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**Assessment of the Hepatoprotective Effects of Aqueous Extract of *Sesamum Indicum* Seeds on Tramadol Induced Liver Toxicity in Wistar Rats.**

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**ABSTRACT**
The liver is vulnerable to drug-related toxicity or injury because it is the primary site of drug metabolism. Additionally, a number of potentially dangerous by-products are generated as drug is broken down in the liver. However, dietary supplements may prevent or relieve some of drug deleterious effects. Therefore, this study was conducted to evaluate the ameliorative effects of aqueous extract of Sesamum indicum (AESI) seeds on tramadol induced hepatotoxicity in rats. 24 Wistar rats were divided into four (4) groups of six (6) rats each, group A (Control), Group B (Tramadol dependent), group C (Tramadol dependent + 500mg/kg of AESI seeds) and group D (Tramadol dependent + 1000mg/kg of AESI seeds). Administrations were via oral gavage for 20 days. Toxicity was induced by administering therapeutic dose (7.2mg/kg) of tramadol hydrochloride and gradual increase in the dose after every day until it reaches a dependent dose of (144mg/kg) by oral gavage. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were monitored and histological examination was carried out. The results revealed that tramadol administration led to significant elevation of ALT, AST and ALP levels. Treatment with two different doses (500mg/kg and 1000mg/kg) of AESI seeds produced significant dose dependent normalization in the hepatic biochemical parameters and reduced histopathological distortion with evidences of liver regeneration. Treatment with two different doses (500mg/kg and 1000mg/kg) of AESI seeds produced significant reduction in histopathological distortions with significant evidence of hepatic tissue regeneration. Hence, the results of this study indicate that the AESI seeds possess significant protection against tramadol induced hepatotoxicity.

**Key words**; Tramadol, Sesamum indicum (SI), ameliorative, hepatotoxicity

## INTRODUCTION

Addiction is an increasing social and health problem worldwide despite all efforts to prevent and control it. Analgesics are among the most popularly abused drugs[1]. The use of drugs could be beneficial or harmful depending on the mode of use[2] .Chronic use of substances can cause serious, sometimes irreversible damage to organs, physical and psychological development. Tramadol is an analgesic that is rapidly absorbed orally especially in the upper part of the small intestine and it has extensive tissue distribution. Its metabolism occurs in the liver cytochrome P450 enzyme system and its by-products are excreted unchanged through the kidneys[3]. Repeated tramadol administration might lead to the accumulation of toxic metabolites in the body characterized with increase in its toxicokinetics effects[4].

Liver diseases are common problems worldwide and some compounds or metabolites promote it in a dose-dependent fashion[5]. The mechanism for injury in hepatic tissue can be directly from the disruption of intracellular function, membrane integrity, damage to endothelial and bile duct cells. It can also occur indirectly through immune-mediated membrane damage[6]. Herbalism practice is becoming more mainstream as improvements in analysis and quality control along with advances in clinical researches showed the value of herbal medicine in the treatment and prevention of many diseases[7].

*Sesamum indicum* belongs to the family Pedalliaceae and contains protein, reducing sugars as well as phenolic antioxidants including flavonoids, cardiac glycosides, anthocyanins, saponins, and lignans[8]. The level and balance of amino acid composition, including the sulfur rich methionine, that is scarcely available in other plant proteins sources makes *Sesamum indicum* seeds very unique traditional health food that is edible as an oil as well as cake[9,10,11].

## MATERIALS AND METHODS

**Ethical clearance:** The animal rights and ethics committee of Kano State ministry of health approved the use of both the study design as presented with an approval letter marked MOH/Off/797/T.I/1676), and animals were handled as stipulated by the guidelines for the use of animals for scientific research purposes.

**Procurement of Animal:** The study animals were procured from the department of Biological sciences of Bayero University Kano and acclimatized for 2 weeks

in the department of Anatomy Bayero University Kano. The animals were maintained before and throughout the experiment period in standard caging with access to water and food (pellets) ad-libitum. At the start of the experiment, the animals were randomly distributed into 4 groups of 6 rats each

**Plant Materials:** *Sesamu indicum* seeds were obtained from a natural habitat at Gezawa, Kano State. The seeds were authenticated and a voucher number of BUKHAN 646 was obtained from herbarium unit in the Department of plant biology, Bayero University, Kano. The seeds were air dried under shade, pulverized into coarse powder before grinding into fine particles. The powder was measured using an electronic weighing balance in which 1500 grams of powder was soaked in 2 liters of distilled water for 24 hours at room temperature. The filtrate was filtered using Whitman's filter paper and the residues were discarded, filtrate was then concentrated using a water bath set at 32º C. A dark sticky oily brown mass of the extract was obtained which weighed 302 grams and was kept air-tight and refrigerated before use.

