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VERNONIA AMYGDALINA ETHANOL LEAF EXTRACT PROTECTS AGAINST TRAMADOL-INDUCED ORGAN DAMAGES THROUGH INHIBITION OF OXIDATIVE STRESS

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Abstract **–** *Vernonia amygdalinaas* has been found to lower blood pressure, reduces body weight, and improve fertility. However, its effects on oxidative stress imposed by opiods has yet to be determined. This research therefore aims to investigate the attenuating potentials of *Vernonia Amygdalina* ethanol leaf extract on oxidative stress biomarkers, following the administration of graded doses of tramadol. Fresh *Vernonia amygdalina* leaf were extracted using ethanol and the extract were stored for use in the experiment. Thirty (30) mature male Wistar rats weighing were used for study. The animals were acclimated for seven days, divided into six groups of five animals in each group. Group 1 received 0.5 ml of normal saline. Group 2, 3, 4, 5 and 6 were given tramadol 30 mg/kg body weight respectively for 12 weeks, however, group 6 was withdrawn after 6 weeks of tramadol administration. Group 3, 4, 5 and 6 also received ethanol extract of *Vernonia amgydalina* at a dose of 250 mg/kg, 500 mg/kg, and 1000 mg/kg body weight respectively for 12 weeks. At the end of the 12 weeks treatment period, the rats were euthanized by cervical dislocation; blood samples were collected and centrifuged to obtain serum for biochemical analysis. Brain, Liver, Pancreas, kidney and testes were excised for biochemical evaluation. The data obtained were analyzed by comparing the values for individual controls for different treatment groups and the results were expressed as mean values ± standard mean error (mean ± SEM). Using the student's t-test, ANOVA variance analysis, and the results were considered significant at P-values of less than 0.01 ($P<0.01$) using SPSS version 23 software, significant differences between control and experimental groups were measured. Results show a significant increase in the activities of non-enzymic antioxidants (Vitamins C, E, and K), carotenoids antioxidants (beta-carotene, lutein, lycopene, and zeaxanthin), thiol antioxidants (Glutathiones, Glutathione peroxidase, and Lipoic acid), oxidoreductase antioxidant (Catalase), metaloenzyme in all tissues of rats given tramadol and treated with *Vernonia Amygdalina* ethanol leaf extract when compared with to the group 2 rats that received only tramadol. Compared to the other groups, group 4 rats likewise show a more pronounced improvement. When compared to group 2 rats that got only tramadol, there was a significant decrease in *Malondialdehyde* activities in all tissues of rats given tramadol and treated with *Vernonia Amygdalina* ethanol leaf extract. *Vernonia amygdalina* was found to be efficacious in reducing ROS-induced tramadol-induced chronic toxicity and organ impairment in Wistar rats. It is suggested that the bioactive chemicals in *Vernonia amygdalina* that function as an adjunct in the chronic therapy of organ harm caused by opioid prescription addiction be identified, isolated, and employed again to validate the findings of this study.

Keywords – Vernonia Amygdalina Extract, Tramadol, Wistar Rat

1.0 INTRODUCTION

The growing non-medical use of prescription drugs is a global health concern (UNODE 2011; Ossai *et al.,* 2021; Ojieh *et al.,* 2022), and such usage can be defined as the taking of prescription drugs, whether obtained by prescription or otherwise, other than in the manner or for the reasons or time period prescribed, or by a person for whom the drug was not prescribed. The real scale of the problem is unknown due partly to lack of data on the non-medical use of prescription drugs, and partly to the existence of many gaps in the monitoring of their legal use for medical purposes as prescribed by health-care professionals (which creates opportunities for the diversion of these drugs to people to whom they were not prescribed). Most studies are on monitoring instruments for substance abuse pertain to the use of illegal drugs especially Opioids, however, the non-medical use of prescription drugs is a unique category of substance use in number of ways and requires attention at different levels (United Nations Office on Drugs and Crime, 2017; Pv *et al.*, 2018).

Advances in the pharmaceutical industry have led to the production of powerful psycho-active medications, which when prescribed appropriately and taken in the manner intended, improve the quality of life of those with specific medical conditions, such as acute pain, palliative care, epilepsy, dependence on opioids and acute anxiety (Usman *et al.,* 2023). However, when used inappropriately, these medications can have serious consequences for health and can lead to dependence (Ossai *et al.,* 2021; Ojieh *et al.,* 2022). In recognition of the problems that may be caused by the inappropriate use of such medication, their use has been regulated by three major drug control treaties which are, the Single Convention on Narcotic Drugs of 1961 as amended by the 1972. Protocol, which was aimed at combating the use of illicit drugs by coordinated international action, the Convention on Psychotropic Substances of 1971, which established an international system of control for the use of psychotropic substances and the United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances (adopted in 1988), which includes legislative and administrative measures against drug trafficking, including provisions against money laundering and the diversion of precursor chemicals. The overall aims of these treaties are to ensure the availability of these medications for medical and scientific purposes only and to prevent their diversion into illicit channels. However drug Poisoning and addiction of illicit drugs most especially Opioids has continue to be the leading causes of mortality and morbidity in many countries (Ossai *et al.,* 2021).

In 1955, poisoning was the most common cause of death in patients aged between 35 and 44 years (Ahmadi *et al.,* 2008), and tramadol had been identified as the most common opoid medication currently been abused by young adult (Ossai *et al.,* 2021, Ojieh *et al.,* 2022).

Tramadol, an opioid analgesic that treats acute or chronic pain (Radbruch *et al.,* 2013), interacts with the brain's reward center by blocking pain signals in the body (Raffa *et al.,* 2012). Long-term use can result in cravings and dependence (Progler, 2010), and People who become dependent on tramadol may suffer from withdrawal, addiction and overdose (Radbruch *et al.,* 2013). Illicit use of tramadol is considered a major public health problem that contributes significantly to the global burden of disease (Whiteford et al., 2013). Long-term use can result in cravings and dependence (Progler, 2010). Tramadol is been used indiscriminately as a sexual stimulants and it has continued to gained wider popularity among young adults without erectile dysfunction (Nna *et al.,* 2016; Ossai *et al.,* 2021). Reasons for the use of tramadol as a sexual stimulants in the absence of erectile disease includes the urge to augment sexual drives, the desire to achieve a harder and long-lasting erection as well as the urge for higher coital frequency and the willingness to delay ejaculation (Bechara *et al.,* 2010, Ossai *et al.,* 2021, Ojieh *et al.,* 2022). People who become dependent on tramadol may suffer from withdrawal, addiction, overdose and several other chronic illness especially related to oxidative stress syndrome (Radbruch *et al.,* 2013)

Recent studies on medicinal plants have shown that they might be effective in treatment of different stages of drug addiction with lower side effects and costs (Sofowora, Ogunbodede and Onayade, 2013). In 2015, Ebrahimie and others review a study on the effect of Iranian herbal medicines on opioid addiction, withdrawal syndrome and confirms that medicinal plants such as *Trachyspermum copticum* L and *Melissa officinalis* decreases the symptoms of withdrawal syndrome in a dose-dependent manner, *Avena sativa*, *Hypericum perforatu*, *Passiflora incarnate*, *Valeriana officinalis*, *Satureja hortensis* L, and *Mentha piperita* have also been proven to have a positive effects on behavior, emotions, and other problems of addicts, decreasing withdrawal symptoms (Ebrahimie *et al.*, 2015). Hence, medicinal plants can be effective in controlling deprivation, decreasing dependency creation, and possibly detoxify opioid addicts, abuse as well as withdrawal symptoms and complications (Ebrahimie *et al.,* 2015).

Vernonia amygdalina commonly referred to as bitter leaf has continued to receive a lot of attention due to the numerous curative potentials and it is purported to possess antioxidant (Iwalewa *et al.*, 2005) and anti-diabetic (Farombi and Owoeye, 2011) activities from radical scavenging (Akah and Ekekwe, 1995; Ayoola *et al.,* 2008; Ojieh *et al.*, 2017). It is one of the most popular herbs in Africa that have been in use for centuries to treat various ailments (Hladik, Krief and Haxaire, 2005; Farombi and Owoeye, 2011). Today, it is still used as a known herbal remedy to treat digestive issues (Akah and Ekekwe, 1995; Farombi and Owoeye, 2011), bleeding, malaria, pile, diabetes and other health issues (Farombi and Owoeye, 2011; Ojieh *et al.,* 2017).

