EFFECTS OF VITAMIN C, E AND GARLIC ON SERUM ENZYMES AND LIPID PROFILE OF TRAMADOL-INDUCED TOXICITY IN WISTAR RATS

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ABSTRACT

This research work was aimed at investigating the effects of vitamin C, E and garlic interaction on tramadol induced toxicity in Wistar rats. Thirty five (35) rats were used for the study and rats were shared into five study groups namely; the positive control group fed with water and rats pellets, the negative control group was induced with tramadol only, the TmVC group in which 0.2mg of tramadol was induced and 0.2ml of vitamin C were administered, The TmVE group in which 0.2 mg of tramadol was induced and vitamin E was administered. The TmG group in which 0.2mg of tramadol was induced and garlic was administered. The body weight, organ weights and serum enzyme activities of the rats were monitored during the experimental period. The enzyme activity level of ALT remained constant in the positive control and negative control groups but began to rise in the TmVC group in which vitamin C was administered and kept rising in the TmVE group and the most significant increase came in the TmG group in which garlic was administered. AST was lower in all of the groups in which tramadol was administered compared to the positive control (group one) with the highest drop in the TmG group in which garlic was administered. ALP, however, showed significant decrease in all the groups except TmG when compared to the positive control at (P<0.05). High density lipoprotein was relatively low in negative control when compared to positive control at (P<0.05). High density lipoprotein was high in antioxidants treated groups compared to negative control at (P<0.05). The low density lipoprotein was high in negative control when compared to positive control. The antioxidant treated groups showed no significant decrease in low density lipoproteins when compared to negative control group.

KEYWORDS: Body Weight Indices, Hepatic Serum Enzymes and Lipid Profile

INTRODUCTION

Tramadol is used to treat acute and chronic pains of moderate to severe intensity. It has a unique dual action of pain relief, acting both as a central opiate agonist and central nervous system (CNS) reuptake inhibitor of norepinephrine and serotonin. Tramadol exist as two enantiomers with analgesic properties, with different mechanisms of action. (+)-Tramadol and it metabolite O-desmethyltramadol (M1) act as selective mu-receptor agonists altering the release of nociceptive neurotransmitters [Grond and Sablotzki, 2004]. The mu activity of tramadol is around 10 fold less than that of codeine with the M1 metabolite having 300 times more affinity for mu receptor compared to its parent compound [Benzon, 2014]. Tramadol is an opioid pain medication used to treat moderate to moderately severe pain. When taken as an immediate-release oral formulation, the onset of pain relief usually occurs within about an hour. It has two different mechanisms. First, it binds to the μ-opioid receptor. Second, it inhibits the reuptake of serotonin and nor epinephrine. Serious side effects may include: seizures, increased risk of suicide, serotonin syndrome, decreased alertness, and drug addiction. Common side effects include: constipation, itchy and nausea, among others. Tramadol is metabolized to O-desmethyltramadol, which is a more potent opioid [Akinola and Ukpanukpong, 2015]. World Health Organization defines tramadol as a centrally acting analgesic with a multiple mode of action. It acts on serotonergic and noradrenergic nociception, while its metabolite O-desmethyltramadol acts on the μ-opioid receptor. Its analgesic potency is claimed to be about one tenth that of morphine. Also (+)-tramadol inhibits serotonin reuptake and (-)-tramadol inhibits norepinephrine reuptake; the reuptake inhibition of serotonin and norepinephrine enhance the inhibitory descending pathways associated with pain transmission in the Central Nervous System [Grond and Sablotzki, 2004]. Tramadol is administered orally, rectally,
have the same high risk for addiction as other opiates, work force of the country. Tramadol is not considered to continuous use and this negatively affects the active active abusers of this substance and suffer most after and otherwise. Mostly the youths of the country are the due to its use as a mild analgesic in preoperative pa in country. It is a substance that can be easily acquir ed government clamping down on the importation into the upring especially in sub Saharan Africa leading to the visiting especially in sub Saharan Africa leading to the government clamping down on the importation into the country. It is a substance that can be easily acquired due to its use as a mild analgesic in preoperative pain and otherwise. Mostly the youths of the country are the active abusers of this substance and suffer most after continuous use and this negatively affects the active work force of the country. Tramadol is not considered to have the same high risk for addiction as other opiates, but it is still addictive. What is common with consumers is that getting pills becomes irrevocable urge for the victim. This goes as far as a person being willing to commit crimes to get pills [Ukpakanuppong et al., 2019].

