharmacological properties such as anti-inflammatory, antimicrobial, and anticancer properties (Calderon-Montano *et al*., 2011). Barringtonia racemosa (L.) leaves and branches water soluble extracts have been used to isolate three acylated flavonoid glycosides which showed moderate anti-inflammatory activity by inhibiting LPS-induced NO production in RAW-2647 cells (Van *et al.,* 2019). The Eucalyptus globulus and Arum palaestinum herbal extract inhibits interleukin 1 alpha with high phenolic and flavonoid content with potential anti-inflammatory and anti-acne agent for Acne vulgaris (Abu-Qatouseh *et al.,* 2019). Several clinical trials showed that flavonoids have anti-inflammatory properties and block several enzymes involved in inflammation pathways.

Antibacterial Action

The increase and spread of multi-drug resistance in pathogenic bacteria resulted into a number of antibiotics that have become ineffective for the treatment of a number of bacterial infections (Badshah and Ulldah, 2018). Flavonoids have the ability to enhance the protective immune systems of humans (Kumar and Pandey, 2013). Several flavonoids act both as bacteriostatic and bactericidal agents by damaging the cytoplasmic membrane, and inhibiting energy metabolism and nucleic acid synthesis of microorganisms (Ahmad *et al.,* 2015). Bridelia is a plant of genus of Phyllanthaceae family, which is used as a pain-relieving medicine in Asia and South Africa. Its extract contains flavonoids like quercetin, gallocatechin-(4′-O-7)-epigallocatechin, myricetin-3-glycosides, and isoflavone. These flavonoids are responsible for its antimalarial, antibacterial, anti-inflammatory activities (Ngueyem *et al.,* 2009). Anthocyanidin plays an important role against tuberculosis and drug-resistant Mycobacterium tuberculosis strains (Chinsembu*et al.,* 2016). Studies have shown that hesperidin and hesperetin, two flavonoids, show very good antimicrobial activity (Iranshahi *et al.,* 2015).There has been extensive literature available on flavonoid rich plants showing antibacterial activities. Chrysin is natural flavone having many health benefits such as antidiabetic, antiallergic, anticancer, and antitumor effects (Kasala *et al.* 2015). Many flavonoids are anti-diabetic by increasing secretion of insulin, improvement of hyperglycemia, reduce resistance to insulin and increase uptake of glucose by skeletal muscles in murine model (Babu *et al.,* 2013). Flavonol rich chocolate enhances insulin sensitivity and reduces insulin resistance in healthy subjects (Latif, 2013). Allium cepa L. (onion) flesh and skin (Milea *et al.,* 2019) are rich in quercetin derivatives (Pucciarini *et al.,* 2019) and display protein tyrosine phosphatase 1B (PTP1B) inhibitory activity, decreases its expression, and increases glucose uptake making it a potential antioxidant and antidiabetic agent (Yang *et al.,* 2019).

Flavonoids have many important functions in gastrointestinal tract i.e. glucose homeostasis and lipid metabolism (Oteiza *et al.,* 2018). Kaempferol, a natural flavonoid, acts as antidiabetic agent by inhibiting cell proliferation, decreases PI3K, P63, SREBP-1 expression and phosphorylates insulin resistance substrates (Imran *et al.,* 2019).

Warioniasaharae is a flavonoid rich plant. Its extract was used on animal model to prove its antidiabetic properties. Treptozotocin (STZ) induced diabetic rats

were used as test subject and normal rats were used as control group. Oral administration of the extract was continued for 15 days and its histopathological examination of liver along with glucose tolerance test was performed. The state of the liver and pancreas was found to be improved with the test group rats and had the potential to act as an antioxidant as well as an efficient antidiabetic agent (Ajebli and Eddouks, 2019).

Although there are several studies in murine models that showed that, the hesperidin controls the blood glucose level, clinical trials in human showed no such observation (Shams-Rad *et al*., 2020). Thus majority of clinical studies hints that hesperidin did not modulate insulin or enzymes of the glucose metabolic pathway. There are some limitations of these clinical trials related to absorption of hesperidin (Shams-Rad *et al.,* 2020).

Methylglyoxal is the metabolite whose concentration increases in the blood during diabetes and causes atherogenesis (a condition where artheromatous plaque develop in arteries, with blockage problems) and it also attaches with nerve ending resulting in nerve damage (Van-den-Eynde *et al.,* 2018). Clinical trials on quercetin indicated a decrease in the plasma level of methylglyoxal while epicatechin has no promising effect. This showed the different potency of various flavonoids against specific disease (Van-den-Eynde *et al.,* 2018).

Antifungal Properties

New antifungal agents are required as the currently available antifungal drugs are not effective completely due the development of resistance and undesirable side effects (Campoy & Adrio, 2016). Leaves of Aquilaria have many bioactive compounds including flavonoids responsible for its antiviral, antifungal, and antitumor activities (Adam *et al.,* 2017). Artemisia sacrorum extract have two flavonoids namely sacriflavone A and sacriflavone B and both of them have antifungal potency (Wang *et al.,* 2015). Moreover, 2′,4′-dihydroxy-5′-(1‴,1‴-dimethylallyl)-8-prenylpinocembrin (8PP) is a natural prenyl flavonoid isolated from Dalea elegans, which shows antifungal effects against Candida albicans biofilms (Peralta *et al.,* 2015).

Hippocampus organ of study

Gross anatomy of the hippocampus

The hippocampus or the Cornusammonis is an important component of the human brain, situated in the temporal lobe. It plays a role in the information processing and the reproductive cycle (Gailard et al., 2014; Juhairiyah et al., 2021; Hu et al., 2019). The hippocampus is a bilaminar gray matter structure located medially in the temporal lobe that protrudes over the temporal horn of the lateral ventricle and occupies the medial region of its floor (Gailard et al., 2014; Juhairiyah et al., 2021; Hu et al., 2019).

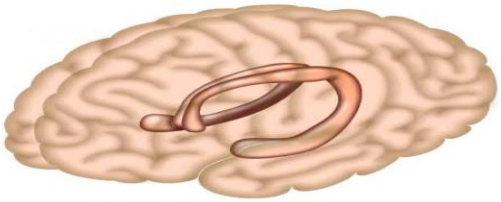


Figure 4: The Hippocampus (adapted from: Gailard et al., 2014; Juhairiyah et al., 2021; Hu et al., 2019)

The hippocampus consists of two interlocking gray matter folds, the cornuammonis (or hippocampus proper) and the dentate gyrus. In the axial plane, the hippocampus resembles a seahorse (hence,

its name) and it arches around the mesencephalon (hence, the term Bmesiotemporal). In the axial and sagittal plane, it can be divided into three parts: (1) the head or anterior segment; (2) the body or intermediate segment; and (3) the tail or posterior segment. White matter fibres from the hippocampus accumulate on its superior surface to form the alveus. White matter fibres from the alveus then gather medially into thickened bundles as the fimbria, which are continuous posteriorly with the fornix (Duvemoy, 2005). Based on its cellular composition, the cornuammonis is divided into four parts, the so-called Sommer's sectors CA1 to CA4. The cornuammonis continues inferomedially in the parahippocampal gyrus, a gray matter structure that forms the transition area between the basal and mesial areas of the temporal lobe. The subiculum is the medial and superior edge of the parahippocampal gyrus and its site of union with the cornuammonis (Duvemoy, 2005). The hippocampus is surrounded by several fissures which are collectively referred to as the perihippocampal fissures. The transverse fissure of Bichat is the lateral extension of the ambient cistern which separates the thalamus superiorly from the parahippocampal gyrus inferiorly. The superolateral extension of the transverse fissure is the choroidal fissure. The inferolateral extension of the transverse fissure is the hippocampal fissure, which extends between the dentate gyrus and the subiculum and is often obliterated and not visible on MRI (Duvemoy, 2005).