In Nigeria various tribes and languages where it is fondly identify in their locale as follows;

Oriwo	Edo
Chusar doki	Hausa
Ewuro	Yoruba
Onugbu	Igbo and Ukwuani
Etidot	Cross River
Onugbo	Urhobo
Ityuna	Tiv

In some other parts of Africa, it is fondly called;

Awonoo, Awonwene, Jankpantire	Ghana
Mululuza	Luganda
Ndolé	Cameroon

Figure 1: Picture of a fresh *Vernonia amygdalina* plant (In its natural habitat)

Taxonomy

The plant is classified under;

Domain:	Eukaryote
Kingdom:	Plantae
Subkingdom:	Viridae plantae
Phylum:	Tracheophyta
Subphylum:	Euphyllophytina
Class:	Spermatopsida
Subclass:	Asteridae
Orders:	Asteraces
Family:	Compositae
Subfamily:	Cichoriodeae
Tribe:	Vernonieae
Subtribe:	Vernoniinieae
Genus:	*Vernonia*
Specific epithal:	*Amygdalina*
Scientific name:	*Vernonia amygdalina* (Bonsi *et al.*, 1995)

In the tropics where it is found, *Vernonia amygdalina*is used for a variety of purposes. Generally, its use in Nigeria is for two major reasons; first is for nutritional purposes and secondly for its health benefits. The leaves are eaten after being washed and crushed, it is used as vegetable in local dishes e.g. *ofe onugbu* especially in Eastern Nigerian states. The plant is also used as herbal preparation for various kinds of diseases and illnesses. All parts of the plant are of pharmaceutical benefits; the roots are chewed against gastro-intestinal diseases, enteritis, and

anthelmintic. The leaves are chewed to stimulate the digestive system, as well as they reduce fever, the dried flowers are used to treat stomach disorders (Burkill, 1985). In Zimbabwe, the chopped roots of *Vernonia amygdalina*are used for the treatment of sexually transmitted diseases (njovhera) (Kambizi and Afolayan, 2001). Its antifertility/abortificientpotency was shown by Desta (1994). In Eastern Uganda, a survey report showed that it was by far the most widely quoted plant for the treatment of malaria (Tabuti, 2003). The leaves of *Vernonia amygdalina* may be consumed either as a vegetable (in soups) or aqueous extracts as tonics for the treatment of various illnesses. In Ethiopia, the leaves of the plant are used to treat skin wounds by Zay people (Giday *et al.,* 2003). The roots of *Vernonia amygdalina*have been used to treat gingivitis, toothache and consequently its antimicrobial activity was established (Elujoba *et al.,* 2005). The herbal plant of *Vernonia amygdalina* have been studied over the years and found to contain several secondary metabolites which points to its use and potency. Leaf extract of *Vernonia amygdalina* was found to contain reducing sugar, polyphenolics, terpenoids, saponins, alkaloids, cardiac glycosides steroids or triterpenes, anthraquinone and coumarins without cyanogenic glycosides (Ayoola *et al.,* 2008). Tannins, glycosides and saponins without flavanoids could be obtained from its root and bark extracts (Nduagu *et al.,* 2008). Its bitter taste was reported to be due to the presence of antinutritional factors such as alkaloids, saponins, tannins and glycosides (Arhoghro *et al.,* 2009). Phenolic compounds identified in *Vernonia amygdalina* can be grouped into flavonoides, tannins and caffeoyl qunic acid (Salawu and Akindahunsi, 2007). Flavonoids protect the cell as antioxidant against free radicals and reactive oxygen species (ROS). Antioxidant activity of *Vernonia amygdalina* was contributed by the flavonoids which can be extracted from the leaves by using methanol extraction. The plant has mild antimicrobial effect on rumen bacteria and protozoa Newbold *et al.,* (1997). Acetone extract of *Vernonia amygdalina* possesses antibacterial activity towards *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Micrococcus kristinae, Staphylococcus aureus, Enterobacter cloacae* and *Escherichia coli* growth according to Kambizi and Afolayan (2001). The methanol extract of *V. amygdalina* possessed antitrichomon as activity with 100% of inhibition against *Trichomonas vaginalis* (Hakizamungu *et al.,* 1992). The ethanol, petroleum ether, dichloromethane, ethylacetate, acetone-water and isoamyl alcohol extracts of *Vernonia amygdalina,* showed antimalarial activity against *Plasmodium falciparum in vitro* (Tona *et al.,* 2004). This antimalarial effect of *Vernonia amygdalina*is contributed by its active compounds or more specifically sesquiterpene lactones such as vernolepin, vernolin, vernolide, vernodalin and hydroxyvernodalin which exhibited antiplasmodial activity (Tona *et al.,* 2004). Awe *et al., (*1998) reported that Methanol extract of *Vernonia amygdalina*at 100 and 200 mg/kg induced 40 and 50% inhibition against thrombosis in mice. *Vernonia amygdalina* caused reduction of blood pressure and Vernolepin isolated from the plant has been shown to have anti-platelet activity and inhibition of platelet aggregation but the mode of action such as the effect on thromboxane A2 formation and on the level of cyclic AMP in platelets is yet to be unraveled. Vlietinck *et al.,* (1995) showed that Ethanol extract of the fruit (which is rarely found on most of the *Vernonia amygdalina* shrub) possessed antiviral effect on polio virus. *Vernonia amygdalina* ethanol extract was also shown to possess antioxidant activity from radical scavenging test (Ayoola *et al.,* 2008). Total flavonoid and phenolic contents was found to be correlated positively with total antioxidant activity of the plant ( ). Boiling of the plant also reduced its reducing capacity and free radical scavenging property but enhances its taste and reduces its toxicity (Oboh, 2005). Aqueous and n-hexane/isopropanol extract of *Vernonia amygdalina* had been reported to enhance the glucose utilization of muscle and liver cell cultures but not on adipose cells (Erasto *et al.,* 2007). Aqueous (hot water) extract of *Vernonia amygdalina* leaf (500mg/kg) reduced blood glucose concentration of both normoglycemic and hyperglycemic rats induced by alloxan (Osinubi, 2007). Consumption of crude *Vernonia amygdalina* and raw chewing by normal human subjects were found to control post prandial blood glucose without inducing severe hypoglycemia (Okolie *et al.,* 2008).

Toxicity studies showed that *Vernonia amygdalina* only had mild toxic effect when administered at very high concentration (Njan *et al.,* 2008). More importantly, safe consumption of dosage needs to be identified for women at different stages or vitality of pregnancy to avoid abortion since it may induce uterine contraction (Ijeh *et al.,* 2008). *Vernonia amygdalina* had been reported to be safe to consume and is good for health unless it is consumed in very large quantities and the potential danger of taking this plant is much lower than the other common vegetables (Ojiako and Nwanjo, 2006).

Since complementary therapies and medicinal herbs therapy appear to be the trend for most of the world's chronic disease management, results of this study may provide understanding and a frame of reference for knowledgeable and directed use of *Vernonia amygdalina* as a good alternative medication in ameliorating oxidative stress induced by tramadol abuse and addiction, this study therefore seek to investigate the attenuating potentials of *Vernonia Amygdalina* ethanol leaf extract on oxidative stress biomarkers in adult wistar rats, following the administration of tramadol.

2.0 METHODOLOGY

Identification of Plant

Fresh leaf of *Vernonia amygdalina* were collected at its growing habitant in Ogbe-Ikolobie's quarter, Obinomba, Ukwuani LGA, of Delta State, Nigeria and were authenticated in Forestry Research Institute of Nigeria, Ibadan, with herbarium number; FHI 124447. The plants were thereafter transported to the Department of Pharmacology and Therapeutics, Delta State University, Abraka, Nigeria for extraction.

Plant Extraction Process

Following the method of extraction by Ossai *et al.* (2021), the leaf of *Vernonia amygdalina* were washed, air-dried and grounded into a fine powder. The weight of the powder extract were measured with a digital weighing balance Made In China Zhengji (Sn. 2034). The powdered extract (1500 g) were soaked in 6 litres of ethanol for 48 hours. The mixture were filtered using Whatman's No. 1 filtered paper. The extracts gotten were allowed to air dry into a paste and stored in universal bottles and kept in the fridge to be used for the experiment.

Experimental Animals

Thirty (30) adult male Wistar rats, weighing between 120-280 g were purchased from the animal facility of Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State.

Location of Study

The research were conducted at the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University of Abraka, Delta State, Nigeria.

Experimental Condition

The experimental condition were maintained in normal and standard laboratory condition of room temp (24±2^{o}C) and a relative humidity (46±6%) with 12/hours light/dark cycle and adequate ventilation.

Acclimatization Period

The animals were allowed a period of 7days for acclimatization with uninhibited access to food and water. They were fed with standard growers mash diet, a product of Top Feed in Sapele, Delta State.

Ethical Consideration

The protocol of the experiments in this study were examined and approved WITH REF NO. REC/FBMS/DELSU/22/09 by the Research and Ethical Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. This research was performed in accordance with the ethical standards on the care and use of animals as laid down. (Helsinki, 1964).

Drug Use

Purchased

Tramadol were purchased from Demaek Pharmacy, Obiaruku, Ukwuani Local Government Area, Delta State.

Preparation
300 g of tramadol were dissolved in 50 ml of distilled water to yield 6 ml of stock solution

Route of Administration
Oral.

Selection of Doses
The regulatory guidelines on selection of dose for treatment that requires minimal toxicity to allow meaningful evaluation of data by Newall *et al.* (2006) were followed.

Since the LD50 of tramadol is 300 mg/kg body weight (Matthiesen *et al.*, 1998) one-tenth of it which is 30 mg/kg body weight were selected, and the volume given to each animal were calculated as follows

$$\text{Vol.} = \frac{\text{Body weight x one-tenth}}{\text{Stock Solution x Dilution Factor}}$$

Experimental Design
Group 1 (n=5): Wister rats received 0.5 ml of normal saline within the period of the study before euthanizing
Group 2 (n=5): Wistar rats received 30 mg/kg of tramadol for 12 Weeks and were euthanize.
Group 3 (n=5): Wistar rats received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks
Group 4 (n=5): Wistar rats received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks.
Group 5 (n=5): Wistar rats received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks.
Group 6 (n=5): Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks before euthanizing

Sample Collection
At the end of the administration period, rats were euthanize by cervical dislocation, each rat were placed on its dorsal surface, and a laparotomy was carried out to expose the internal organs. Blood were collected via cardiac puncture, using 5ml syringes and 23G needle into plain blood sample containers. The Brain, Pancreas, liver, Kidney and testes were excised for biochemical analysis.