Vitamins are a class of nutrients that are essentially required by the body for its various biochemical and physiological processes. Mostly, the human body does not synthesize them; therefore, they must be supplied by the diet in the required amount. Vitamins are subdivided into fat-soluble and water soluble vitamins. Fat-soluble vitamins are those that are soluble in fat solvents. Water-soluble vitamins are those, which are soluble in water and include vitamin C and vitamin B complex [Lawani et al., 2014]. Vitamin C (Ascorbic Acid) is a water-soluble antioxidant. It is an unstable, easily oxidized acid and can be destroyed by oxygen, alkali and high temperature. Body requires vitamin C for normal physiological functions. It helps in the metabolism of tyrosine, folic acid and tryptophan. It helps to lower blood cholesterol and contributes to the synthesis of the amino acids carnitine and catecholamine that regulate nervous system [Ukpakanuppong et al., 2018]. It is needed for tissue growth and wound healing. It helps in the formation of neurotransmitters and increases the absorption of iron in the gut. Being an antioxidant, it protects the body from the harmful effects of free radicals and pollutants [Lawani et al., 2014]. Mega doses of vitamin C is used in the treatment and prevention of large number of disorders like diabetes, cataracts, glaucoma, macular degeneration, atherosclerosis, stroke, heart diseases and cancer. Ascorbic acid is metabolized in the liver, and to some extent in the kidney, in a series of reactions. The principal pathway of ascorbic acid metabolism involves the loss of two electrons. The intermediate free radical reversibly forms dehydroascorbic acid, leading to the irreversible formation of the physiologically inactive 2,3-diketogulonic acid. Diketogulonic acid may be either cleaved to oxalic acid and threonic acid, or decarboxylated to carbon dioxide, xylose, and xylulose, leading eventually to xylonic acid and lyxonic acid [Ejoba et al.,2013]. In addition to its anti-scorbutic action, vitamin C is a potent reducing agent and scavenger of free radicals in biological systems [Dua and Luneć, 2005]. Briefly, mono-anion form (ascorbate) is the predominant chemical species at physiological pH. Ascorbate readily undergoes two consecutive, reversible reaction as one-electron oxidations to generate dehydroascorbate (DHA) and an intermediate, the ascorbate free radical. The ascorbate free radical (AFR) is however, a relatively non-reactive free radical, with a reduction potential considerably low compared to the α-tocopherol radical, the glutathione radical and virtually all reactive oxygen and nitrogen species that may be involved in human disease conditions are superoxide anion, hydroxyl radical, hydroperoxyl radicals, singlet oxygen, nitrogen dioxide, nitroxide radicals and hypochlorous acid [Ejoba, 2014].

Vitamin E consists of two families of compounds, the tocopherols and tocotrienols, characterized by a 6-chromanol ring and an isoprenoid side chain. The members of each family are designated alpha, beta, gamma, or delta according to the position of methyl groups attached to the chroman nucleus. Therefore, eight stereoisomers of the large vitamin E family are possible but only the RRR-form occurs naturally. Unlike most nutrients, a specific role for vitamin E in a required metabolic function has not been found. Vitamin E’s major function appears to be as a non-specific chain-breaking antioxidant that prevents the propagation of free-radical reactions. The vitamin is a peroxyl radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) within membrane phospholipids and in plasma lipoproteins. The efficiency of vitamin E absorption is low in humans [IOM, 2000]. In the event that peroxyl radicals (ROO) do form, the action of a chain breaking antioxidant is required to inhibit propagation. Typically, inhibitors are sterically hindered phenols, of which c-tocopherol is a special example. c-Tocopherol (c-TOH) short-circuits the destructive propagative cycle and can intercept the peroxyl radical (ROO.) more rapidly than polyunsaturated fatty acids. The c-TOH donates its phenolic hydrogen atom to the radical and converts it to a hydroperoxide product. The tocopheroxyl radical (a-TOH) that is formed is sufficiently stable to be unable to continue the chain and is removed from the cycle by reaction with another peroxyl radical to form inactive, nonradical products. Although polyunsaturated fat is vulnerable and the most probable target, proteins may undergo free radical peroxidative attack [Graham and Maret, 1990].