Location

It lies in the hippocampal sulcus immediately below the floor of the temporal horn of the lateral ventricle, and in cross section (coronal) has appearance that are reminiscent of a seahorse. Hippocampus has a head (posterior to the amygdale), a body, and a tail (which follows the upwardly curving lateral ventricle).

The term hippocampal formation generally applies to the dentate gyrus, fields CA1-CA3 (CA4 is frequently called the hilus and considered part of the dentate gyrus), and the subiculum (parahippocmpal gyrus). The hippocampus proper is made up of CA1, CA2, and CA3 fields (El-Falougy *et al.*, 2008).

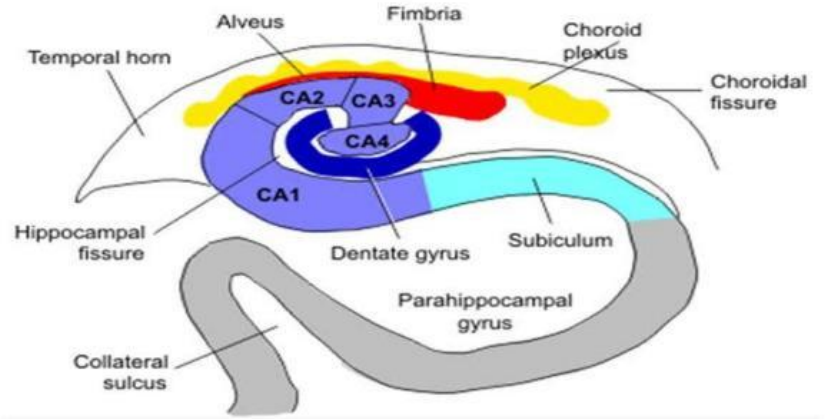


Figure 5: The gross features of the hippocampus (adapted from: El-Fayoug*et al.*, 2008)

Blood supply and drainage

Arterial Supply

Usually, three arteries (or groups of arteries) arising from the main or branches of the

posterior cerebral artery vascularize the hippocampus: the anterior, middle and posterior hippocampal arteries. The anterior hippocampal artery supplies the hippocampal head, whereas the middle and posterior hippocampal arteries vascularize the hippocampal body and tail. The middle and posterior hippocampal artery are richly interconnected with another through the so-called longitudinal terminal segments that run parallel to the course of the hippocampal body. The uncal branch of the anterior choroidal artery is usually anastomosed with the anterior hippocampal artery in the uncal sulcus (Duvemoy, 2005).

Venous Drainage

Intrahippocampal veins, draining in the superficial hippocampal veins, forming two arches, which ultimately drain into the basilar vein. The posterior end draining in the medial arterial vein and the anterior end, inferior ventricular vein, draining in the medial arterial vein.

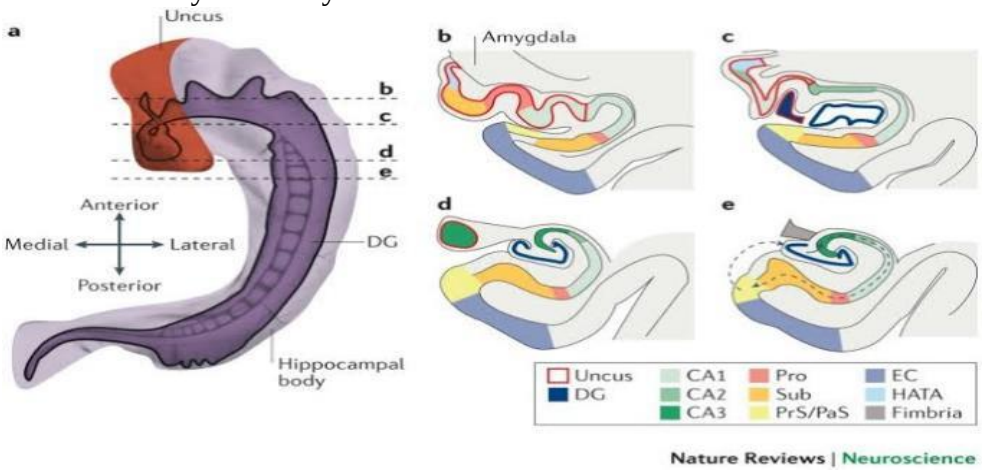


Figure 6: The development of the hippocampus (adapted from: Duvemoy, 2005)

Embryology of the hippocampus

It originates in the isocortex as part of the fifth limbic lobe of the brain in the cerebral hemisphere's medial surface. The hippocampus is considered as part of the olfactory cortex.

The development of the hippocampus

According to Duvemoy (2005), the development of the Hippocampus is thus:

Weeks 13 to 14- unfolded hippocampus, on the medial surface of the widely open hippocampal sulcus (hippocampal fissure).

Weeks 15 to 16- dentate gyrus and cornuammonis start to infold. The hippocampal sulcus remains open. The parahippocampal gyrus is larger and more medially positioned. The CA1, CA2, and CA3 fields of the cornuammonis are arranged linearly. The dentate gyrus has a narrow U shape.

Weeks 18 to 20 – fetal hippocampus begins to resemble the adult hippocampus. The dentate gyrus and cornuammonis is folded into the temporal lobe. The hippocampus and subiculum approximate each other across a narrow hippocampal sulcus. The CA1-3 fields form an arc and the CA4 field increase in size within the widened arch of the dentate gyrus.

3 months – a longitudinal fasciculus of high signal intensity is seen in the white matter beneath the subiculum.

Birth to 2 years - volume increases rapidly.

After 2 years – volume increases slowly thereafter.

Three important changes are necessary for the complex shape and location of the hippocampus:

i. Rotation of the lateral parts of the developing telencephalon dorsocaudally, then ventrally and rostrally, forming the parietal, occipital, and temporal lobes.
ii. The hippocampal sulcus then invaginates into the medial wall of the temporal lobe.
iii. The hippocampal sulcus rotates along a longitudinal axis of the hippocampus, forming a complex structure that is present in the medial aspect of the temporal lobe.

Histology of the hippocampus

The hippocampus consists of the following neural structures: the subiculum (Sb), the regio superior (Rs), and regio inferior (Ri). The most peripheral part of the hippocampus is the subiculum, which connects it with the neocortex. It has a preserved laminar structure, with a marginal layer and cellular layer I and II. These layers are represented by nerve fibers and glial cells. Cellular layer I of the subiculum is formed of relatively well stained neurons of average size, whereas cellular layer II consists of small, multipolar, triangular or oval cells that are slightly less intensively stained. The regio superior is located dorsally with respect to the dentate area and consists of 5 layers.

The stratum oriens is the most external layer, made up of numerous small neurocytes with nerve fibers and glial cells. The second layer is the stratum pyramidale, which is composed of different numbers of layers over its length in the various hippocampal areas. In CA1, for example, there are 3-4 layers, compared to 5-6 in CA3. Difference in the shape, size, and intensity of neuron staining make it possible to determine the boundaries between regions.

