HEPATOPROTECTIVE EFFECT OF TRYPTOPHAN IN CARBONTETRACHLORIDE-INDUCED HEPATOTOXICITY IN MALE WISTAR RATS

T.O. Ayinde[1],L.A. Olayaki[1], L.S. Ojulari[1], A. Oluwasola[2], H.A. Abdulraheem[3], A. Z. Lawal[3], A. Alli-Oluwafuyi[4]

[1]Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria, [2]Department of Physiology, Faculty of Basic Medical Sciences, Al-Hikmah University, Ilorin, Nigeria, [3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria

Abstract: In the present study, tryptophan was evaluated for its hepatoprotective effects against carbontetrachloride-induced hepatocellular injury in rats. Hepatotoxicity was induced in male Sprague-Dawley rats by intraperitoneal injection of CCl_4 (4ml/kg) in olive oil (1:1). Tryptophan at doses of 100mg/kg and 200mg/kg was administered orally for 28 days. The hepatoprotective effect of tryptophan was evaluated by the assay of biochemical parameters viz.: alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), total protein, albumin and lipid peroxidation. Tryptophan produced a dose-dependent significant increase ($p<0.001$) in serum ALP (41% & 60%), a dose-dependent decrease ($p<0.001$) in serum Malondialdehyde (61% & 65%), and a significant increase ($p<0.001$) in levels of serum protein and serum albumin, in CCl_4induced hepatotoxic rats, following administration of 100 mg/kg bwand 200 mg/kg bw, respectively. The toxic effect of CCl_4 in tryptophan treated groups was controlled significantly by restoration of the levels of enzymes, total protein and albumin as compared to the CCl_4 treated groups. The results suggest that tryptophan is able to significantly alleviate the hepatotoxicity induced by CCl_4 and may be attributed to the antioxidant property of tryptophan.

Keywords: Carbontetrachloride, Hepatotoxicity, Lipid peroxidation, Tryptophan

***Corresponding Author**: Ojulari Lekan Sheriff, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria. Tel.:234-8032429067; Email: Ojulari.ls@unilorin.edu.ng

INTRODUCTION:

Reactive oxygen species (ROS)including oxygen free radicals are involved in the development of many degenerative diseases. Enhanced production of free radicals and oxidative stress can be induced by a variety of factors such as radiation or exposure to heavy metals and xenobiotics (such as carbontetrachloride)[1].Carbontetrachloride (CCl_4) intoxication in laboratory animals has become an established experimental model that mimics oxidative stress in many pathophysiological situations[2].Studies have demonstrated that CCl_4administration induces the generation of free radical in many tissues such as liver, kidney, heart, lung, testis, brain and blood[3]. The toxicity of CCl_4 probably depends on formation of the trichloromethyl radical (CCl_3) which interacts with oxygen to form the more toxic trichloromethylperoxyl (CCl_3O_2) radical[4]. Oxidative stress resulting from increased free radical production after CCl_4 intoxication may also play an important role in the degenerative process in the tissues. Although toxic effects of CCl_4 were shown in the brain, heart or kidney, the major injuries after CCl_4 intoxication were investigated in the liver[5].

Tryptophan is an essential amino acid required for the biosynthesis of proteins involved in a many metabolic processes. It is also the main precursor of serotonin, melatonin secreted by pineal gland and niacin[6,7,8].Melatonin, a neurohormone with a range of functions[9,10]is mainly known for its ability to scavenge for free radicals during endotoxic shock[11,12].Tryptophan deficiency leads to a negative tryptophan balance leading to reduction in the concentrations of its metabolites such as serotonin, picolinic acid, niacin and melatonin[13]. Melatonin plays a crucial role in the activation and suppression of immune responses[14]and is produced by a wide range of organism including animals, plants and microbes. In addition, direct free radical scavenging, indirect antioxidative as well as immunomodulatory actions of melatonin have been reported[15,16,17,18,19,20].However, it is not yet clear if tryptophan, the precursor of melatonin will produce similar protective effects on the liver during intoxication with liver damaging chemicals. Therefore, this studyinvestigated the effect of tryptophan on liver enzymes, serum protein and lipid peroxidation following the administration of CCl_4 in rats.

MATERIALS AND METHODS:

Laboratory Animals

This work was carried in The Department of Physiology, University of Ilorin, Ilorin, Nigeria. Twenty four male Sprague Dawley rats were obtained from Animal Breeding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. Rats (average weight of 180.5±7.4g) were allowed to acclimatise to the laboratory condition for one week and were maintained under standard laboratory conditions in photoperiod-controlled chambers. The rats were fed with commercial pelleted diet (Bendel Feeds, Edo State, Nigeria) and water *ad libitum*. All experimental procedures were approved by the Animal Ethical Committee of the Faculty of Basic Medical Sciences, University of Ilorin.