Biochemical Analyses

Estimation of Non-enzymic Antioxidants
The assay for Vit C, E and K activities were carried out using a method adopted by Bergin *et al.* (2021).

Estimation of Carotenoids antioxidants
The assay for beta- carotene, Lutein, Lycompene and Zeaxanthin activities were carried out using a method adopted by Wang *et al.* (2022).

Estimation of Thiol antioxidants
The assay for Glutathione and Glutathione peroxidase activities were carried out using a method adopted by Dorion *et al.* (2021) while that of Lipoic acid were carried out following the method of Prathima *et al.* (2017).

Estimation of Oxidoreductase antioxidant (Catalase) and in Metaloenzyme (Superoxide dismutase) and *Malondialdehyde* (MDA)
The assay for Catalase (CAT), Superoxide Dismutase (SOD) and Malondialdehyde (MDA) activities were carried out using a method adopted by Ojieh (2020).

Statistical Analysis
The data were analyzed by comparing the values for individual controls for different treatment groups and the results were expressed as mean values ± standard error mean (mean ± SEM). Using the student's t-test, ANOVA variance analysis, the results were considered significant at P-values of less than 0.01 ($P<0.01$) using SPSS version 23 software, significant differences between control and experimental groups were measured.

3.0 RESULT

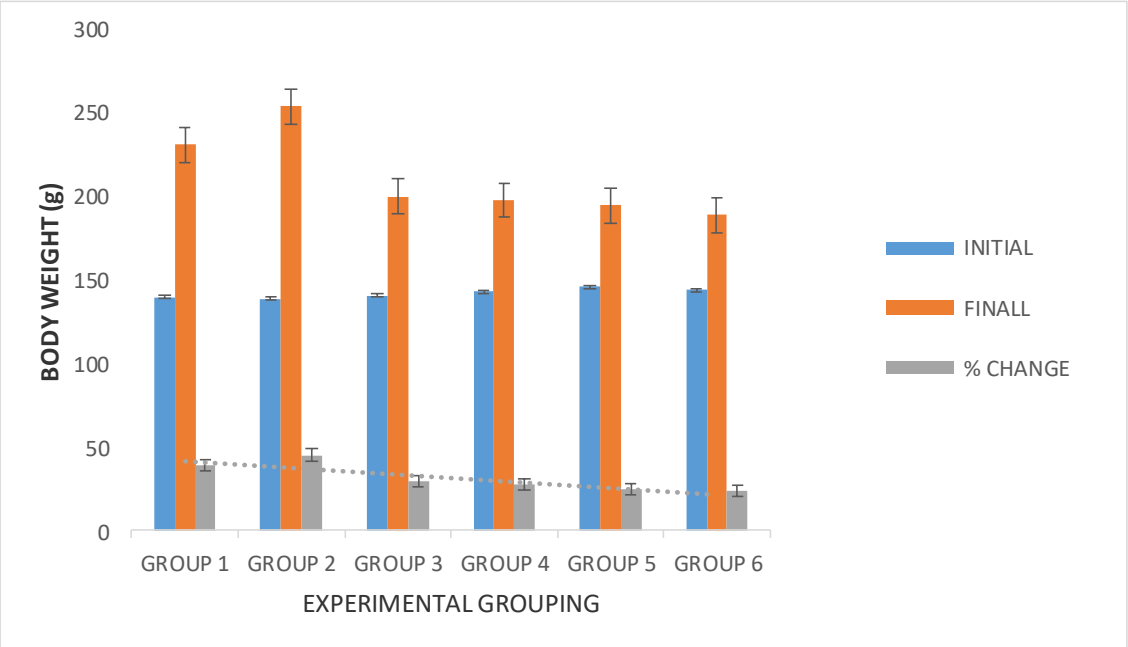

Figure 3.1: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on body weight in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 ($P<0.01$) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

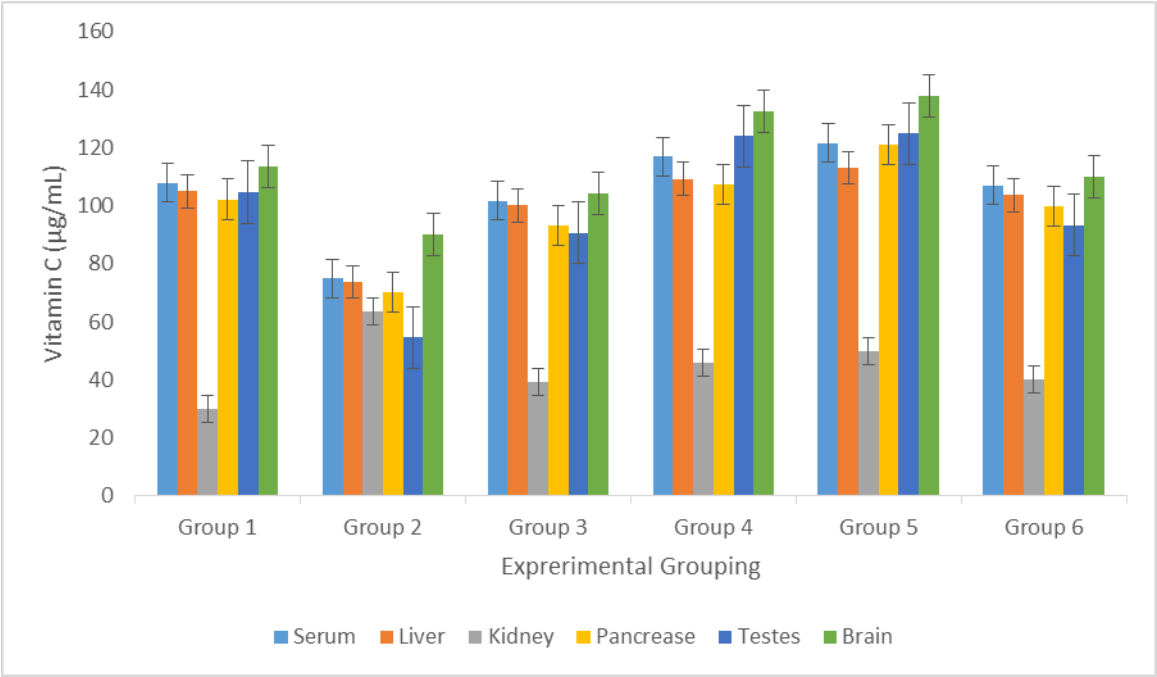

Figure 3.2: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Vitamin C in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

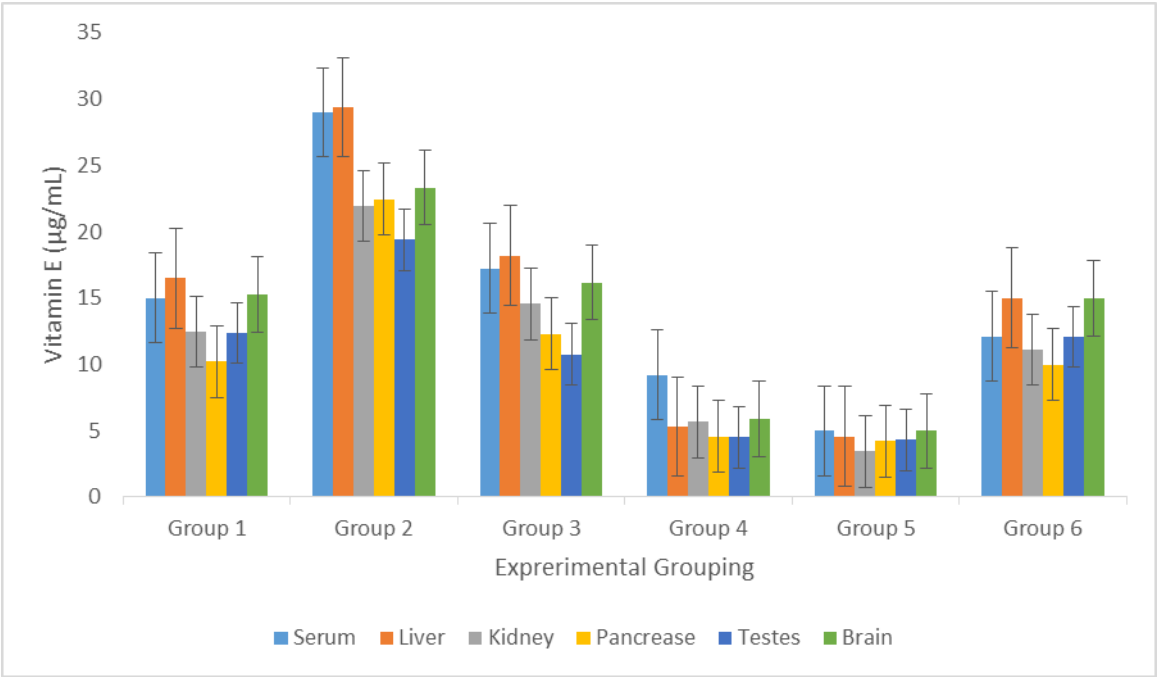

Figure 3.3: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Vitamin E in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