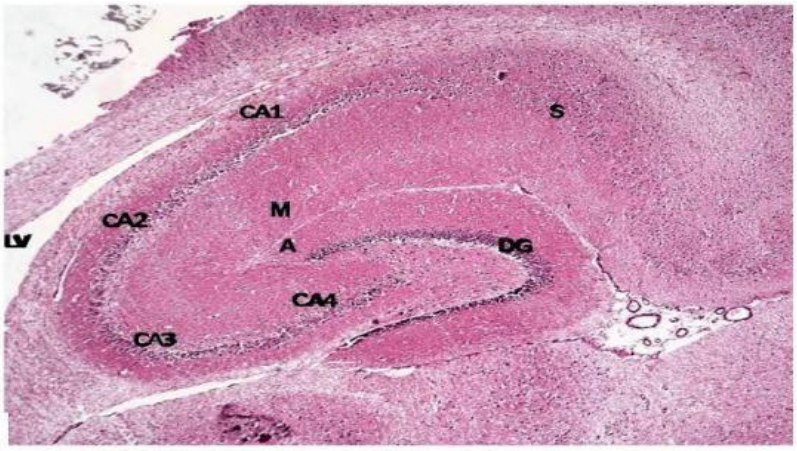


Figure 7: showing the different areas of the hippocampal formation where the hippocampus proper is formed of the cornusAmmonis (CA) as CA1, CA2, CA3 & CA4 regions, and is continued as subiculum (S). Dentate gyrus (DG), is seen surrounding CA4 by its upper & lower limbs. Lateral ventricle (LV) related to CA1 & CA2. M denotes molecular layer inside concavity of CA and of DG (adapted from: El-Fayoug*et al.*, 2008))

Frontally, the regio superior passes into the subiculum and from the other side into the regio inferior. The boundary with the subiculum is made clearly visible by intensively stained cells arranged in layers. This region is composed of small and medium sized, densely packed nerve cells. In CA1 the cells are loosely scattered, whereas in CA2 and CA3/CA4 they adhere closely to

one another. The stratum radiatum is a narrow band of small, loosely scattered nerve cells, between which the neuroglia is localized. There is a relatively thin stratum lacunosum behind the stratum radiatum, composed of single neurons, numerous nerve fibres, and glial cells. The last layer of the regio superior is the stratum molecular. Single small and medium-sized neurons occur between a dense network formed of numerous plexus of nerve fibres and accompanying glial cells. The regio inferior is located between the regio superior and the hilus of the dentate gyrus. Transively, it has the shape of a crescent whose terminal arm faces the dentate area. The regio inferior, like the regio superior, has a laminar structure and consists of the stratum oriens (SO), stratum pyramidale (SP), stratum radiatum (SR), stratum lacunosum (SL), stratum moleculare (SM). The stratum oriens slightly narrows the lumen of the lateral ventricle of the brain. In the external part of this layer nerve fibres compose the alveus of the hippocampus, which passes into the fimbria of the hippocampus. There are numerous glial cells here, and between them single small, triangular or multipolar neurons. The stratum pyramidale is made up of large nerve cells whose size and intensity of staining make it possible to demarcate the boundary between the regio superior and regio inferior. This layer has the characteristic appearance of a crescent over the entire length of the regio superior. Pyramidal neurons of triangular or multilateral shape are larger and more intensively stained in comparison to the stratum pyramidale of the regio superior. The cells are smaller and more densely packed near the hilus (Hil) of the dentate area. The cell has various forms: pyramidal, which is the most prevalent shape, oval, round, granular, extended, stellate, fusiform, multilateral or triangular. The hippocmapal neurons form bands located close to another or loosely scattered. Pyramidal neurons are generally the largest cells (El-Falougy *et al.*, 2008).

Functional anatomy of the hippocampus

The hippocampus plays important roles in the consolidation of information from short-term memory to long-term memory, and in spatial memory that enables navigation. Damage to the hippocampus can lead to anterograde amnesia (loss of the memories). Human have to two hippocampi, one on each side of the brain. The hippocampus has three distinct zones: the dentate gyrus, the hippocampus proper, and the subiculum. The dentate gyrus and hippocampus proper form two C-shaped rings that interlock. The subiculum is thus a trasition zone, linking the hippocampus proper with the dentate gyrus. The hippocampus is closely associated with the amygdale, hypothalamus, septum and mammillary bodies such that any stimulation of the nearby body parts also marginally stimulates the hippocampus. The hippocampus is also very hyperexcitable, meaning it can sustain weak electrical stimulation into long, sustainable stimulation that helps in encoding memory from olfaction, visual, auditory and tactile senses. In lower animals, the hippocampus helps them determine if they will eat certain foods, based on olfactory discernment, avoid danger, respond to sexual invites through pheromones, or react to life and death decisions. Therefore, the hippocampus is a site for decision making and committing information to memory for future safety uses (El-Falougy *et al*, 2008).

Materials and methods

Collection and identification of plant materials

The *Phyllanthus amarus* leaves was collected from farmlands in Awgu local government

area in Enugu state and it was identified and authenticated at the Department of Plant Sciences and Biotechnology, Enugu State University of science and Technology, south East Nigeria.

Preparation of extract

Four thousand five hundred (4,500) grams of *Phyllanthus amarus* leaves was cleaned and air-dried under room temperature until leaves became dry and crispy. Dried leaves were ground to a fine powder in a manual blender. The fine powdered leaves were then used for the flavonoid extraction through column chromatography at Nsukka Local Government Area of Enugu State.

Carbamazepine and Pentylenetetrazol drugs

The Carbamazepine drugs used in the study was obtained from Open Haven Pharmacy, GRA in Enugu metropolis opposite Enugu state university teaching Hospital, Parklane, Enugu State, Nigeria. The pentylenetetrazol was purchased from the Neurophysiology unit of the University of Port Harcourt, Choba, South-south Nigeria.

Routes of administration

Administration of flavonoid was via oral route while PTZ was via intraperitoneal route. Pilot study for dose response trial was carried out to ascertain the appropriate dose for flavonoid extract using Lorke's method (Enegide *et al.,* 2017). Basal parameters was carried out in line with the outcome measures of this study prior to commencement of administration

Experimental animal

Forty-two (42) adult female Wistar rats weighing 140-2000g respectively was purchased from breeding house in Animal House, Faculty of Basic Medical Sciences, University of Cross River State, Okuku campus, Yala Local Government Area, Cross River State. They were housed in a well-ventilated iron cage in the Animal House of the Department of Anatomy, Enugu State University College of Medicine Parklane, Enugu. The rats were maintained under controlled atmospheric pressure, humidity and acclimatized to the environment for 14 days prior to experimental use and were allowed free access to clean water and standard livestock pellets (Guinea Feed Nigeria Limited).

Figure 8: One the researchers handling the experimental animals

Ethical Approval

Ethical approval was obtained from Faculty of Basic Medical Science research ethics committee to carry out this research.

Experimental Design

Thirty-Forty (42) adult Wistar rats was used for this study and they were divided into seven (7) groups with six (6) animals in each (groups A, B, C, D, E, F and G). Group A is the control group, group B was given PTZ only, group C was given PTZ and low dose of Flavonoid fractions, D was given PTZ and medium dose of Flavonoid fractions, E was given PTZ and high dose of flavonoid fractions, F was given PTZ and high dose of flavonoid fractions while animals in group G were given Carbamazepine (a control drug for convulsion) and PTZ. Flavonoid fractions of *Phyllanthus amarus* was administered orally to animals in groups C, D, E, and F for 17 days prior to administration of PTZ from the 18^{th} day to the 21^{st} day. At the commencement of administration of PTZ on the 18^{th} day, the thresh-hold for commencement of convulsion were taken with the aid of a stop-watch. This will measure the interval between administrations of PTZ to commencement of convulsion. It was recorded in seconds. Also, the latency of convulsion was be taken with the aid of a stopwatch. This will measure the duration of convulsion (from onset to termination). It was recorded in seconds.

Table 2: Summary of experimental design

Groups	No of rats	Duration for flavonoid	Flavonoid dosage (mg/kg)	PTZ dosage	Duration for administration of PTZ
A (normal control)	5	NIL	FEED & WATER	NIL	NIL
B (PTZ only)	6	NIL	NIL	33mg/kg	4 days
C (Low dose + PTZ)	6	17 days	100mg/kg	33mg/kg	4 days
D (medium dose + PTZ)	6	17 days	200mg/kg	33mg/kg	4 days
E (High dose + PTZ)	6	17 days	400mg/kg	33mg/kg	4 days
F (High dose)	6	17 days	400mg/kg	NIL	NIL
G (PTZ & drug)	6	17 days	6mg/kg of Tegretol	41.2mg/kg	4 days

Sacrifice and collection of specimen

At the end of the experiment, the animals were anasthesized using 0.5ml of ketamine injection. Sacrifice was via perfusion method and the brain was removed using surgical blades and scalpel. The brain was preserved in 10% formal saline for 24 hours after which the hippocampus was carefully harvested and prepared for histological examination.

