Original Research Paper

Anatomy

COMPARATIVE ASSESSMENT OF ZINC SULPHATE AND ESSENTIALE FORTE® IN MANAGING BIOCHEMICAL ALTERATIONS OF CARBON TETRACHLORIDE –INDUCED HEPATOTOXICITY ON ADULT WISTAR RATS.

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ABSTRACT The main **objective** of this study was to assess and compare the effect of Zinc Sulphate and Essential Forte ® on the biochemistry of carbon tetrachloride (CCl_4) induced hepatotoxicity in adult Wistar rats. **Materials and Method:** Twenty five adult Wistar rats, weighing between 150 g and 170 g, were used for the study. They were housed in plastic cages fed on standard laboratory rat pellets and given water *ad libitum*. The animals were divided into five groups A, B, C, D and E (n=5). Group (A) received 0.7 ml/kg of olive oil orally. Groups B, C, D and E were administered CCl_4 (0.7 ml/kg orally) for 1 week in 1:1 dilution with Olive Oil. After CCl_4 administration Group C was treated with Essentiale Forte® (4.5 mg/kg bw orally) for four weeks. Group D was treated with Zinc Sulphate (7mg/kg bw orally) daily for four weeks. Group E received Zinc Sulphate (7 mg/kg bw orally) and Essentiale Forte ® (4.5 mg/kg bw orally) for a period of 4 weeks, while group B was left untreated. Animals were left for another one week and afterwards sacrificed under ether anaesthesia. Blood samples were obtained via cardiac puncture and centrifuged to obtain serum. Markers of liver function such as, Aspartate Amino Transferase(AST), Alanine Amino Transferase(ALT) as well as Alkaline Phosphatase(ALP) were estimated in the serum using enzyme colorimeter assay kit (Randox). Data obtained were analyzed by one way ANOVA, then Student Newman- Keuls (SNK) test for multiple comparisons. The **result** of Treatment with Zn and or Essentiale Forte® restored the hepatic function as the biochemical analysis with Mean± SEM showed significant reduction in the levels of markers of liver function and protected the liver tissue from fatty and degenerative changes. In **conclusion**, this study showed that combination of Essential Forte® and Zinc supplement offered better ameliorative effect on the markers of liver function of Wistar rats following CCl_4 induced - hepatotoxicity compared with separate administration of either Essentiale Forte® or Zinc supplement.

KEYWORDS : Wistar rats, Carbon tetrachloride, Zinc Sulphate, Essentiale Forte, Liver.

INTRODUCTION

Most routine tests reflect liver damage the tests of function are those which reflect synthetic capacity for instance albumin and prothrombin time, liver function tests may be grossly deranged when function is normal and may be normal when function is grossly deranged for it has enormous functional reserves, such that early liver impairment is clinically masked and the progression of the deranged liver function makes the condition life threatening(Cotran *et al*.,1999). Morphologically, liver responds to injurious events in 5 different ways, irrespective of the cause viz; Degeneration and intracellular accumulation, Necrosis and Apoptosis, Inflammation, Regeneration, Fibrosis (Cotran *et al*.,1999).

CCl_4 is a colourless liquid, ether-like in odour with a density of 1.6 gcm^{-3} melting point is 22.9°C, boiling point is 76.7°C and soluble in water at 0.08 g/100ml (25°C). It is also soluble in alcohol, ether, chloroform, benzene, naptha and carbon sulfide. The vapour pressure is 11.94kPa at 20°C and refractive index of 1.5, it has crystal structure with tetrahedral shape . It is not flammable, its auto-ignition tempt is 982°C and LD$_{50}$ is 2350 mg/kg. International programme on chemical safety (IPCS) (1999). It has been reported to produce free radical which affects the cellular permeability of the liver cells leading to altered level of serum biochemistry and liver enzymes(walker *et al.,*1992). In time series,it was found to have an atmospheric life time of 85 years and liver damage inflicted byCCl4 has lethal consequences. Walker *et al.,* (2000).

For so many years, liver disease remains one of the major health concern despite discovery and development of new drugs, morbidity and mortality accompanying hepatic pathology is still clinically significant. (Bojuwoye *et al.,* 2009). Chronic exposure of liver to CCl_4, has been researched with consequent spectrum of chronic liver disease, such as fatty liver, hepatitis, liver cirrhosis and hepatoma

(Bojuwoye *et al.,* 2009). Liver damage can occur after 24 hrs of exposure to CCl_4 and in serious cases this can result in hepatitis,ascites, hemorrhages, hepatic coma and even death. International programme on chemical safety(IPCS) (1999).