**Drug purchase and preparation:** (Tramadol hydrochloride) capsules manufactured by Hovid were purchased from Lamco pharmacy, Kano. Its chemical name is (+) cis-2-[(dimethylamino) methyl]-1-(3-m ethoxyph-enyl) cyclohexanol hydrochloride**.** The LD50 of tramadol for Wistar rats adopted was 300 mg/kg[12].

**Experimental design:** Twenty-four (24) experimental Wistar rats weighing from 130g – 210g were divided randomly into four (A, B, C, and D) comprising of six (6) rats each. Group A (Control group) received 1 ml/day of distilled water. Group B (Tramadol dependent group) received tramadol hydrochloride in gradually increasing doses from 7.2 mg/kg (therapeutic) to 144 mg/kg (dependence) adapted from Heba YS and Azza HM [3] for twenty (20) days. Group C (Tramadol dependent group and extract I) received the same dose as that of group B and at the same time received 500 mg/kg of AESI seeds. Group D (Tramadol dependent group and extract II) received the same dose as that of group B and at the same time received 1000 mg/kg of AESI seeds. All administrations were via oral route and lasted for twenty (20) days.

**Biochemical analysis:** Blood sample were collected in sterilized EDTA samples bottles via cardiac puncture. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were determined using enzymatic kits (CYPRESS® Diagnostics, Langdorp, Belgium) according to the manufacturer's instructions.

**Histological studies:** The liver tissues of the experimental Wistar rats were harvested by making a median incision through the abdominal cavity, the harvested liver tissues were fixed in 10% buffered formalin solution for tissue processing and staining (H & E). The stained sections were examined using light microscope according to Bancroft JD and Gamble M[13].

**Statistical analysis:** All data obtained were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was employed to compare the means between and within the groups, and a $P$ value $< 0.05$ was considered significant. A post-hoc test (Bonferonni) was also applied to assess significant differences between the groups. Statistical analysis was done using SPSS version 20.

**RESULTS**

In the current study, the histological features of the studied experimental Wistar rats from the different groups were evaluated after careful processing of the liver tissue. The histological sections of the hepatic tissues obtained from the studied Wister rats in the different groups in the current study were presented in Plate 1. The result for the serum levels of liver function related enzymes were also evaluated and documented for presentation for each of the study groups.