Histological studies

The rats' brains were carefully dissected out obtaining the hippocampus and processed through the stages of fixation in formalin. After fixation, they were dehydrated in ascending grades of alcohol and embedded in molten paraffin wax. Coronal paraffin sections cut and sections was mounted onto a gelatin coated slides then stained using Hematoxylin & Eosin (H&E) stain and other special stain. Thereafter, slides were viewed with digital light microscope.

Statistical studies

The data collected were recorded at M ± SED (standard error difference) and were analyzed using SPSS package version 23. ANOVA was used to compare the differences in the means of the various variables. Where significant, a post-hoc analysis using turkey test was carried out.

Results and discussion

Thresh-hold and latency of PTZ

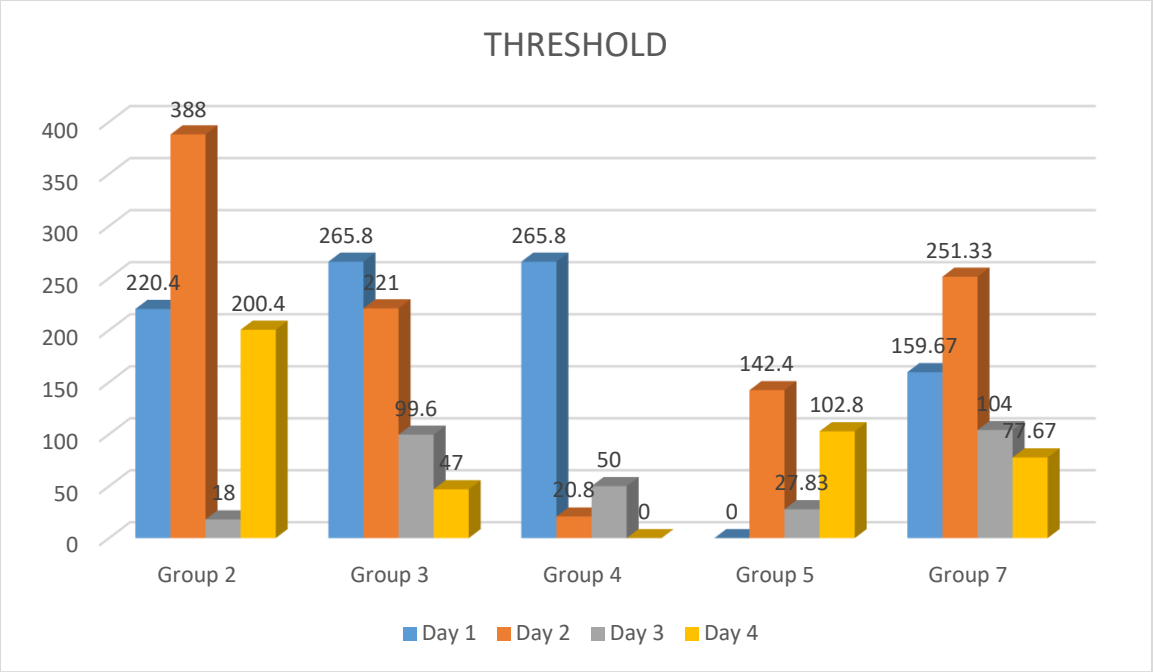


Fig 9: Showing comparison of thresh-hold response of animals across groups 2, 3, 4, 5 and 7 following Pentylenetetrazol induced convulsion. Mean difference is significant at the 0.05 level. P > 0.05 (P=0.305)

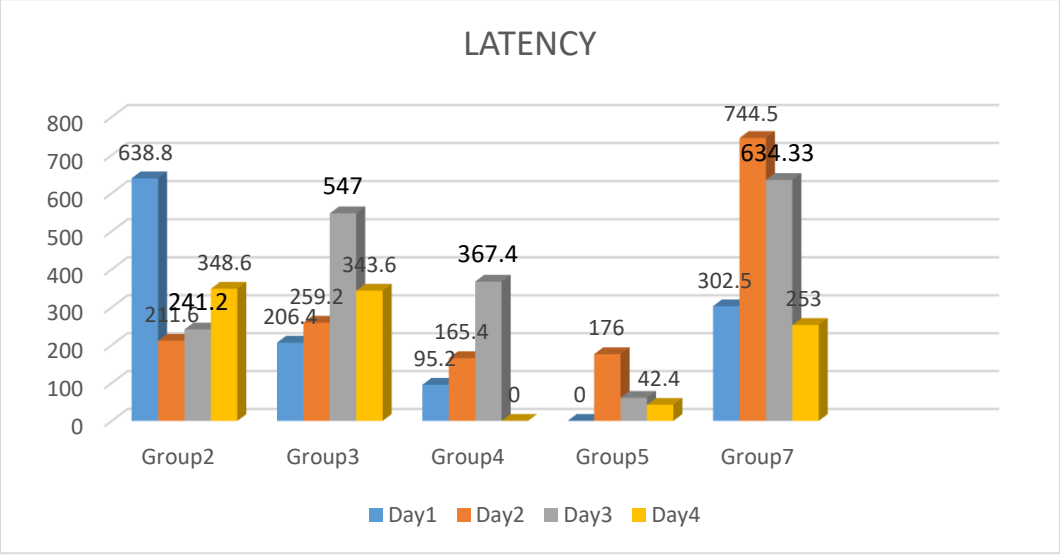


Fig 10: Showing comparison of latency response of animals across groups 2, 3, 4, 5 and 7 following Pentylenetetrazol induced convulsion. Mean difference is significant at the 0.05 level. P < 0.05 (P=0.023).

Table 3: Summary description of thresh-hold and latency scores following Pentylenetetrazol induced convulsion

ONE WAY ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Threshold	Between Groups	1131354.329	19	59544.965	1.166	.305
	Within Groups	4339227.233	85	51049.732		
	Total	5470581.562	104			
Latency	Between Groups	4836639.424	19	254559.970	1.920	.023
	Within Groups	11267776.767	85	132562.080		
	Total	16104416.190	104			

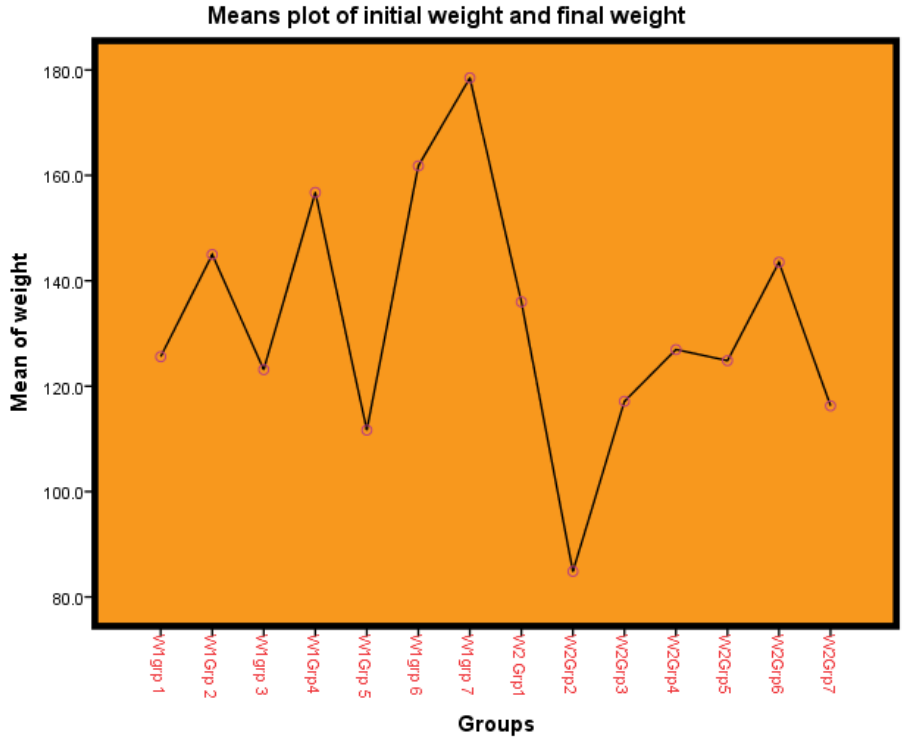


Fig. 11: Showing the mean comparison of weight of animals across all groups

Table 4: Showing description of mean weight of all groups used for this study. P < 0.05.