Essential phospholipid has since been used in the cases and managements of chronic liver disease. It is a300 mg hard gel capsule that contains De-oiled enriched phospholipids from neutral soya beans. Its mechanism of work by phospholipids replacement and regeneration in the liver cell membranes, which have been damaged by various means especially through liver toxin. The pharmaceutical excepients are chiefly ethanol, hard fat, Hydrogenated, Castor oil, Ethyl vanillin,4-methoxylphenylethanone, Alpha-tocopherol ,Gelatin, Colouring agents, Sodium laurylsulfate, Purified water. Sonafi avensis pharmaceutical (2007).

Zinc is known as an essential trace element necessary for protein metabolism, as well as membrane integrity and also involved in the structure and function of numerous metalloenzymes. It has important functions in skin and connective tissue, metabolism as well as in wound healing. (Berger, 2002). It exerts its antioxidant effects indirectly by maintaining membrane structures, involving in the structure of SOD, increasing the metallothionein concentrations and, competing with redox reactive metals, iron and cuprous for critical binding sites (Yardic *et al.,* 1989).It is shown that hepatic and serum zinc levels of patients in liver disease decreased depending on the degree of liver damage (Zhou 2010).

MATERIALS AND METHODS

Animal Care and Management:

Twenty five adult Wistar rats, weighing between 150 g and 170 g (6–10 weeks old) obtained from Animal Holding of International Institute of Tropical Agriculture Ibadan Oyo State were used for the

research. The animals were housed in plastic cages in a clean environment of 12 hours day/light cycle, at room temperature, in the animal holding of the Department of Anatomy and Cell Biology. They were fed on standard laboratory rat pellets and have free access to water. Ethical clearance for the study was obtained from Health Research Ethical Committee (HREC),Institute of Public Health(IPH) Obafemi Awolowo University(OAU) Ile-Ife. The animals were given humane care according to the guidelines of HREC, IPHOAU.

Preparation of the Chemicals/Drugs

2.5 litres of CCl_4 was obtained from the central research laboratory of Obafemi Awolowo University, Olive Oil, Zinc Sulphate(ZnSO4) tablets and Essentiale Forte® capsules were of the best grade commercially available. 60ml of $CCl_4$was diluted with 60ml of olive Oil in 1:1 equivalent, and this was administered at a dose of 0.7ml/kg p.o. 3 tablets of ZnSO4 was dissolved in 60ml of distilled water and this was administered at a dose of 7ml/kg, Essentiale Forte® capsule was prepared by dissolving a capsule in 60 ml of distilled water, and was administered at a dose of 4.5mg/kg all being freshly prepared on each day of administration.

Animal Treatment

The rats were divided into five groups A, B, C, D and E of five rats each (n=5).Group (A) normal control, received oral administration of olive oil only. Group B negative control received daily administration of CCl_4 (0.7 ml/kg p . o) for 1 week in 1:1 dilution with olive oil without treatment. Groups C test group I received Essentiale Forte® 4.5 mg/kg/day for four weeks after the administration of CCl_4.Group D test group II received Zinc Sulphate ® (7mg/kg/day p .o) daily for four weeks after the administration of CCl_4. Group E test group III received Zinc Sulphate® 7mg/kg/day for four weeks and Essentiale Forte (4.5mg/kg/day p .o) for a period of 4 weeks concurrently, after CCl_4 administration. All administrations were via oral route for four weeks (Essentiale Forte® and Zinc Sulphate®) while CCl_4 was for one week.

Animal Sacrifice and Sample Collection

At the end of the experimental procedure the animals were sacrificed after one week of recovery period. Animals were euthanized by ether anaesthesia a midline incision was made along the anterior abdominal wall. The blood was taken by cardiac puncture, the abdomen of the sacrificed animal were cut open quickly and the liver perfused with isotonic saline, excised, blotted dry, weighed, and divided into samples.

The degree of hepatic necrosis and fibrosis were determined by a semi-quantitative method (Pilette et al., 1998). Some portions of the liver tissues were homogenized for biochemical assay (ALT,AST,ALP) and the rest fixed in 10% formal saline for subsequent routine histological procedures.

Serum and homogenate Alanine, Aspartate Aminotransferases and Alkaline Phosphatase (ALT, AST and ALP)Whole blood was centrifuged at 4700 rpm for 10 min at 4°C and ALT, AST and ALP were determined spectrophotometrically with an automatic analyzer (Cobas Mira; Roche, Rotkreuz, Switzerland) using commercially available kits (Randox Diagnostics). Their activities were expressed as an international unit (IU/L). ALT,AST and ALP activities in liver homogenates were also determined using a quantitative, colorimetric end-point Randox assay kit (Procedure No. 104, Sigma Diagnostics, St. Louis, MO) that used α-ketoglutaric acid as the substrate and that detected production of pyruvic acid. Results were expressed as international units(IU).