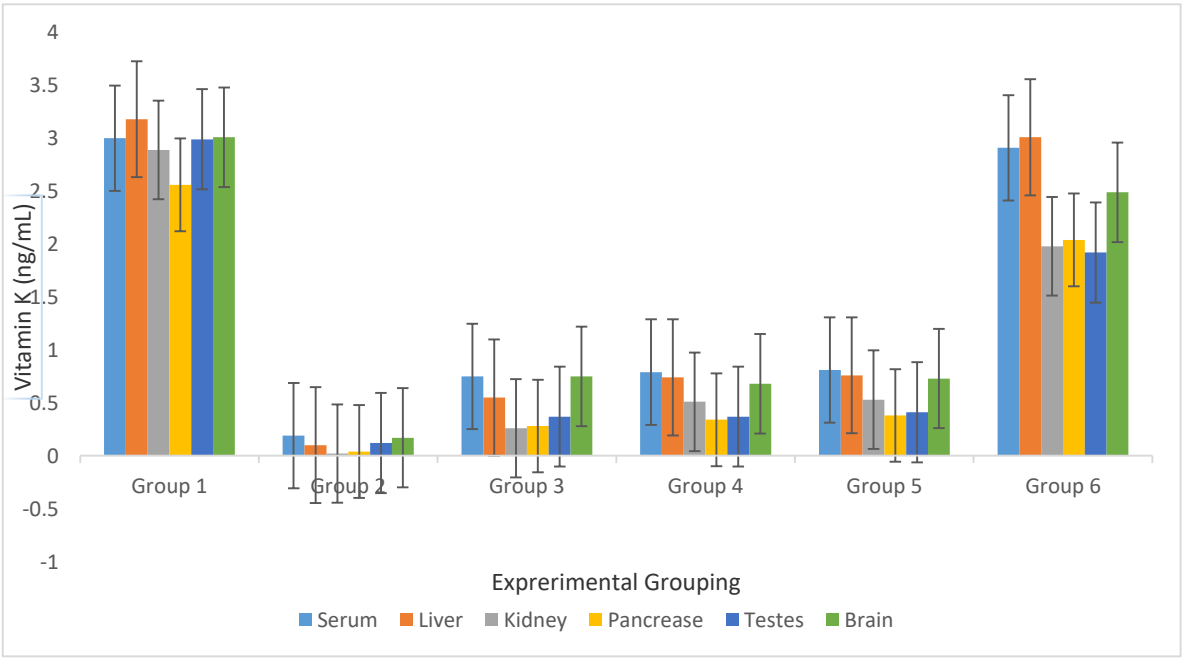

Figure 3.4: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Vitamin K in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

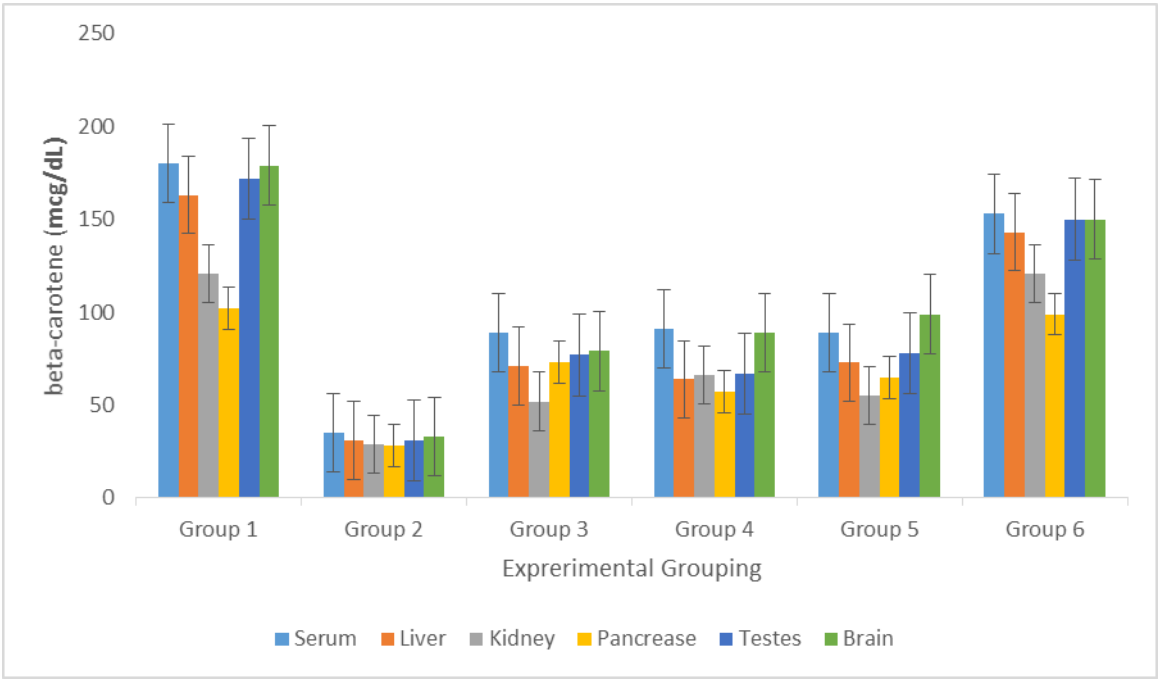

Figure 3.5: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Beta carotene in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 ($P<0.01$) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

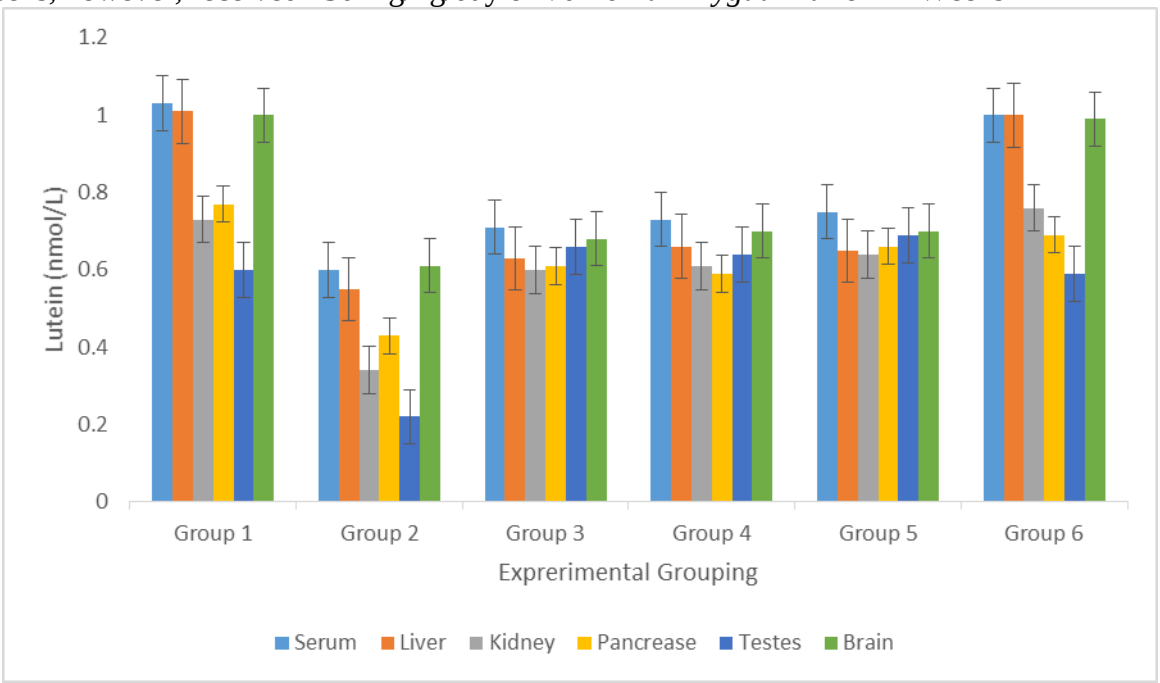

Figure 3.6: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Lutein in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