GROUPS	MEAN	STD. DEVIATION	STD. ERROR	P-Value
1 INITIAL WEIGHT	125.600	3.5777	1.6000	0.545
2INITIAL WEIGHT	144.980	7.9773	3.5675	
3 INITIAL WEIGHT	123.140	69.9136	31.2663	
4 INITIAL WEIGHT	156.760	17.4254	7.7929	
5 INITIAL WEIGHT	111.660	65.3522	29.2264	
6 INITIAL WEIGHT	161.800	5.9749	2.6721	
7 INITIAL WEIGHT	178.500	14.2014	5.7977	

1 FINAL WEIGHT	136.000	4.5277	2.0248	
2 FINAL WEIGHT	84.840	77.6130	34.7096	
3 FINAL WEIGHT	117.120	66.7797	29.8648	
4 FINAL WEIGHT	126.940	72.0401	32.2173	
5 FINAL WEIGHT	124.850	83.5500	41.7750	
6 FINAL WEIGHT	143.517	70.9391	28.9608	
7 FINAL WEIGHT	116.267	91.1027	37.1925	
TOTAL	132.962	56.7332	6.6861	

Histological results

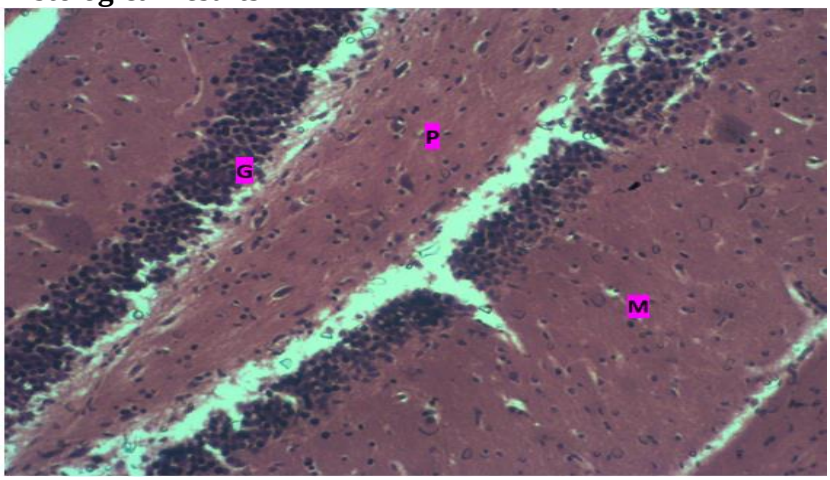
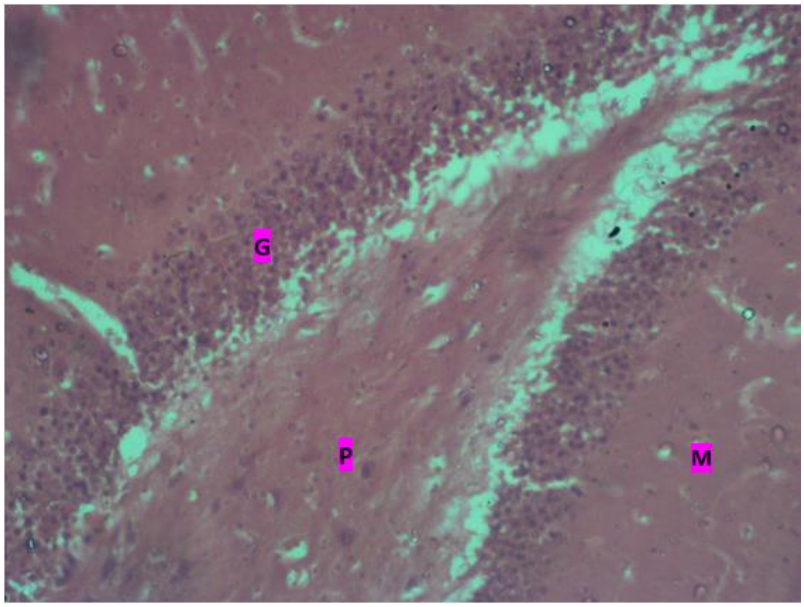


Plate 1: Normal control Group (normal saline)

Group 1A Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture appears normal. H & E. X200



Group 1B Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture appears normal. H & E. X200

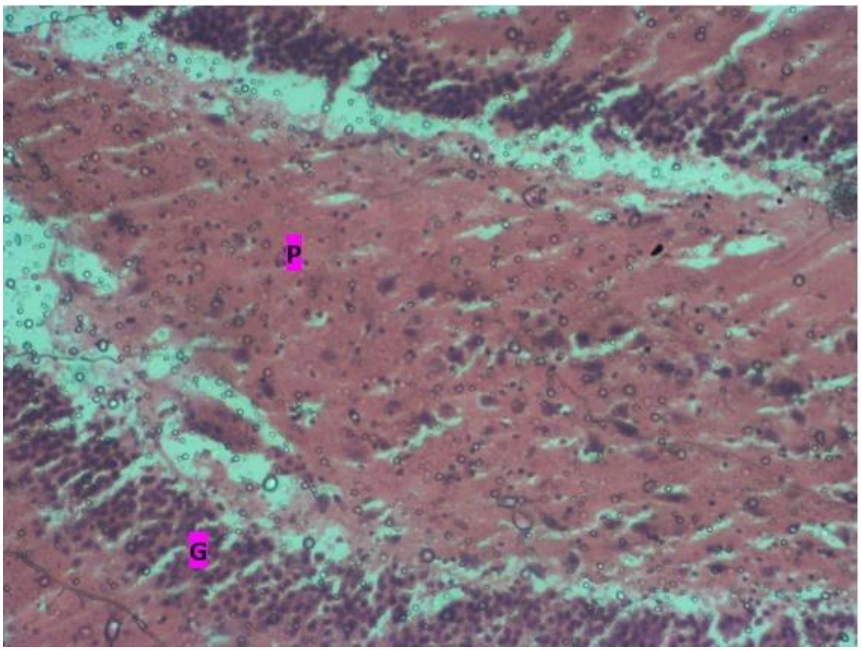
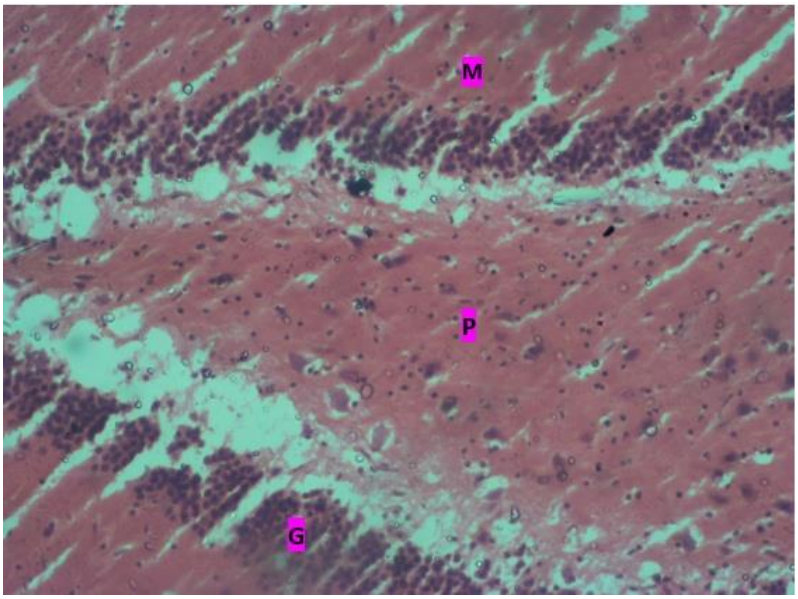