The rate at which phenolic antioxidants react with peroxyl radicals is a direct measure of their antioxidant efficiency. It has been determined after a comprehensive survey that c-tocopherol is one of the most efficient
chain-breaking antioxidants available [Graham and Maret, 1990]. For example, it reacts approximately 200 times faster with peroxyl radical than commercial antioxidant, butylated hydroxytoluene (BHT). Furthermore, a chroman head group is entirely responsible for the near-optimal antioxidant properties of c-tocopherol; the phytol tail has shown no influence on antioxidant activity [Atamgba et al., 2015]. Because c-tocopherol can compete for peroxyl radicals much faster than polyunsaturated fatty acids, a small amount of c-tocopherol is able to protect a large amount of polyunsaturated fat. Concentrations of C-tocopherol in biological membrane are approximately one part per 1000 lipid molecules. Therefore, it is regenerated via the tocopheroxyl radical by vitamin C which has been shown to be feasible in vitro or by some other means [Atamgba et al., 2015]. The importance of vitamin E for protecting the integrity of lipid structures membranes in vivo is underscored by the finding that it is the only major lipid-soluble, chain-breaking antioxidant that has been found in plasma, red cells, and tissues [Ukpanukpong et al., 2013]. This finding holds true even in the plasma of children with chronic, severe vitamin E deficiency. Although 3-carotene has chain-breaking antioxidant activity also, it is less efficient than vitamin E and is expected to be important only in regions of very low oxygen partial pressure. Vitamin E absorbed together with lipids, packed into chylomicrons, and transported to the liver with the chylomicrons and the remnants derived thereof [Dasofunjo et al., 2014]. The first phase of the digestion—absorption process is the dissolution of vitamin E in the lipid phase of the meal. This phase is then emulsified into lipid droplets at both gastric and duodenal levels. No vitamin E degradation or absorption appears to exist in the stomach. In addition, the size of the droplets does not seem to have any effect on the efficiency of the subsequent absorption of the vitamin E in healthy humans [Ekam et al., 2019]. In the duodenum, vitamin E is incorporated, along with lipid digestion products, in mixed micelles, structures that are theoretically essential for its absorption by the enterocyte. Indeed, mixed micelles can solubilize hydrophobic components and diffuse into the unstirred water layer (glycocalix) to approach the brush border membrane of the enterocytes [Eteng et al., 2018]. This process is similar for all forms of vitamin E tested. Only after passage through the liver does α-tocopherol preferentially appear in the plasma. Most of the ingested β-, γ-, and δ-tocopherol is secreted into bile or not taken up and excreted in the feces. The reason for the plasma preference for α-tocopherol is its specific selection by the hepatic α-tocopherol transfer protein (α-TTP) [Oduotuga et al., 2016]. The α-TTP not only specifically sorts out the a form of all tocopherols but also has a preference for 2R-stereoisomers. Supplementation studies with differentially deuterated α-tocopherols revealed that the 2R epimers compared with the 2S epimers are preferentially retained in all tissues except the liver [Dasofunjo et al., 2014]. Vitamin E functions as a chain-breaking antioxidant that prevents the propagation of free radical reactions [Aliyu et al., 2016]. Vitamin E, like every redox-active compound, may exert anti- and pro-oxidative effects depending on the reaction partners present. Prooxidative functions of α-tocopherol have been demonstrated in LDL isolated from healthy volunteers [Ukpanukpong et al., 2014]. The role of vitamin E in cellular signaling, especially in relation to protein kinase C, has been studied intensively by Azzi’s group. α-Tocopherol inhibits smooth muscle cell proliferation, decreases protein kinase C activity, increases phosphoprotein phosphatase activity, and controls expression of the α-tropomyosin gene [Atamgba et al., 2015]. Vitamin E prevents loss of spermatogenesis in males and the failure to retain zygotes in female rats. Male infertility also results from selenium deficiency, and could thus be envisaged to support a general antioxidant function of vitamin E in the reproductive system. A synergistic effect of vitamin E and selenium in the protection of biomembranes from oxidative attack has been widely discussed [Berena et al., 2016].