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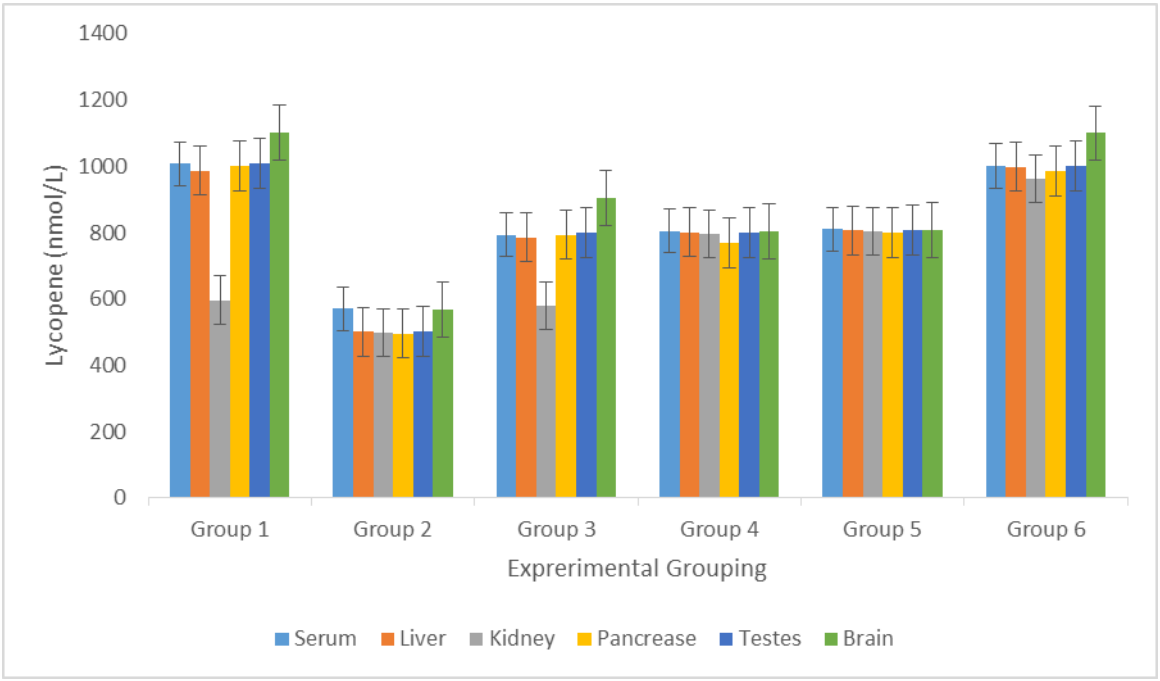

Figure 3.7: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Lycompene in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

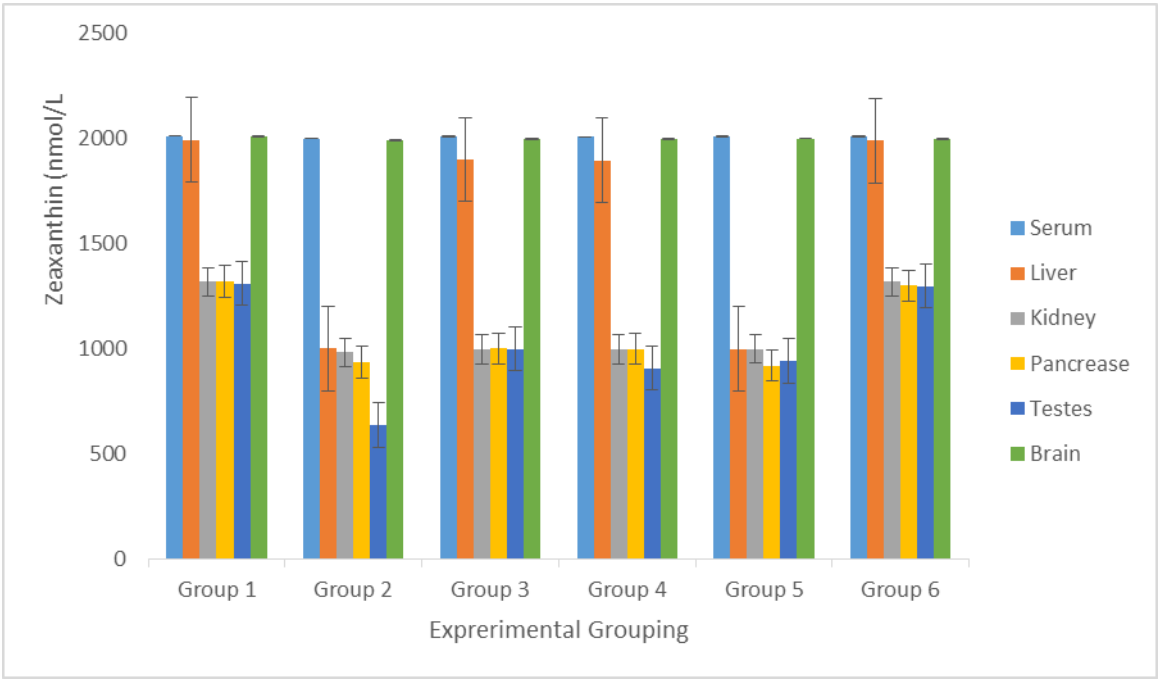

Figure 3.8: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Zeaxanthin in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 ($P<0.01$) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

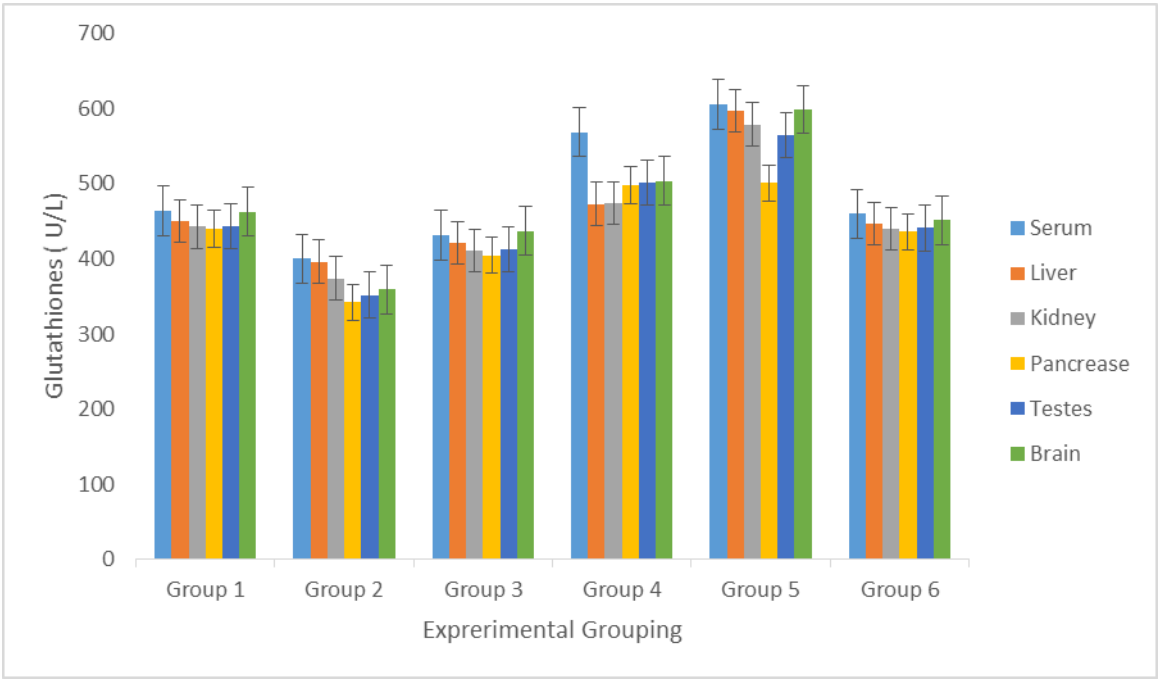

Figure 3.9: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Glutathiones in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

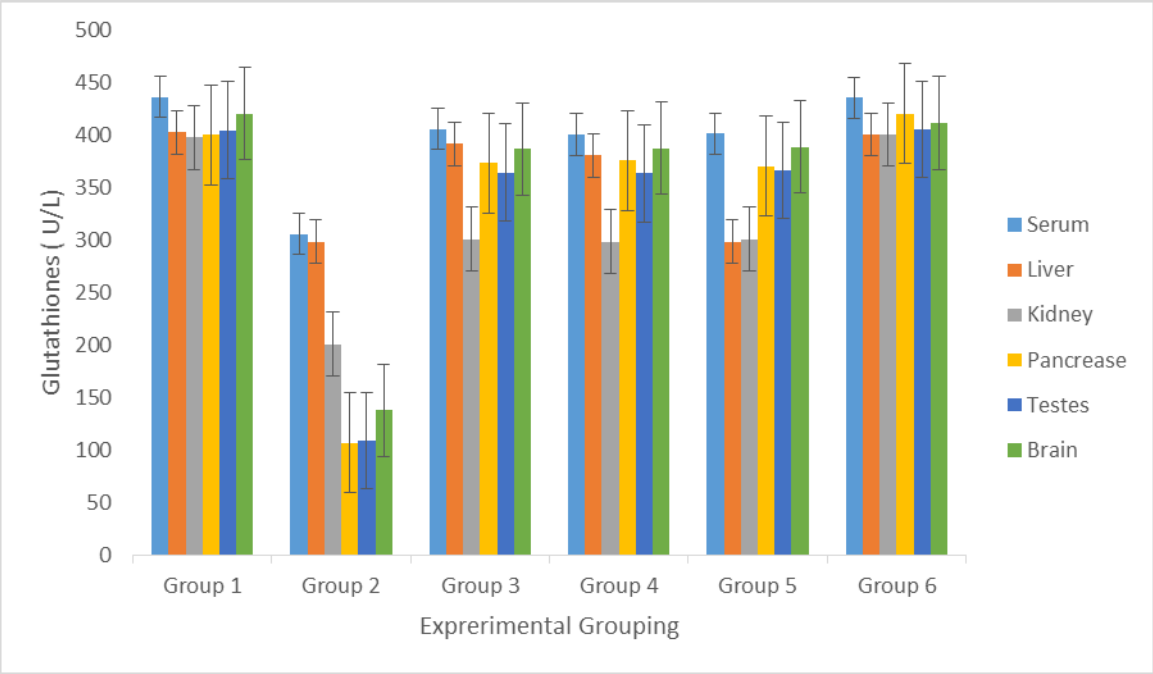

Figure 3.10: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Glutathione peroxidase in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