Plate 2: Negative control Group (pentylenetetrazol only)
Group 2A Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture shows general tissue traumatic encephalopathy. H & E.X200



Group 2B Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture shows general tissue traumatic encephalopathy and mild degranulation of the dentate granular layer. H & E. X200

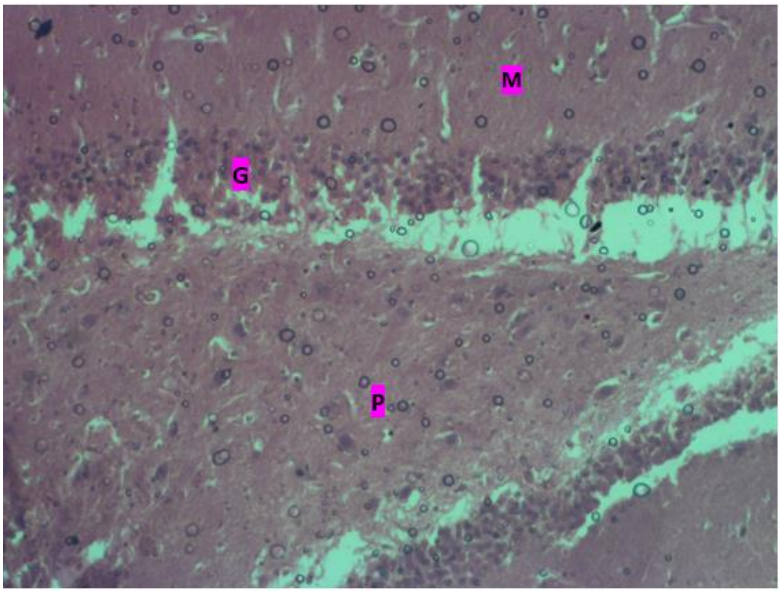
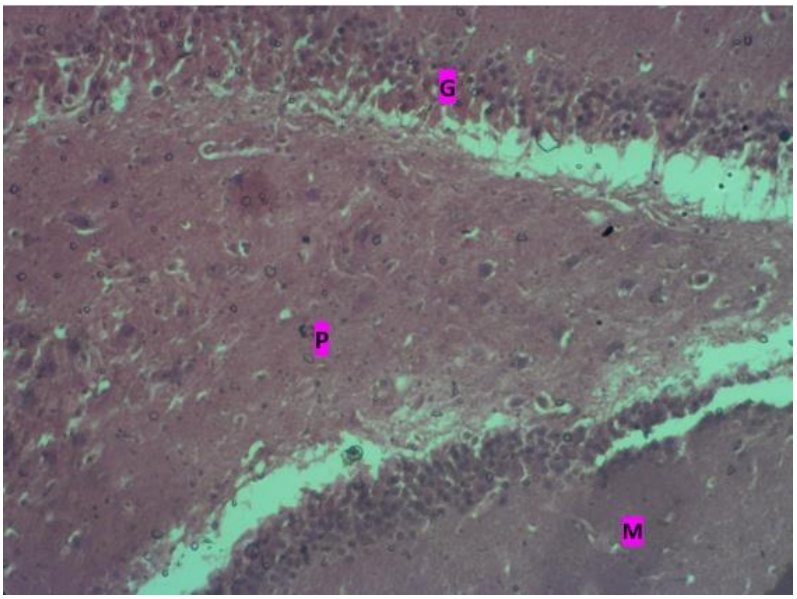


Plate 3: Treatment group (High dose flavonoid and pentylenetetrazol)
Group 3A Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture shows mild degranulation of the dentate granular layer. H & E. X200



Group 3B photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture shows mild degranulation of the dentate granular layer. H & E. X200

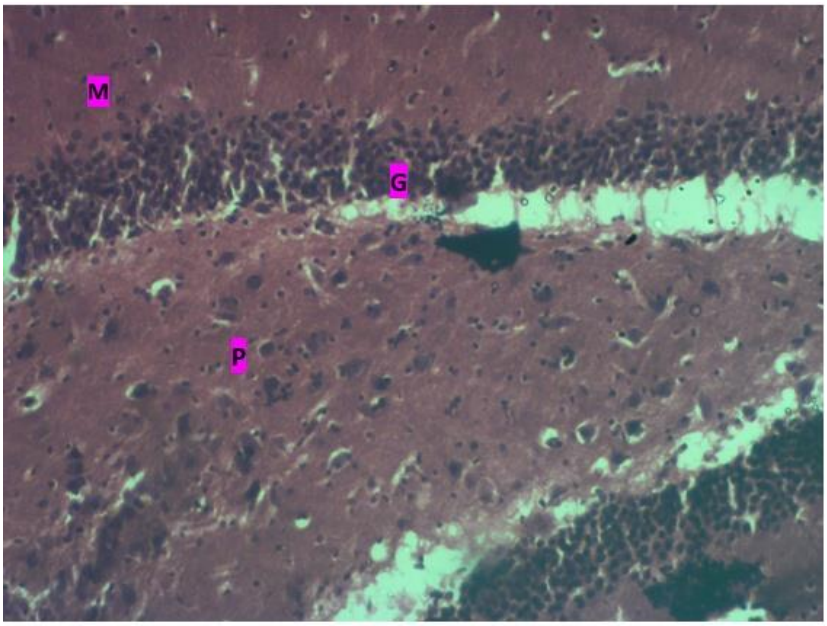
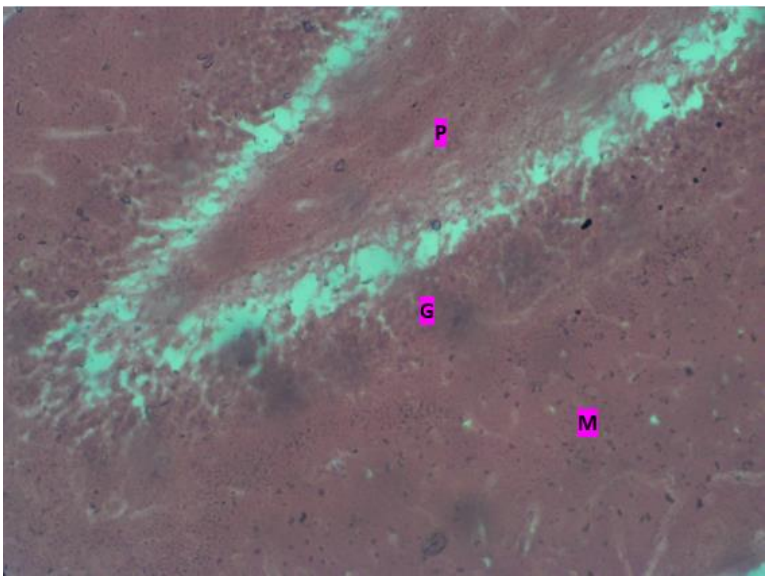


Plate 4: Treatment group (Medium dose flavonoid and pentylenetetrazol)
Group 4A Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture appears normal. H & E. X200



Group 4B Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture shows degranulation of the dentate granular layer. H & E. X200