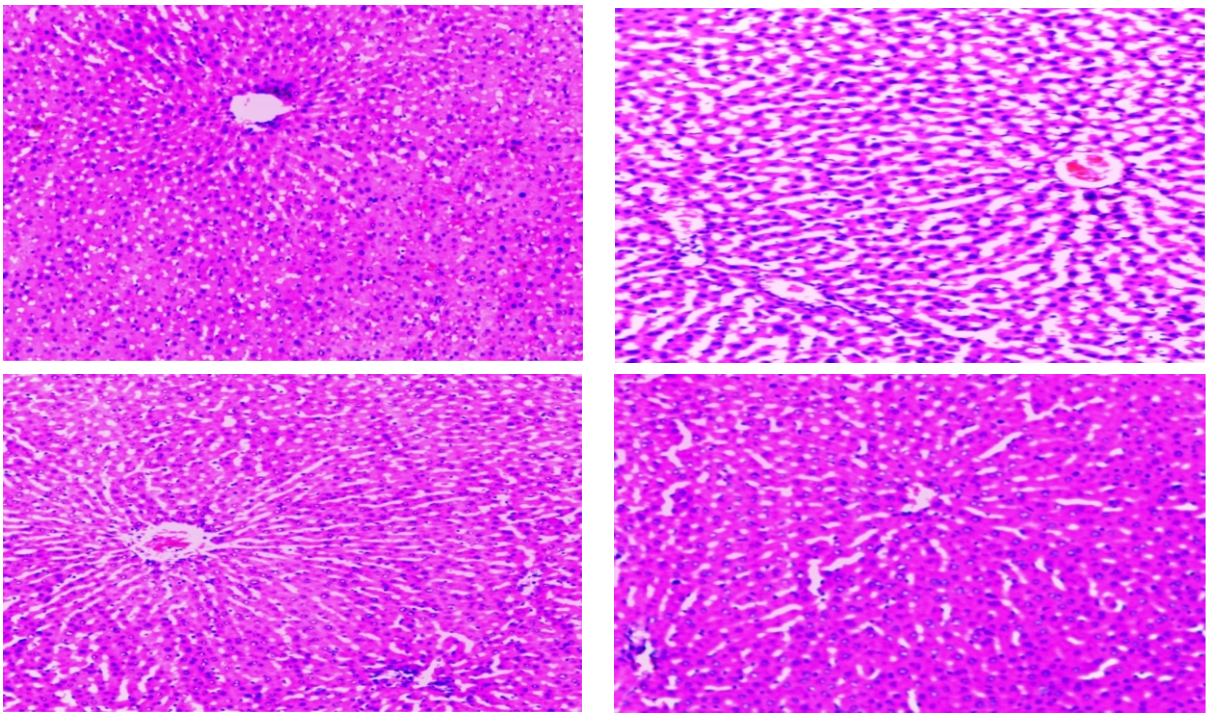

**Plate I**. Photomicrograph of the liver section from Wister rats in the; control group (A) showing preserved histoarchitecture, central vein (C), hepatocytes (H), and sinusoids (S); hepatic tissue from Wister rats with chronic exposure to tramadol (B) showing congested central vein (C), severe sinusoidal dilatations (S), and portal triad (P); hepatic tissue from Wister rats with chronic exposure to tramadol with low dose (500 mg/kg body weight) (C) or high dose (1000 mg/kg body weight) (D) of *AESI* seeds showing preserved histoarchitecture of the tissue, the central vein (C), hepatocytes (H), sinusoids (S), and portal triad (P) - best restoration observed at the higher doses of *Sesamum indicum* (H&E X100).

The level of the circulating liver enzymes is usually assessable as part of a liver function test. In the current study, the levels of the liver enzymes were assessed with the individual values obtained for each group. The entire individual values from each group were computed together to obtain a representative average value for the group. The same procedure was followed to obtain the average representative values for the other groups and all the average representative values including their measure of dispersion are presented in Figure 1 to 3.

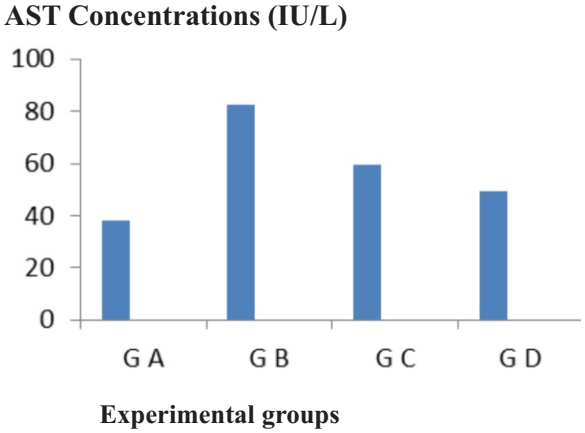


**Figure 1** Mean concentrations expressing level of liver aspartate aminotransferase AST (IU/L) in all experimental groups. The GB (Group B) and GC (group C) differed significantly with GA (group A), whereas no significant difference was observed between GD (group D) and GA (group A) ($P$<0.05) when assessed using Bonferonni post-hoc test.

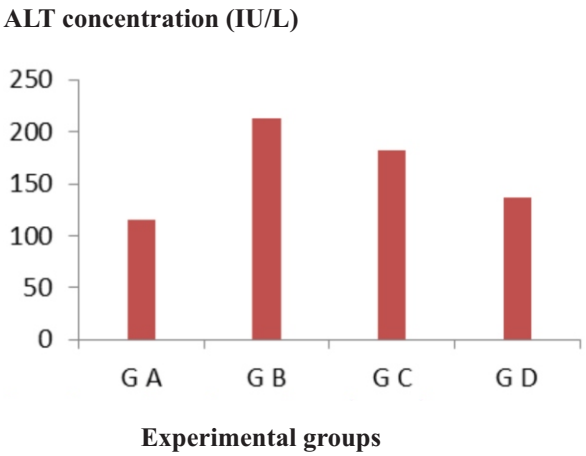


**Figure 2** Mean concentrations of the liver alanine aminotransferase ALT (IU/L) level in all experimental groups. The level of the enzyme in all the groups (GA, GB and GC) was higher and the difference was statistically significantly when compared with the level in GA (group A) (P<0.05) and this evaluation was done using the Bonferonni post-hoc test.

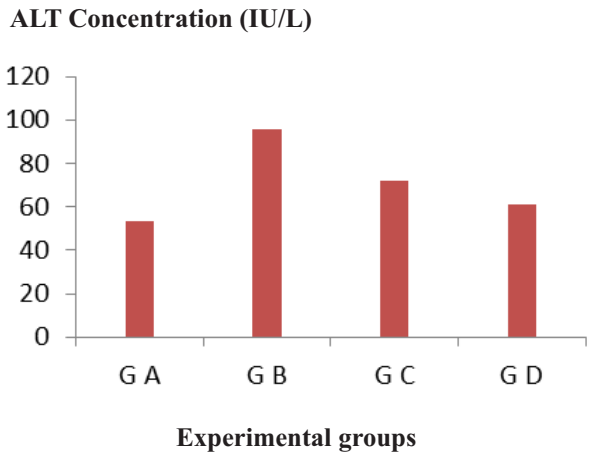


**Figure 3** Mean concentrations expressing level of liver alkaline phosphatase ALP (IU/L) in all experimental groups. GB (Group B) and GC (group C) differed significantly with GA (group A), whereas no significant difference was observed between GD (group D) and GA (group A) (*P*<0.05) and this evaluation was done using the Bonferonni post-hoc test.