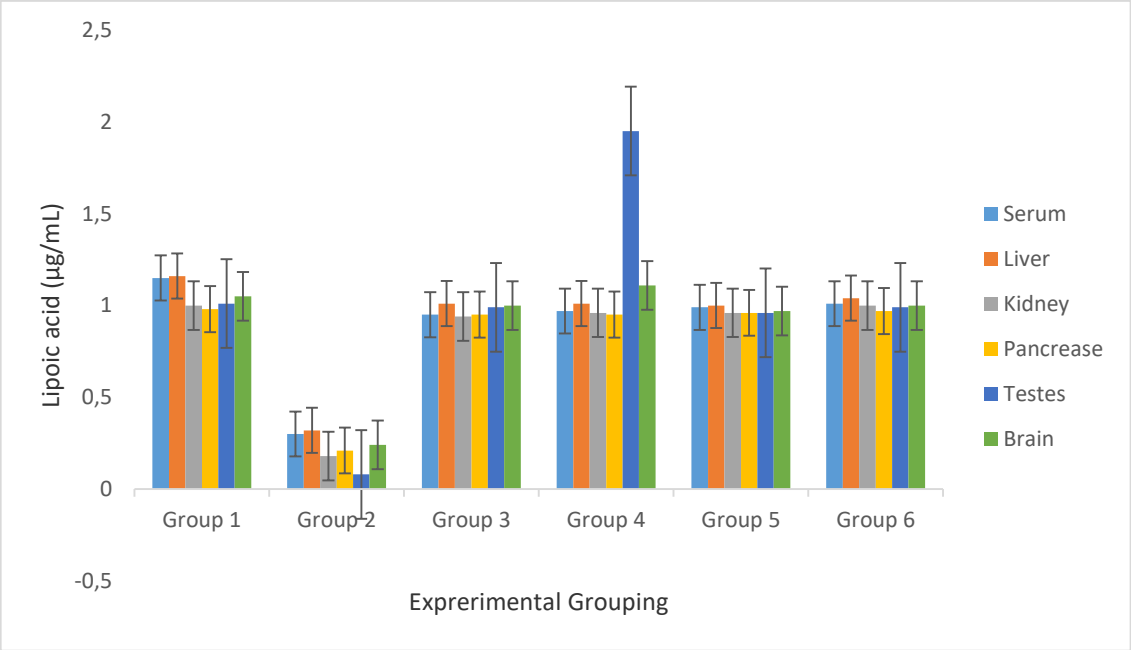

Figure 3.11: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Lipoic acid in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 ($P<0.01$) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

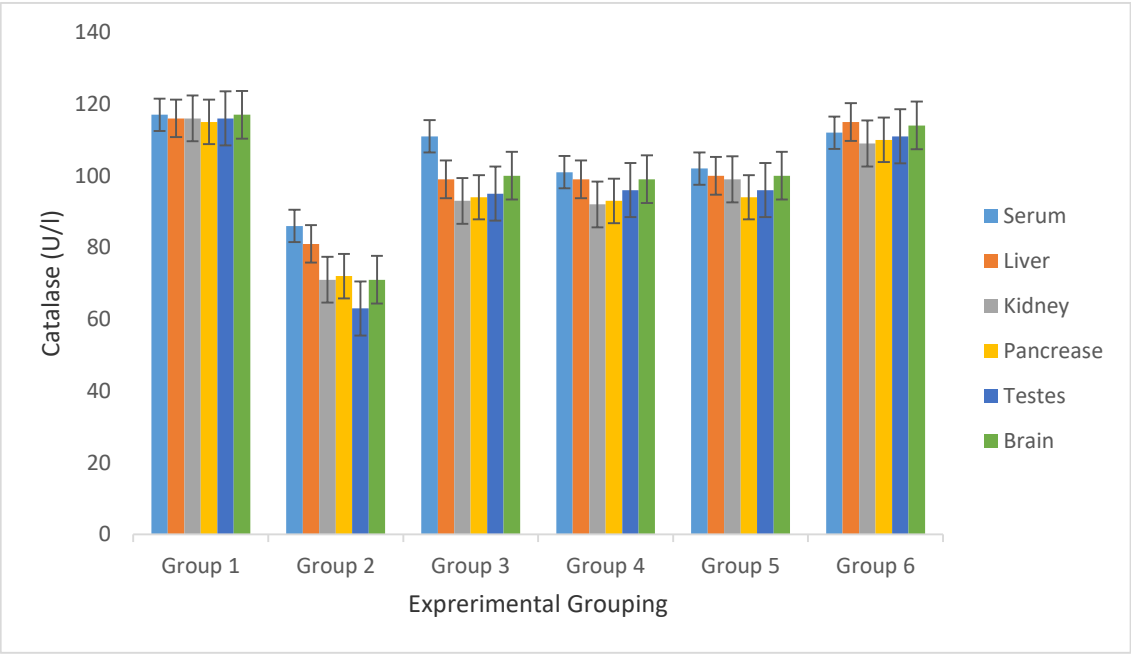

Figure 3.12: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Catalase in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

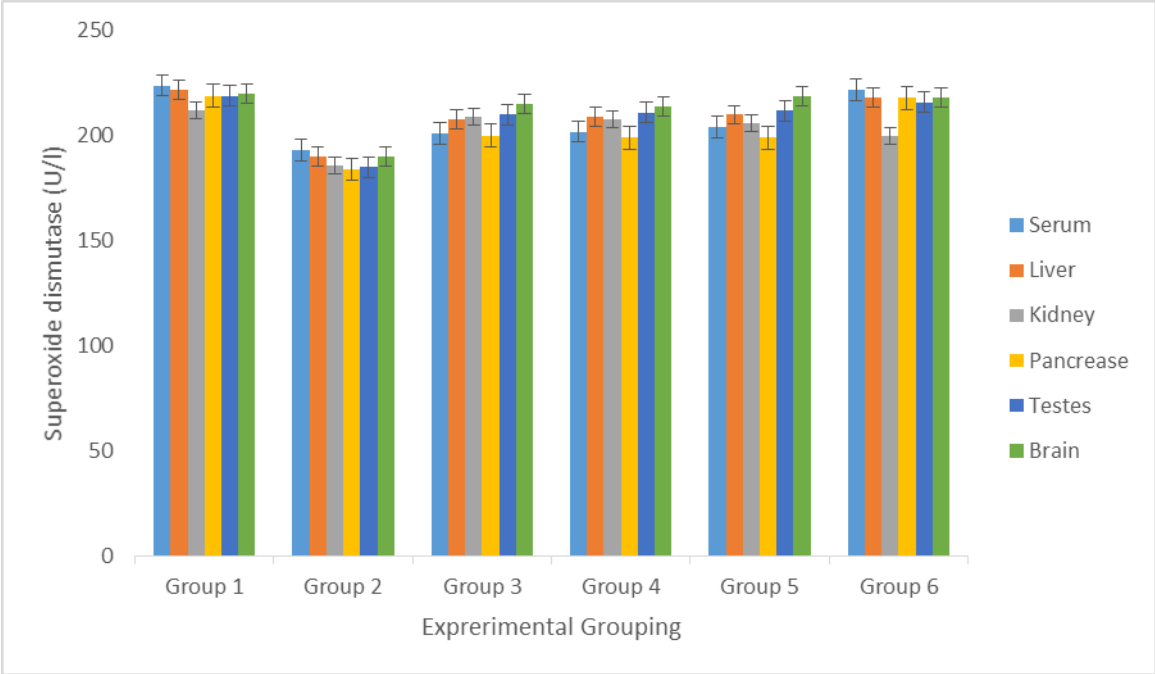

Figure 3.13: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on Superoxide dismutase in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 (P<0.01) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

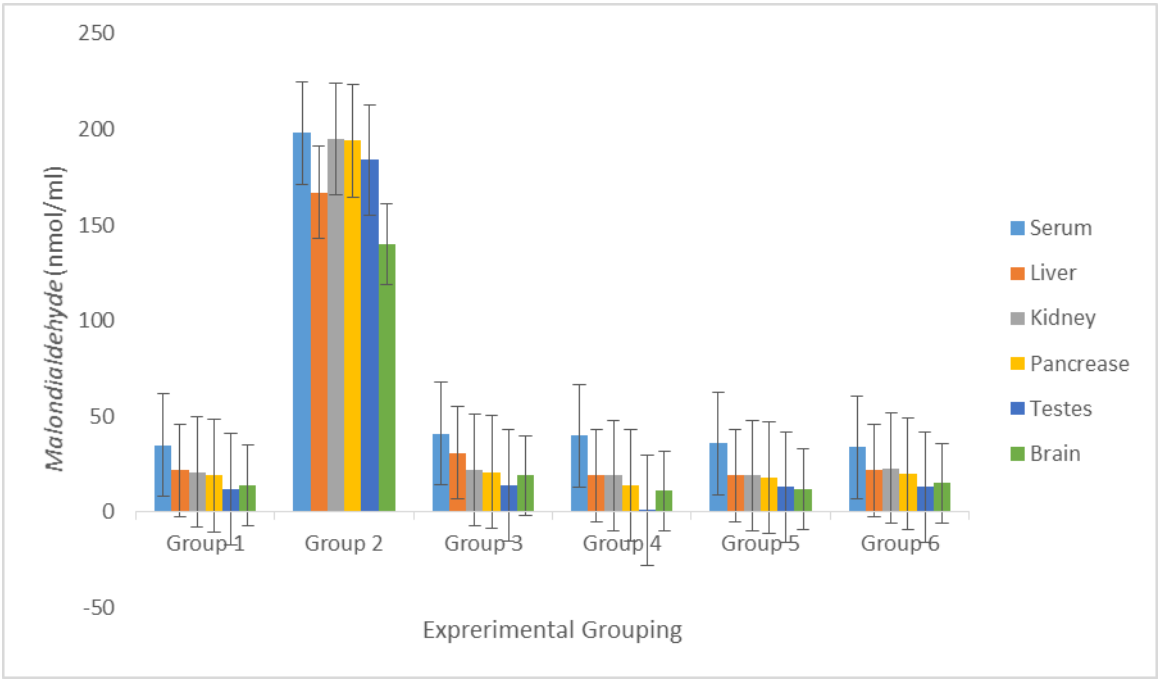

Figure 3.14: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on *Malondialdehyde* in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 ($P<0.01$) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