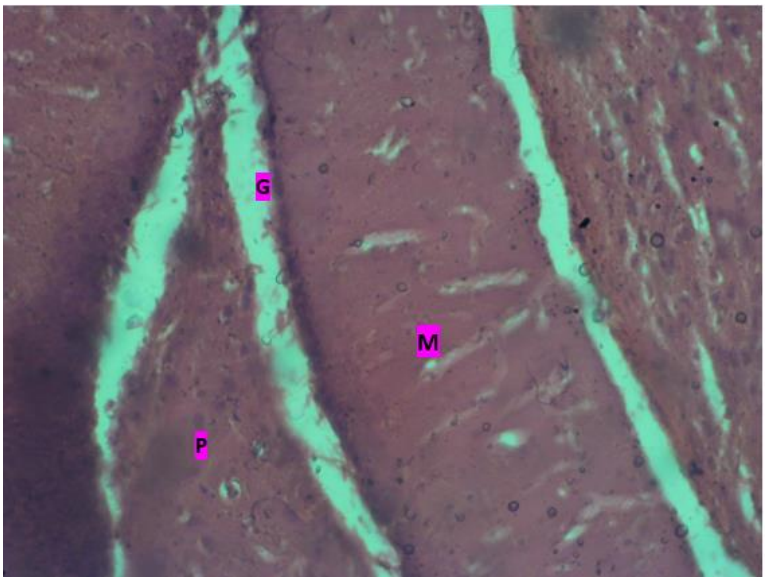
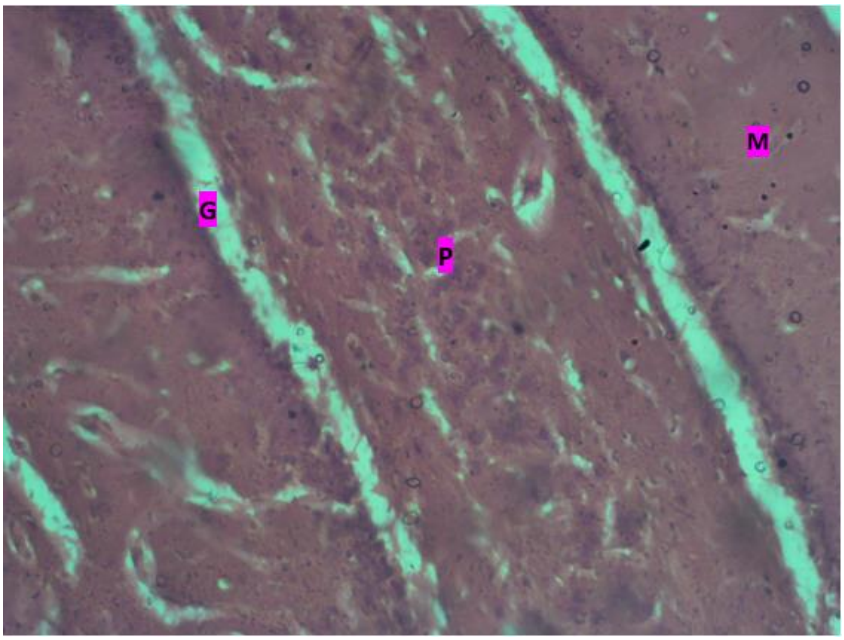


Plate 5: Treatment group (Low dose flavonoid and pentylenetetrazol)
Group 5A
Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture shows degranulation of the dentate granular layer. H & E. X200



GP 5B Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture shows mild degranulation of the dentate granular layer. H & E. X200

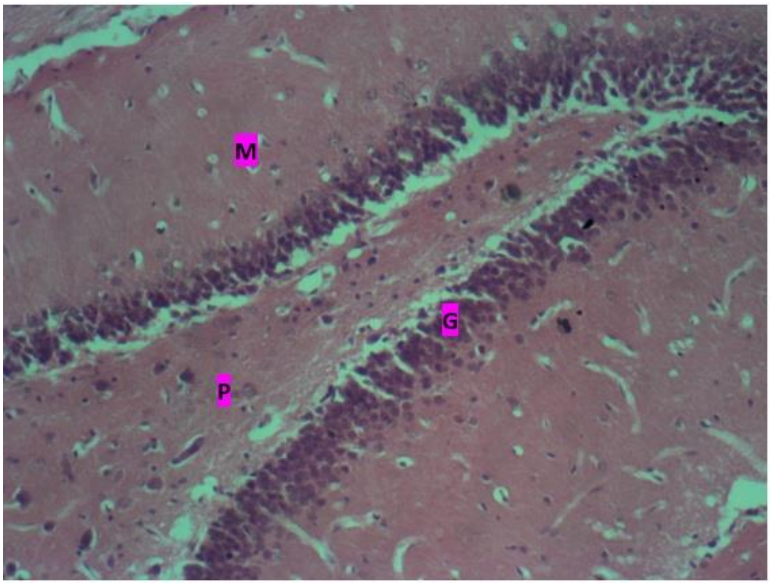
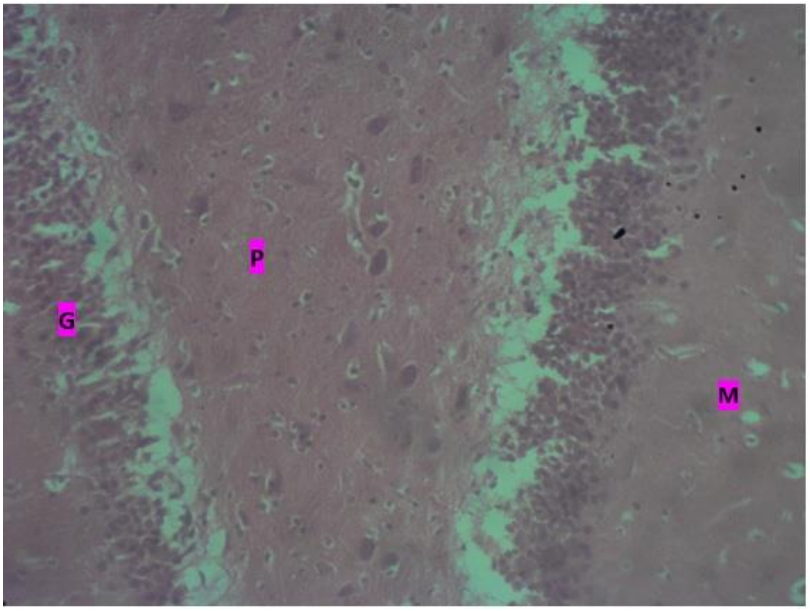


Plate 6: Flavonoid group (*P. amarus* only)
Group 6A Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture appears normal. H & E. X200



Group 6B photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture appears normal. H & E. X200