Research Design

The rats were randomly divided into four groups with six animals per group. Rats in the control groups received oral administration of saline while other animals received once dailyadministration of tryptophan (100 or 200 mg/kg body weight) for 28 days. On day 28, rats received a single intraperitoneal administration of saline or CCl_4 (4ml/kg body weight) suspended in olive oil (1:1v/v) as indicated in the Figures. Rats were sacrificed by cervical dislocation 24 h following CCl_4 administration and liver samples were excised immediately and homogenised. Blood samples were collected via cardiac puncture and serum separated by centrifugation was stored at -20^{o}C until used for analysis. Samples of the specimen were deposited in the departmental laboratory, Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin.

Biochemical measurements

Serum levels of alanine aminotransferase (ALT), alkaline phosphate (ALP), aspartate aminotransfrase (AST), total protein and albumin were determined using commercially available kits (Agape Diagnostics, Switzerland Gmbh) according to manufacturers recommended protocols. Absorbance readings were obtained using an automated blood chemistry analyser (URIT-810 Chemistry Analyzer, URIT Medical Electronic Co., Ltd. Guangxi, PR China).Tissue malonaldedyde (MDA) levels were estimated as described byOhkawaet al.[21]. Liver samples were homogenized using a cold buffer containing 100mM KCl and 0.003M EDTA. Following centrifugation at 600g for 15min, the supernatant (400µl) was added to 0.2ml 8.1% SDS, 1.5ml 20% acetic acid (pH 3.5), 1.5ml 0.8% thiobarbituric acid and 0.6ml water. The solution was heated to 95^{o}C for 60 minutes followed by the addition of water (1.0ml) and of n-butanol-pyridine mixture (5.0ml, 15:1, v/v), the mixture was vigorously shaken and centrifuged at 2,000g for 15minutes. The absorbance of the upper layer was read at 532nm with Acurex Microplate Reader. MDA bis-dimethyl acetal was used as the external standard. Results were expressed as nanomoles MDA per milligram protein. The intra-assay variability was determined in five sets of triplicate samples, and the coefficient of variation given.

STATISTICAL ANALYSIS

Data were analysed using GraphPad Prism Version 3 and expressed as mean ± SEM. More than two variables were compared by using one-way ANOVA test and Duncan's test was performed for post-hoc analysis.

RESULTS:

Effects of tryptophan on serum levels of AST, ALT and ALP in rats with carbontetrachloride-induced hepatotoxicity

Administration of CCl_4 to saline treated rats resulted in marked elevation of serum levels of AST (129%, P<0.001), ALT (121%, P<0.001) and ALP(249%, P<0.001) (Figure 1). However, CCl_4-induced elevation of serum AST and levels was reduced by 77% (P<0.001) and 37% (P<0.01) respectively in both groups of rats treated with tryptophan. Tryptophan treatment in rats intoxicated with CCl_4 produced dose dependent reduction of elevated serum ALP with 41% (P<0.001) and 60% (P<0.001) reduction observed for rats treated with 100 and 200 mg/kg body weight of tryptophan respectively.