MATERIALS AND METHODS

Experimental animals

Thirty five female Wistar rats were obtained from the animal house of the college of medical sciences, University of Calabar, Calabar, Nigeria. The rats were kept under normal laboratory conditions in cages and were allowed free access to clean water and rat pellets [NIH, 2008]. The animals were allowed two weeks acclimatization and their weights measured before treatment commenced.

Experimental design

The rats were assigned into five study groups of seven rats each. The grouping and treatment given to the rats in each groups were as follows; Group A: Designated NT consisted of positive control rats without any treatment Group B: Designated Tm consisted of negative control rats administered 0.2mg of tramadol Group C: Designated TmVC consisted of rats administered 0.2mg of tramadol and 0.2ml of vitamin C Group D: Designated TmVE consisted of rats administered 0.2mg of tramadol and 0.2ml of vitamin E Group E: Designated TmG consisted of rats administered 0.2mg of tramadol and 0.2ml of garlic. The experimental animals in negative and treatment groups were orally administered 0.2mg of tramadol concomitantly with their respective antioxidants for 28 days.

Sacrifice of animals

At the end of the experimental period, the rats in each study group were fasted overnight and sacrificed under anesthesia.

Enzyme assay

Determination of L-alanine aminotransferase (ec 2.6.1.2) activity

L-alanine aminotransferase (ALT) activity was estimated by the method of [Reitman and Frankel, 1957]. The method measures spectrophotometrically the intensity of the coloured hydrazine formed from the reaction of pyruvate with 2, 4-dinitrophenylhydrazine at 546nm. The assay was based on the reaction of the enzyme below

\[
\text{Glutamate + pyruvate} \rightarrow \alpha\text{-ketoglutarate + Alanine}
\]

The keto acid reacts with 2,4-dinitrophenyl hydrazine to form hydrazine

Determination of L-aspartate aminotransferase (ec 2.6.1.2) activity

The assay was based on the reaction of the enzyme below

\[
\text{L-Aspartate + α-ketoglutarate} \rightarrow \text{Oxaloacetate + L-Glutamate}
\]

\[
\text{L-Glutamate + pyruvate} \rightarrow \alpha\text{-ketoglutarate + Alanine}
\]
The oxaloacetate produced by transmission activity of AST is spontaneously decarboxylated to pyruvate which then reacts with 2,4-dinitrophenyl hydrazine. The intensity of the red colour formed is a measure of transaminase activity.

**Determination of alkaline phosphate activity**

Alkaline phosphate activity was assayed according to the method described by [Bassey et al., 1946] and modified by [Wright and Plummer, 1974]. Phenol was released by enzymatic hydrolysis from disodium phenylphosphate under defined conditions of time, temperature and pH. This reacts with 4-aminoantipyrine in the presence of alkaline oxidizing agent to give a red coloured compound, which is estimated at 405nm against a blank reagent. Colour development is rapid and stable for at least an hour in bright light. Sodium hydroxide was added immediately after incubation to raise the pH and stop the reaction.

**LIPID PROFILE**

**Total Cholesterol Determination**

Cholesterol was enzymatically measured in a series of coupled reactions that hydrolyze cholesterol esters and oxidize the 3-OH group of cholesterol. One of the reactions by products, $\text{H}_2\text{O}_2$ was quantitatively measured in a peroxidase catalyzed reaction that produces colour while absorbance was measured at 500nm. The colour intensity was proportional to the cholesterol concentration. The reaction sequence is as follows;

\[
\text{Cholesteryl ester} + \text{H}_2\text{O} \rightarrow \text{Cholesterol} + \text{Fatty acid}
\]

\[
\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminophenazine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} 4\text{-} \left(\text{p-benzoquinone-monoimino}\right)\text{-phenazone} + 4\text{H}_2\text{O}
\]

**Triacylglycerol (TAG) Determination**

Triacylglycerol was enzymatically measured in serum or plasma using series of coupled reactions in which triglycerides were hydrolysed to produce glycol. Glycerol was then oxidized using glycerol oxidase and $\text{H}_2\text{O}_2$. One of the products was measured as described above for cholesterol. Absorbance is measured at 500nm. The reaction sequence is as follows.