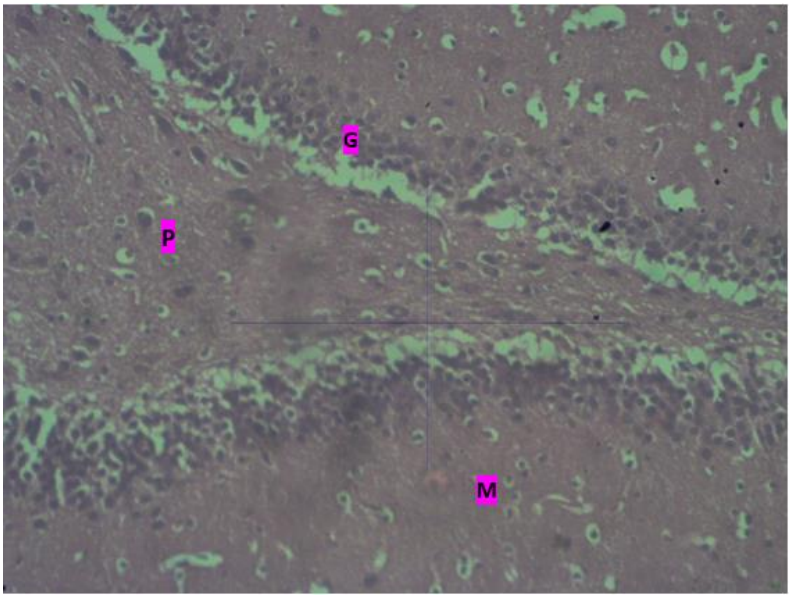
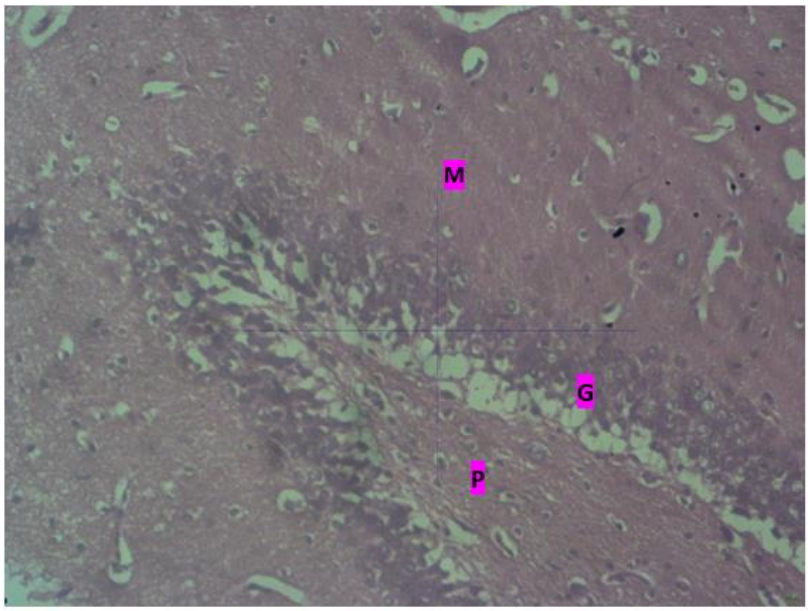


Plate 7: Standard drug group (Carbamazepine)
Group 7A Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture appears normal. H & E. X200



Group 7B Photomicrograph of the hippocampus (dentate gyrus) showing neuronal cells in the dentate polymorphic layer (p), dentate molecular layer (m) and in the dentate granular layer (g). Cyto-architecture appears normal. H & E. X200

Conclusion

This study was carried out to investigate the Anti-convulsive properties of Flavonoid fractions of *Phyllanthus amarus* (PA) leaf on the hippocampus of adult Wistar

rats following/after administration of pentylenetetrazol (PTZ). This study grouped animals used into seven (7) groups with six (6) animals per group. Group 1 were given standard animal feed and water only, group 2 were given 33mg/kg of PTZ only, group 3 were given 100mg/kg of Flavonoid fractions and 33mg/kg of PTZ, group 4 were given 200mg/kg of Flavonoid fractions and 33mg/kg of PTZ, group 5 were given 400mg/kg of Flavonoid fractions and 33mg/kg of PTZ, group 6 were given 400mg/kg of Flavonoid fractions only and group 7 were given 6mg/kg of carbamazepine (a control drug for convulsion) and 41.2mg/kg of PTZ. All data generated in the course of this study were analyzed using SPSS V.23 analytical software with 0.05 considered as level of significance. Mean levels of all quantitative specific objectives were gotten.

Threshold is the interval between immediate removal of syringe used to administer PTZ from the intraperitoneal region and the onset of convulsion. Normally, a drug that has the ability to either reduce or stop convulsion will have a shorter threshold time. Only animals in group 2,3,4,5 and 7 received PTZ. Animals in group 1 were the normal control while those in group 6 were the negative control which were given 400mg/kg of Flavonoid fractions only. Pentylenetetrazol (PTZ) was given for four (4) days following 16 days pre-treatment with Flavonoid fractions.

From findings gotten from this study, animals in group 3 and group 4 had higher threshold response compared to other groups (group 2, 5 and7) and animals in group 5 (given 400mg/kg of Flavonoid) did not show any sign of convulsion and had no threshold response in day one.

In day two, animals in group two had the higher threshold response compared to animals in groups 3, 4, 5 and 7. Also, animals in group 3 and 7 which were treated with 100mg/kg bwt of Flavonoid fractions and 6mg/kg bwt of carbamazepine respectively had higher threshold response compared to animals in group 4 and 5 which were treated with 200mg/kg bwt and 400mg/kg bwt of Flavonoid fractions respectively. Also, in day two of PTZ administration, animals in group 4 given 200mg/kg bwt of Flavonoid fractions had lower PTZ threshold response compared to animals in group 5 treated with 400mg/kg bwt of Flavonoid fractions.

In day three of PTZ administration, animals in group 7 had higher threshold response than animals in group 2, 3, 4 and 5 pre-treated with PTZ only and 100mg/kg bwt, 200mg/kg bwt and 400mg/kg bwt of Flavonoid fractions respectively.

In day four of PTZ administration, animals in group two had a higher threshold response than animals in group 3, 5 and 7. Also animals in group 4 did not show any sign of convulsion and thus had no threshold response.

Inferentially, flavonoid fractions reduced thresh-hold time for onset of convulsion following PTZ induced convulsion. In day one, animals in group 2 showed higher latency response compared to other animals in pre-treatment groups (group 3, 4, 5 and 7). Among the animals in the treatment group, animals in group 7 pre-treated with 6mg/kg of tegretol showed more latency response followed by animals in group 3 pre-treated with 100mg/kg of Flavonoid fractions.

In day two, animals in group 7 showed a higher latency response followed by animals in group 3. In day three, animals in group 7 showed a higher latency response followed by animals in group 3.

In day four, animals in group 2 which were given PTZ showed a higher latency response followed by animals in group 3 which were pre-treated with 100mg/kg of Flavonoid fractions. From all these, it shows that animals in group 2, 3 and 7 has higher

latencies more than animals in group 4 and 5 which were pre- treated with 200mg/kg and 400mg/kg of Flavonoid fractions respectively.

Neuronal loss is a characteristic feature following epileptic seizures. Administration of PTZ induces histological aberration in the brain hippocampus reflected by general tissue trauma encephalopathy and mild degranulation of the dentate granular layer (plate 2,3 and 5) as compared to normal control (plate 1). Histological analysis of the hippocampal region of brain tissue from normal control did not show any tissue trauma encephalopathy and mild degranulation of the dentate granular layer rather cytoarchitecture appears normal (plate 1). For carbamazepine (6 mg/kg) treatment after PTZ-induction in the groups, histological features in the hippocampal region appears normal (Plate 7) as compared to normal control (Plate 1). Treatment with flavonoid of Phyllanthus amaraus (100 and 400 mg/kg) did not produce any significant protection against PTZ-induced histological aberrations in the hippocampal region (Plate 3 and 5). However, the administration of flavonoid (200 and 400 mg/kg) significantly attenuated tissue trauma encephalopathy and mild degranulation of the dentate granular layer in the hippocampal region (Plate 4 and 6) when compared with normal control and drug control group (7) (Plate 1 and 7).

Neuroinflammation is thought to be an essential pathophysiological pathway for the induction of epileptogenesis (Vezzani *et al.*, 2019). An array of stimuli initiate various responses such as activation of endothelial cells astrocytes, microglia, peripheral immune cells, and inflammatory influx, which contributes to the pathophysiology of epilepsy (Webster *et al.*, 2017). Increasing evidence suggests that elevated expressions of various chemokines and proinflammatory cytokines caused structural alteration in the blood–brain barrier and thus cause neuronal hyperexcitability and elevated susceptibility to epileptic seizures (Gorter *et al.*, 2019).

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