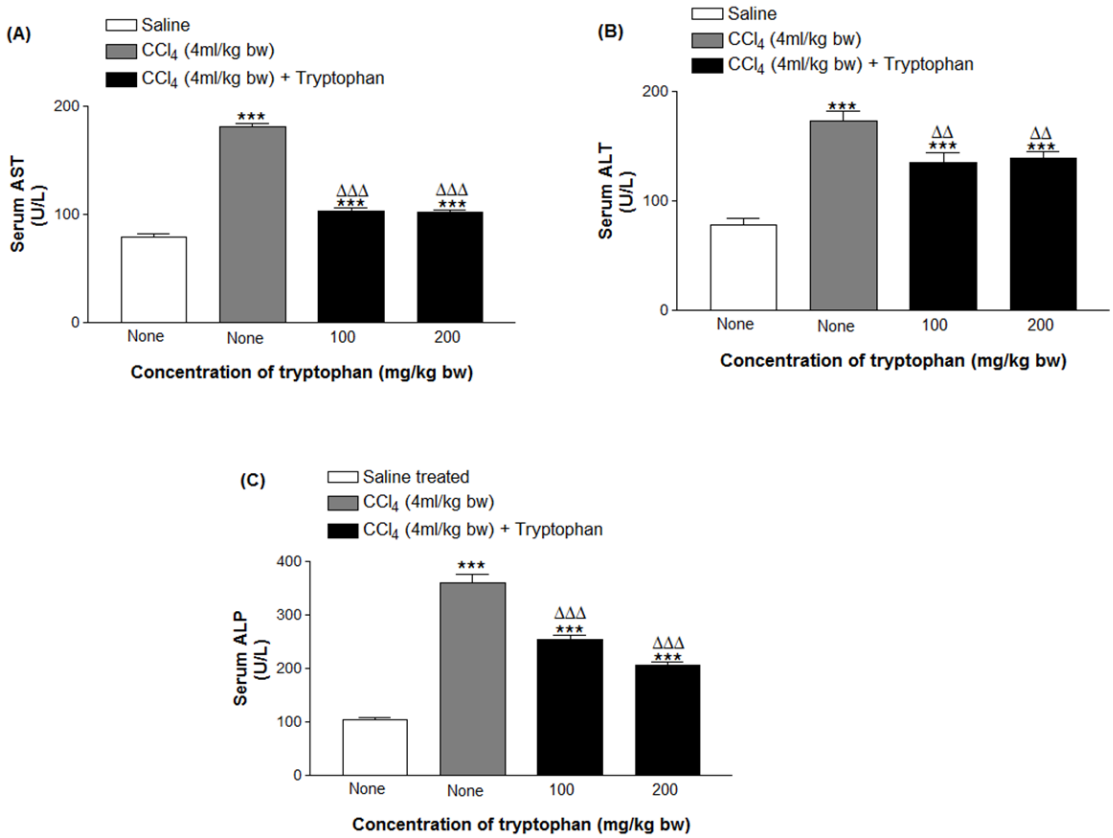

Figure 1: Effects of trytophan on serum levels of (A) aspartic transaminase, (B) alanine transaminase and (C) alkaline phosphatase in rats with carbontetrachloride-induced hepatotoxicity. Values are mean ± SEM with n=6. *P<0.05 and ***P<001 compared with saline treated control rats with no carbontetrachloride administration. $^{\Delta\Delta}$P<0.01, $^{\Delta\Delta\Delta}$P<0.001 compared with carbontetrachloride-intoxicated rats treated with saline.

Effects of tryptophan on lipid peroxidation in rats with carbontetrachloride-induced hepatotoxicity

Serum malondialdehyde concentration in saline-treated rats intoxicated with CCl_4 was significantly elevated by 125% (P<0.001, Figure 2). This increase was inhibited in a dose-dependent manner by treatment with tryptophan. Elevation of serum malondialdehyde was inhibited by 61% (P<0.001) and 65% (P<0.001) in rat treated with 100 and 200 mg/kg body weight of tryptophan respectively.

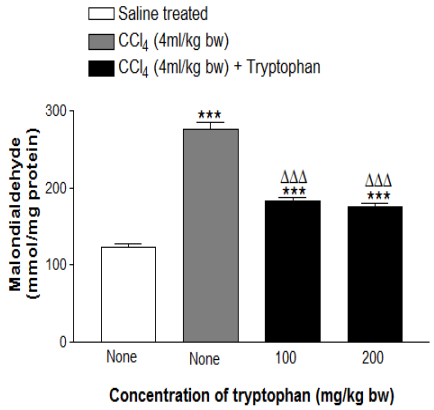

Figure 2: Effects of trytophan on serum levels of malondialdehyde in rats with carbontetrachloride-induced hepatotoxicity.Values are mean ± SEM with n=6. ***P<001 compared with saline treated control rats with no carbontetrachloride administration. $^{\Delta\Delta\Delta}$P<0.001 compared with carbontetrachloride-intoxicated rats treated with saline.

Effects of tryptophan on serum total protein and albumin in in rats with carbontetrachloride-induced hepatotoxicity

Serum total protein decreased by 70% (P<0.001) in rats intoxicated with CCl_4 compared with control rats (Figure 3A). Serum total protein was significantly improved by treated with 100mg/kg body weight of tryptophan (2.1-fold, P<0.001) and was almostly completely restored to normal levels in rats receiving 200mg/kg body weight of tryptophan (Figure 3A). Similarly, reduced serum albumin was observed in rats intoxicated with CCl_4 (44%, P<0.001) but treatment with tryptophan increased serum albumin by 1.5-fold (P<0.001) in rats intoxicated with CCl_4 (Figure 3B).