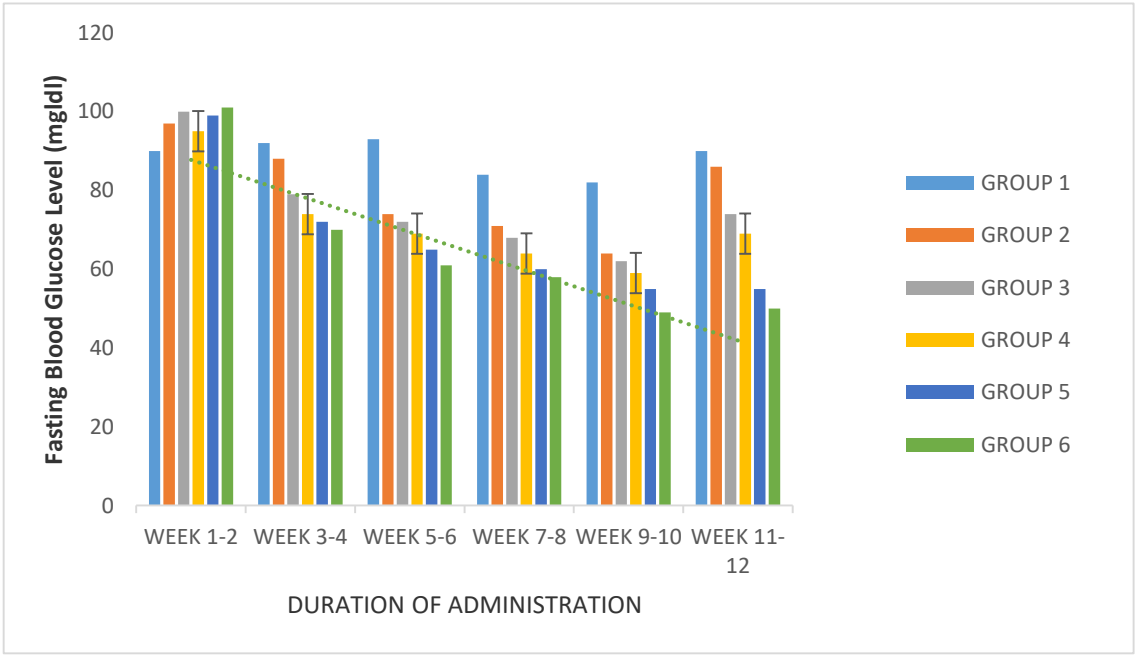

Figure 3.15: The effect of Tramadol and co-administration of different dosage of *Vernonia Amygdalina* ethanol leaf extract on fasting blood glucose level in adult Wistar rats

Data are expressed in mean ± SEM. (n=5) and results were considered significant at P-values of less than 0.01 ($P<0.01$) and the error bars indicates the level of significance.

Key: Group 1: Normal Control received 0.5 ml of normal saline, Group 2 Rats Received 30 mg/kg of tramadol for 12 Weeks, Group 3 received 30 mg/kg of tramadol and 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 4 received 30 mg/kg of tramadol and 500 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 5 received 30 mg/kg of tramadol and 1000 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks, Group 6 rats was Withdrawn for 6 weeks after receiving tramadol 30 mg/kg for 6 weeks, however, received 250 mg/kg/day of *Vernonia Amygdalina* for 12 Weeks

DISCUSSION

Vernonia amygdalina leaves are used in Nigeria as a green vegetable or as a spice in soup, especially in the popular bitter-leaf soup. Such preparation includes freshly harvested leaves which are macerated with either cold or hot water to reduce the bitterness of the leaves to a desirable level. The latter are then added with other condiments for the soup while the water extract may be taken as a tonic to prevent certain illnesses. The leaves can be taken as an appetizer and the water extract as a digestive tonic (Singha, 1965). These are largely consumed by the female Hausas in their belief that it makes them more sexually attractive. *Vernonia amygdalina* had been reported to possess several important medicinal properties which could be helpful in the management of chronic metabolic diseases as well as complications caused by opioids addiction (Aruoma *et al.,* 2006). Predictors of different classes of drugs, such as tramadol is an important research topic considering the soaring global rates of overdoses and abuse among young adults. Amidst the outcry to curb drug overdoses, a comparison of various factors that predict licit or illicit drug misuse/use is warranted. Tramadol use had been reported to cause an overall increase in the body weight as well as organ weight in users (Mohamed and Mahmoud, 2019; Ossai *et al.,* 2021), a report which is in agreement with the result in **figure 3.1** of this study which confirms a significant increase in the body weight of groups 2 rats administered with tramadol (30 mg/kg) only when compared to the normal control group 1 rats. However, administration of *Vernonia Amygdalina* ethanol leaf extract (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively) significantly decreases the body weight in group{s} 3,4, 5 and 6 rats respectively when compared with the negative control group 2 rats that received only tramadol and the group 1 control rats.

Vernonia amygdalina leaves had been reported to possess antioxidant properties against acetaminophen-induced hepatotoxicity and oxidative stress in mice (Iwalokun *et al.,* 2006). The biologically-active compounds of *Vernonia amygdalina* believed had been reported to include saponins and alkaloids (Muraina *et al.,* 2010), terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones and anthraquinone (Cimanga et al., 2004), edotides (Izevbigie, 2003) and sesquiterpenes (Kupchan *et al.,* 1969).

Vitamin C (ascorbic acid) is a required nutrient for a variety of biological functions (Traber and Stevens, 2011). Humans and other primates have lost the ability to synthesize ascorbic acid due to a defect in L-gulono-1,4-lactone oxidase, an enzyme that catalyzes the conversion of L-gulonolactone into ascorbic acid (Traber and Stevens, 2011), however, depend on the diet as a source of vitamin C to prevent the vitamin C deficiency disease, scurvy, and to maintain general health (Traber and Stevens, 2011). The health-promoting effects of vitamin C can be attributed to its biological functions as a co-factor for a number of enzymes, most notably hydroxylases involved in collagen synthesis, and as a water-soluble antioxidant (Traber and Stevens, 2011). Vitamin C can also function as a source of the signaling molecule, hydrogen peroxide, and as a Michael donor to form covalent adducts with endogenous electrophiles in plants (Traber and Stevens, 2011). In **figure(s) 3.2, 3.3 and 3.3, t**here is a significant increase of vitamin C, E as well as vitamin E in serum, liver, pancreas, testes and brain, and significant decrease in kidney of rats in groups 3,4 and 5 administered with *Vernonia Amygdalina* ethanol leaf extract

when compared with the negative control group 2 rats that received only tramadol. However, Group 5 rats that received 1000 mg/kg body weight of *Vernonia Amygdalina* ethanol leaf extract shows a higher concentration of vitamin C when compared to group 3 rats that received 250 mg/kg body weight of *Vernonia Amygdalina* ethanol leaf extract and the group 4 rats that received 500 mg/kg body weight *Vernonia Amygdalina* ethanol leaf extract respectively. No significant difference was observed in the group 6 rats that received 30 mg/kg tramadol for 4 weeks and 250 mg/kg of *Vernonia Amygdalina* ethanol leaf extract when compared with the group 1 positive control rats, however, significant increase was observed when compared with group 2 and group 3 rats respectively.

Beta-carotene, Lutein, Lycopene, Zeaxanthin are important carotenoids potent antioxidants and offer a range of health benefits especially in the eyes (Koo *et al.*, 2014). Overdose as well as abuse of opioids had been reported to cause free radicals generation which can damage the cells of the retina, contribute to aging and lead to the progression of diseases like heart disease, cancer, type 2 diabetes and Alzheimer's disease (Kochhar and Gujral, 2020; Roberto and Dall'Osto, 2021). However, Beta-carotene, Lutein, Lycopene, Zeaxanthin protect the body's proteins, fats and DNA from stressors and can even help recycle glutathione (Nolan *et al.*, 2013). Additionally, Beta-carotene, Lutein, Lycopene, Zeaxanthin antioxidant properties may reduce the effects of "bad" LDL cholesterol, thus decreasing plaque build-up in the arteries and reducing the risk of heart disease (Evans and Lawrenson, 2017).