## DISCUSSION

In this study, chronic administration of tramadol hydrochloride resulted in a significant elevation of some serum liver enzymes (AST, ALT and ALP). It also affected the histoarchitecture of the section of liver lobules and the distortion were characterised with dilation in the sinusoidal spaces, infiltration by inflammatory cells and features of hepatocytes membrane degeneration. A dose related reinstatemet of the hepatic histoarchitecture towards normal with features of hepatic tissue regeneration as well as normalization of the elevated serum liver enzymes levels were also observed following treatment with AESI seeds. Thus, the liver histopathological changes in the current study suggested that chronic tramadol administration has a hepatotoxic effect in Wistar rats while AESI seeds is adduced to have a hepatoprotective effect against tramadol toxicity in them.

Free radicals are inherently unstable because they contain "extra" energy. To reduce their energy load, free radicals react with certain chemicals in the body, and in the process, they interfere with the cells' ability to function normally. Antioxidants work in several ways which may include reducing the energy of the free radical or halting the formation of free radicals abintio. It may also include interrupting the oxidative-chain reaction so as to minimize the damage caused by free radicals. The flavonoids, which are richly found in AESI have been found to possess potent antioxidant activity by scavenging for free radicals[8,14]. It has also been demonstrated in the literature that lycopene, which is also very abundant in the AESI seeds has antioxidant activity and almost completely prevented oxidative damage to DNA in the liver of rats, thus forestalling necrotic changes in the tissue[15,16]. The findings from this study is in line with results from earlier studies that evaluated the hepatotoxic effect associated with chronic tramadol administration[17,18,19,20].

Although there were differences in the route of administration, dosing for tramadol as well as the duration of exposure to it, the hepatotoxic effect of tramadol remained similar. Specifically, the earlier protocols included a 3 days 10 mg/kg body weight treatment with tramadol in rabbits, a 7.2 mg/kg body weight 3 daily interval treatment with progressively increasing dose of tramadol (by 7.2 mg/kg body weight) for 60 days among rats and a 30 days study which was characterized by intraperitoneal dosing of 20, 40 and 80 mg/kg/day on the first, second and third ten days of the study with the rats. In all these studies, tramadol had a potent disruptive effect on the hepatic tissue sections as well as the level of liver enzymes. The hepatotoxic damages elicited by tramadol reported in the current study were similar to the histoarchitectural disruptions reported following exposure to carbon tetra chloride (CCL4) in another study[20]. At very high doses (300 µm) tramadol had a ß1 adrenoceptors stimulatory effect which was irreversible to ß adrenoceptor antagonist like propranolol and irreversible to opioid receptor antagonist like naloxone. The attenuated contractility associated with the activity of tramadol may synergistically improve the visceral analgesia reported with tramadol. Nevertheless, this preceding description may explain the ease with which tramadol toxicity can develop with its associated propensity for hepatotoxicity.

The effect of the extract of Sesamum *indicum* seeds on

tramadol induced liver toxicity observed in this study is in line with the finding in the literature, that sesame lignans oil feeds reduces Fe2+ induced oxidative stress in Wistar rats[18]. These sesame oil-fed rats had lower levels of hepatic thiobarbituric acid reactive substances, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) indicating protection against Fe-induced oxidative stress. Similar findings were also reported in another study in which the aqueous extract of Sesamum *indicum* seeds had antioxidant and hepatoprotective properties, against ethanol, especially when administered orally two hours before and/or simultaneously with ethanol[21]. In another literature, it was reported that rats treated with ethanolic extract of Sesamum *indicum* seeds and silymarin were protected against paracetamol induced hepatotoxicity[20]. In another study, ethanolic extract of Sesamum *indicum* seeds was not hepatoprotective against carbon tetrachloride induced liver toxicity when administered intraperitoneally (Nureddin*et al.,* 2012)[22].

However, the hepatoprotective effect was observed on CCL4 induced hepatotoxicity when the ethanolic extract of *Sesamum indicum* was administered orally[20]. The reason for the difference in finding appears to be mainly attributed to the route of administration of the extract rather than the hepatotoxic agent or the formulation of the extract (oral administration bequeathed a better protection).

## CONCLUSION

The present study suggest that orally administered aqueous extract of *Sesamum indicum* (AESI) seeds have a dose dependent hepatoprotective effect against liver toxicity of addictive dose of orally administered tramadol hydrochloride.

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