\[
\text{Triglycerides} + 3\text{H}_2\text{O} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol – 3 – phosphate} + \text{ATP}
\]

\[
\text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{Glycerophosphate oxidase}} \text{Dihydroacetonephosphate} + \text{H}_2\text{O}
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminophenzone} + \text{chloro Phenol} \xrightarrow{\text{Peroxidase}} 4\ \left(\text{p-benzoquinone-monoimino}\right)\text{-phenazone} + 4\text{H}_2\text{O} + \text{HCl}
\]

**High Density lipoprotein Determination**

HDL was measured directly in serum. The apoß containing lipoproteins in the specimen reacted with a blocking reagent that rendered them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoß containing lipoprotein were excluded from the assay and only HDL – cholesterol was detected under the assay conditions. The method uses sulphated α-cyclodextrin in the presence of Mg$^{2+}$, which formed complexes with apoß containing lipoproteins, polyethylene glycol-esterase and cholesterol oxidase for the HDL-cholesterol measurement. The reactions are as follows;

1. Apoß controlling lipoproteins + α cyclodextrin + Mg$^{2+}$ + Dextran SO$_4$ \xrightarrow{\text{PEG- cholesterol esterase}} Soluble non-reactive complexes with apoß containing lipoproteins

2. HDL –cholesteryl esters \xrightarrow{\text{PEG- cholesterol oxidase}} HDL- unesterified cholesterol fatty acid

3. Unesterified Cholesterol + O$_2$ \xrightarrow{\text{HDL-Cholestenone} + \text{H}_2\text{O}_2}

4. H$_2$O + 5-aminophenone + N-ethyl-N-(3-methylphenyl)-N-succinyl ethylene diamine + H$_2$O +

5. H$^+$ peroxidase \xrightarrow{\text{Quinoneimine dye} + \text{H}_2\text{O}} Absorbance was measured at 600nm.

**Low Density Cholesterol Determination**

Most of the circulating cholesterol was in three major lipoprotein fractions, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density cholesterol (HDL).

\[
\text{[Total cholesterol]} = \text{[VLDL cholesterol]} + \ [\text{LDL cholesterol}] + \ [\text{HDL cholesterol}]
\]

LDL was calculated from measured values of total cholesterol, TAGs and HDL-cholesterol according to the relationship:

\[
[\text{LDL-cholesterol}] = \ [\text{Total Cholesterol}] + \ [\text{HDL} – \text{cholesterol}] – \ [\text{TG}]/5
\]
where \[\text{TG}/5\] was an estimate of VLDL-cholesterol and all values were expressed in mg/dl. LDL carries most of the circulating cholesterol in man and when elevated contributes to the development of atherosclerosis. LDL-cholesterol was measured to assess risk for Coronary Heart Diseases and to follow the progress of patients being treated to lower LDL-cholesterol concentrations. Desirable levels of LDL-cholesterol are those 130mg/dl in adults and 110mg/dL in children.

**Statistical analysis** The data collected were analyzed using students t-test and values expressed as Mean ± SEM.

**RESULTS**

The result of the effect of tramadol-induced toxicity on body weight indices, lipid profile and selected serum enzymes in Wistar rats is presented in the figures below.

![Graph showing the effect of tramadol and antioxidants vitamin C, E and garlic interactions on the body weight.](image1)

**Figure 1.0:** Graph showing the effect of tramadol and antioxidants vitamin C, E and garlic interactions on the body weight.

**Value are expressed in mean ± SEM of 7 determinations**

NT: Group I, positive control, no tramadol administered
TM: Group II, negative control, only tramadol administered
TmVC: Group III, tramadol and vitamin C administered
TmVE: Group IV, tramadol and vitamin E administered
TmG: Group V, tramadol and garlic administered

![Graph showing organ weight indices.](image2)

**Figure 2.0:** Graph showing the effect of tramadol and antioxidants vitamin C, E and garlic interactions on organ weight.
Figure 3.0: Graph showing the effect of tramadol and antioxidants vitamin C, E and garlic interactions on the serum enzymes.