The human eyes are continually exposed to both oxygen and light, which in turn promote the production of harmful oxygen free radicals (Fernandez and Afshari, 2008). Beta-carotene, Lutein, Lycopene, Zeaxanthin had been reported to have the properties of cancelling out these free radicals and prevent damage to the retina (Edwards, 2016). Results in **figure(s) 3.4, 3.5, 3.6 as well as 3.7** shows a significant decrease of beta-carotene, Lutein, Lycopene, Zeaxanthin, in groups 2 rats administered with tramadol (30 mg/kg) only when compared to the normal control group 1 rats. However, administration of *Vernonia Amygdalina* ethanol leaf extract significantly increases the concentration of beta-carotene, Lutein, Lycopene, Zeaxanthin in group{s} 3,4 and 5 rats respectively when compared with the negative control group 2 rats that received only tramadol. No significant difference of beta-carotene was observed in the group 6 rats that received 30 mg/kg tramadol for 4 weeks and 250 mg/kg of *Vernonia Amygdalina* ethanol leaf extract 12 weeks when compared with the group 1 positive control rats, however, significant increase was observed when compared to group 2 rats respectively

Figure 3.9, figure 3.10 as well as **figure 3.11** showed a significant decrease in Glutathiones, Glutathione peroxidase and lipoic acid levels in groups 2 rats administered with tramadol (30 mg/kg) only when compared to the normal control group 1 rats, however, there is a significant increase of Glutathiones, Glutathione peroxidase and lipoic acid levels in serum, liver, pancreas, testes and brain, and significant decrease in kidney of rats in groups 3,4 and 5 administered with *Vernonia Amygdalina* ethanol leaf extract when compared with the negative control group 2 rats that received only tramadol. However, Group 5 rats that received 1000 mg/kg body weight of *Vernonia Amygdalina* ethanol leaf extract shows a higher concentration of Glutathiones when compared to group 3 rats that received 250 mg/kg body weight of *Vernonia Amygdalina* ethanol leaf extract and the group 4 rats that received 500 mg/kg body weight *Vernonia Amygdalina* ethanol leaf extract respectively. No significant difference was observed in the group 6 rats that received 30 mg/kg tramadol for 4 weeks and 250 mg/kg of *Vernonia Amygdalina* ethanol leaf extract when compared with the group 1 positive control rats, however, significant increase was observed when compared with group 2 and group 3 rats respectively.

In **Figure(s) 3.12 and 3.13,** a Significant decrease in Catalase and Superoxide dismutase activities were observed in groups 2 rats administered with tramadol (30 mg/kg) only when compared to the normal control group 1 rats, however, there was a significant increase of in all the excised tissues and organs of rats in groups 3,4 and 5 administered with *Vernonia Amygdalina* ethanol leaf extract

(250 mg/kg, 500 mg/kg and 1000 mg/kg respectively) when compared to the negative control group 2 rats that received only tramadol (30 mg/kg) for 12 weeks. No significant different was observed in the Group 3 rats that received 30 mg/kg of tramadol and 250 mg/kg *Vernonia Amygdalina* ethanol leaf extract when compared to the group 4 rats that received 500 mg/kg body weight and 500 mg/kg *of Vernonia Amygdalina* ethanol leaf extract and that of the group 5 that received received 30 mg/kg of tramadol and 1000 mg/kg *Vernonia Amygdalina* ethanol leaf extract respectively. No significant difference was observed in the group 6 rats that received 30 mg/kg tramadol for 4 weeks and 250 mg/kg of *Vernonia Amygdalina* ethanol leaf extract when compared to the group 1 positive control rats.

Result in figure 3.14 showed a significant increase of *Malondialdehyde* in groups 2 rats administered with tramadol (30 mg/kg) only when compared to the normal control group 1 rats. However, administration of *Vernonia Amygdalina* ethanol leaf extract (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively) significantly decreases the activities of *Malondialdehyde* in group{s} 3,4 and 5 rats respectively when compared with the negative control group 2 rats that received only tramadol. No significant difference was observed in the group 6 rats that received 30 mg/kg tramadol for 4 weeks and 250 mg/kg of *Vernonia Amygdalina* ethanol leaf extract 12 weeks when compared with the group 1 positive control rats, however, significant decrease was observed when compared to group 2 rats respectively.

Pre-administration of *Vernonia amygdalina* resulted in a dose-dependent had been reported to cause reversal of acetaminophen-induced alterations of all the liver function parameters and suppressed acetaminophen-induced lipid peroxidation and oxidative stress. The study suggested that *Vernonia amygdalina* protected against acetaminophen-induced hepatic damage in mice by antioxidant mechanisms as previously reported by Adesanoye and Farombi (2010).

In this study, *Vernonia amygdalina* protected against tramadol organ toxicity by inducing antioxidant and phase enzymes. The antioxidant activity of *Vernonia amygdalina* may be attributed to the presence of flavonoids, as reported by Igile *et al.* (1994). Using spectroscopic techniques, the study had isolated and characterized the flavonoids occurring in *Vernonia amygdalina*. Three flavones were identified with chemical and spectroscopic techniques namely: luteolin, luteolin 7-*O*-*β*-glucuronoside, and luteolin 7-*O*-*β*-glucoside (Farombi and Owoeye, 2011). Determination of the antioxidant activity of the three flavones had shown that luteolin showed greater activity than the other two. Since flavonoids are established as possessing antioxidant activity (Cook and Samman, 1996; Prabhakar *et al.*, 2006; Farombi and Owoeye, 2011). It can be speculated that the antioxidant properties of *Vernonia amygdalina* can be attributed to the presence of these flavonoids. The advantage of this antioxidant property has been revealed in neurotoxic studies since it has been established that flavonoids can traverse the blood brain barrier (Youdim *et al.*, 2003; Farombi and Owoeye, 2011).

Figure 3.15 confirms the report of Ojieh et al. (2017) on the ability of *Vernonia Amygdalina* to bring about hypoglycemic condition, as a significant decrease in the blood sugar level in groups 2 rats administered with tramadol (30 mg/kg) only when compared to the normal control group 1 rats were observed. However, administration of *Vernonia Amygdalina* ethanol leaf extract (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively) significantly caused a further decrease in blood sugar level in group{s} 3,4, 5 and 6 rats respectively when compared with the negative control group 2 rats that received only tramadol and the group 1 control rats.

Summary of Findings

1. Treatment with *Vernonia Amygdalina* ethanol leaf extract (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively) significantly decreases the body weight in group{s} 3,4, 5 and 6 rats respectively when compared with the negative control group 2 rats that received only tramadol (30 mg/kg).

2. Treatment with different doses of *Vernonia Amygdalina* ethanol leaf extract significant increase of the Non-enzymic antioxidants

(vitamin C, E and K) in groups 3,4 and 5 when compared with the negative control group 2 rats that received only tramadol.

3. Treatment with different doses of *Vernonia Amygdalina* ethanol leaf extract significant increase in Carotenoids antioxidants (beta-carotene, Lutein, Lycompene and Zeaxanthin) in group{s} 3,4 and 5 rats when compared to the negative control group 2 rats that received only tramadol (30 mg/kg) only.
4. Treatment with different doses of *Vernonia Amygdalina* ethanol leaf extract significant increase Thiol antioxidants (Glutathione, Glutathione peroxidase and Lipoic acid) in group{s} 3,4 and 5 rats when compared to the negative control group 2 rats that received only tramadol (30 mg/kg) only

5. Treatment with different doses of *Vernonia Amygdalina* ethanol leaf extract significant increase Oxidoreductase antioxidant (Catalase) and in Metaloenzyme (Superoxide dismutase) of group{s} 3,4 and 5 rats when compared to the negative control group 2 rats that received only tramadol (30 mg/kg) only.

6. Treatment with different doses of *Vernonia Amygdalina* ethanol leaf extract significant decrease in *Malondialdehyde* (MDA) in group{s} 3,4 and 5 rats when compared to the negative control group 2 rats that received only tramadol (30 mg/kg) only.

7. Treatment with different doses of *Vernonia Amygdalina* ethanol leaf extract significant decrease the blood sugar level in group{s} 3,4, 5 and 6 rats respectively when compared to the negative control group 2 rats that received only tramadol and the group 1 control rats.

Contribution to Knowledge

1. Findings from this study confirmed the ethanol leaf extract of *Vernonia Amygdalina* a potent substance that protect the body against oxidative stress caused by Opioids drugs with the 250 mg/kg dose demonstrating the most potent activities.

2. The study also established that one pathway of carrying out this antioxidant action is via increasing the activities of non-enzymic antioxidants, Carotenoids antioxidants, Thiol antioxidants, and Metaloenzyme, while inhibiting *Malondialdehyde (MDA)* activities

3. The study also confirm findings of Ojieh *et al.*, 2017 which claims *Vernonia Amygdalina* as a potent hypoglycemic substance

Conclusion

Our study concludes that ethanol leaf extract of *Vernonia Amygdalina* demonstrated significant antioxidant and hypoglycemic properties and could be used to manage oxidative stress caused by Opioids medications.

Recommendation

Findings from this study shows that *Vernonia Amygdalina* possesses potent antioxidant properties, hence a chromatographic (GCMS-Gas Chromatogram Mass Spectrometric) study is necessary to isolate and estimate the specific compounds present in *Vernonia Amygdalina* extract that may be responsible for these beneficial properties.

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