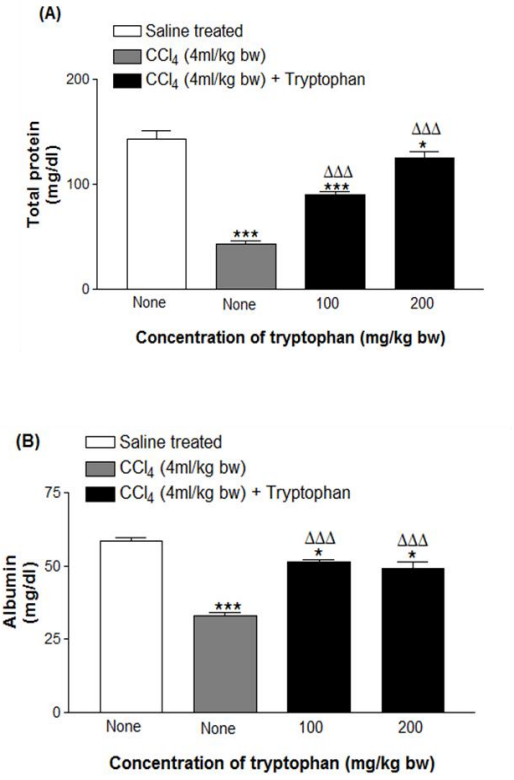

Figure 3: Effects of different photoperiods on serum levels of total protein and albumin in rats with acetaminophen-induced hepatotoxicity. Values are mean ± SEM with n=6. *P<0.05 and ***P<001 compared with saline treated control rats with no carbontetrachloride administration. $^{\Delta\Delta\Delta}$P<0.001 compared with carbontetrachloride-intoxicated rats treated with saline.

DISCUSSION:

Carbontetrachloride (CCl_4) is bio-transformed by cytochrome P450 system to produce trichloromethyl free radicals. These free radicals may again react with oxygen to form trichloromethlperoxyl radical, which may attack lipids on the membrane of endoplasmic reticulum to elicit lipid peroxidation finally resulting in cell necrosis and consequently cell death[22].Hepatocellular necrosis leads to the release of liver enzymes such as AST, ALT and ALP into the blood stream, thus making them good biomarkers of hepatotoxicity[23].Hepatic cells participate in avariety of metabolic activities and contain a host of enzymes. In tissues, AST and ALT are found in higher concentration in cytoplasm and AST also exists in the mitochondria. In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in the serum. The elevated levels of AST and ALT in serum are indicative of cellular leakage and loss of functional integrity of cell membrane in liver.

In this study, the administration of CCl_4 significantly raised serum levels of AST and ALT, which is consistent with previous observations in rats intoxicated with carbontetrachloride[24].Oral administration of tryptophan at doses of 100mg/kg and 200mg/kg caused a decrease in the activity of the above enzymes, which may be consequence of the stabilisation of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. The activity of the serum alkaline phosphatase (ALP) was also elevated during CCl_4 administration. Reduction in serum ALP levels in tryptophan treated group could be due to hepatoprotective effect of tryptophan.

Malondialdehyde (MDA) is a major reactive aldehyde produced as a result of the peroxidation of biological membrane polyunsaturated fatty acid. MDA concentration is used as an indicator of tissue damage involving a series of chain reactions. Lipid peroxidation of hepatocyte membranes caused by free radical derivatives of CCl_4 has been implicated in the pathogenesis of CCl_4-induced hepatotoxicity[25]. Elevated serum levels of MDA in rats administered CCl_4 in the present study is consistent with this hypothesis. Moreover, the significant reduction in the level of MDA following tryptophan administration in CCl_4 intoxicated rats provides evidence that the hepatoprotective effect of tryptophan might be due to its ability to stabilise the plasma membrane in hepatocytes.

Previous studies have also reported reduced serum total protein and albumin following CCl_4 administration[26,27]. The reduction shows the level of damage to the liver which is the major organ involved in protein metabolism. It has been reported that tryptophan alleviates oxidative stress by increasing antioxidant capacity through the stimulation of the activities of superoxide dismutase and glutathione peroxidise[28,29]. A direct correlation between evening administration of tryptophan and nocturnal circulating melatonin levels has also been reported, suggesting that the synthesis of serotonin and melatonin, as well as the associated innate immune responses, can be modulated by oral ingestion of tryptophan[30].

CONCLUSION:

In conclusion, the present study show that tryptophan confers protection against liver

damage induced by CCl_4 via a mechanism that may involve the ability of tryptophan to improve plasma membrane stability in hepatocytes and its effects on the synthesis of serotonin and melatonin. However, further studies are required to understand exact molecular changes that occur during these processes in the presence of tryptophan. Results obtained in this study also strongly suggest that tryptophan may have potential clinical application in the treatment of liver injury.

Conflict of interests:

Authors declared no conflict of interests.

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