Value expressed in mean ±SEM of 7 determinations
NT: Group I, positive control, no tramadol administered
TM: Group II, negative control, only tramadol administered
TmVC: Group III, tramadol and vitamin C administered
TmVE: Group IV, tramadol and vitamin E administered
TmG: Group V, tramadol and garlic administered

![SERUM ENZYME ACTIVITY](image)

Figure 4.0: Graph showing the effect of tramadol and antioxidants vitamin C, E and garlic interactions on the lipid profile

Value expressed in mean ±SEM of 7 determinations
NT: Group I, positive control, no tramadol administered
TM: Group II, negative control, only tramadol administered
TmVC: Group III, tramadol and vitamin C administered
TmVE: Group IV, tramadol and vitamin E administered
TmG: Group V, tramadol and garlic administered

![LIPID PROFILE](image)
DISCUSSION

The research was carried out to evaluate and compare the effects of Vitamin C, vitamin E and garlic on some selected hepatic serum enzymes and lipid profile of Tramadol induced toxicity in Wistar rats within the experimental period. The body weight of the negative control group decreased significantly when compared to other groups where there was an increase in the final body weight. There was a significant increase in the positive control group while the antioxidants vitamin C, E and garlic treated group showed significant increase in body weight compared to the negative control group. There was a significant increase in the weight of the liver of Tramadol (TM) administered rats from that of the rats in the positive control (NT) group. With the addition of vitamin C in the TmVC group, the weight remained constant but decreased when compared with vitamin E was added to tramadol in the TmVE group to below the weight recorded in the positive control group. In the TmG group the weight of the liver rose again after garlic was added. The weight of the heart remained fairly constant when all five groups were compared and the weight of the kidneys of the rats in the positive control group remained lower than the weights of the kidneys in the other four groups in which tramadol was administered with the most significant increase coming in the negative control (TM) group. The study agreed with [Bassey et al, 2020] who reported that intervention of alcohol on rats under experiment showed significant decrease in body weight indices as there will be obvious physiological changes occasion by loss of appetite. Serum enzyme activity of ALT remained constant in the positive control and negative control groups but began to rise in the TmVC group in which vitamin C was administered and kept rising in the TmVE group and the most significant increase came in the Tm G group in which garlic was administered. The serum enzyme activity of AST was lower in all of the groups in which tramadol was administered compared to the positive control (group one) with the biggest drop coming in the TmG group in which garlic was administered. Serum enzyme activity of ALP was lower in all of the groups except TmG when compared to the positive control. There was significant decrease in the cholesterol level of the negative control compared to the positive control group at (p<0.05). This was due to the fact that the rats in that group lost a lot of weight due to the effect of tramadol toxicity. The total cholesterol content of the antioxidants vitamin C, E and garlic increased significantly when compared to that of the negative control at (p<0.05). The level of TAG in the negative control group rose significantly when compared to the positive control group at (p<0.05). HDL also decreased in the negative control group when compared to the positive control at (p<0.05) but LDL increased in the negative control group compared to the positive control group at (p<0.05). And extracts treated groups showed no significant increase in LDL at (P<0.05) when compared with negative control. This findings is in line with Ukpanukpong et al, [2017] who reported that Laranthus bengweusis leaf extract was able to decrease LDL in treatment groups but with an attendant increase in negative control group.

CONCLUSION

Vitamin C, E and Garlic had varying effects on the serum enzyme levels of Wistar rats in tramadol induced toxicity. The ALT levels remained the same both in rats that were not administered tramadol and in those that were. However, the levels rose significantly when vitamins C, E and also garlic were added. AST levels on the other hand dropped from the value for the rats that were not given tramadol and continued dropping even after vitamin C, E and garlic were administered. ALP levels dropped upon the administration of tramadol in the negative control group, it rose very little when vitamin C was added in the TmVC and then dropped upon the addition of vitamin E in the TmVE group before rising finally when garlic was administered in the TmG group. So, findings in this experiment showed that tramadol administration may compromise the integrity of cell membrane and this amount sharp rises in enzyme activity level but antioxidant vitamins C, E and garlic exhibited ameliorating effect over tramadol.

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