Statistical Analysis

One way ANOVA was used to analyze the data, followed by Student Newman-Keuls (SNK) test for multiple comparisons. Graph Pad Prism 5 (Version 5.03 Graph Pad Inc.) was thestatistical package used for data analysis. Significant difference was set at $p<0.05$.

RESULTS

Hepatic Enzyme Levels In the Control and Test Groups.

GROUPS	AST (IU/L)	ALP(IU/L)	ALT(IU/L)	PCV(%)
A(Olive Oil)	67.80±3.50	100.4±7.86	17.60±1.94	41.00±2.00
B (Ccl4)	100.20±4.42α	322.2±29.12αδ	49.80±5.41αδ	32.00±1.00αδ
C (Ccl4+EPL)	65.60±3.08β	72.60 ±4.86β	09.00±0.71β	42.00±1.50
D (Ccl4+ ZnSO4)	69.00±4.55β	130.6±18.49β	20.60±1.50β	40.00±2.00
E (Ccl4+ ZnSO4+EPL)	53.60±3.83β	96.20±12.71β	13.20±1.07β	43.00±2.50

Results presented as mean ± SEM (n= 5)
α Significantly different from normal control at $p < 0.05$
β Significantly different from toxic control at $p < 0.05$
δ Significantly different from C,D and E at $p<0.05$

   EPL -   Essentiale forte®.
   CCl4 -   Carbon tetrachloride.
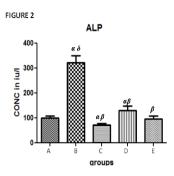   ZnSO4   -   Zinc Sulphate.

FIGURE 2

Fig 2:The graph showing the ALP levels of rats across the group after treatment.
Results presented as mean ± SEM, n=5 (p<0.05)
α Significantly different from normal control at p < 0.05
β Significantly different from toxic control at p < 0.05
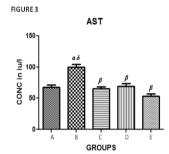δ Significantly different from C,D and E at p<0.05

FIGURE 3

Fig 3:The graph showing AST levels of the rats across the group after treatment.
Results presented as mean ± SEM, n=5 (p<0.05)
α Significantly different from normal control at p < 0.05
β Significantly different from toxic control at p < 0.05
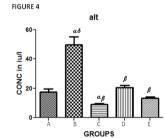δ Significantly different from C,D and E at p <0.05

FIGURE 4

Fig 4: The graph showing ALT levels of the rats across the group after treatment.
Results presented as mean ± sem, n=5 (p<0.05)
α Significantly different from normal control at p < 0.05
β Significantly different from toxic control at p < 0.05
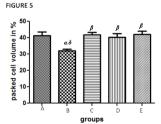δ Significantly different from C,D and E at p<0.05

FIGURE 5

Fig 5: The graph showing the packed cell volume of the rats across the group after treatment.
Results presented as mean ± sem, n=5 (p<0.05)
α Significantly different from normal control at p < 0.05
β Significantly different from toxic control at p < 0.05
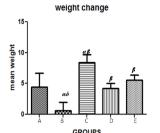δ Significantly different from C,D and E at p<0.05

FIGURE 6

Fig 6 :the graph showing the mean weight change of the rats across the group after treatment.

Results presented as mean ± sem, n=5 ($p<0.05$)

α Significantly different from normal control at $p < 0.05$
β Significantly different from toxic control at $p < 0.05$
δ Significantly different from C,D and E at $p<0.05$

DISCUSSION

Serum hepatobiliary enzymes, are present in high concentrations in the liver in stressful conditions. When there is hepatocyte necrosis or membrane damage, these enzymes are released into the circulation, as indicated by elevated serum enzyme levels. Zn was not able to decrease the levels of AST, ALT and ALP as compared to the standard drug essentiale forte but when combined, the enzyme levels were markedly reduced .

Significant reduction in the levels of these enzymes in the double treated groups indicated that Zn in combination with Essentiale forte was able to offer better protection to the liver against CCl_4 induced hepatotoxicity.

Zn treatment was able to ameliorate CCl4- induced hepatocellular damage as evidenced by reversal of increased serum transaminase (AST and ALT) levels subsequent to exposure. The enzyme levels in group B was found to be markedly elevated to almost three folds however, this enzyme level was quickly reverted to normal after treatments. Moreover, the finds of this research was contrary to the result of the work by Alumot et al., (1976) which reported no significant effects on serum enzyme levels or hepatic fat content of rats exposed to doses of Ccl4.

Ccl_4 toxicity was also found to have led to relative reduction in the haematocrit in group B unlike the other groups which is in keeping with the work done by Guild et al., (1958) and Stewart et al., (1991) which found that focal hemorrhagic lesions in the gastrointestinal tract and mild anemia were observed in humans who have ingested Carbon tetrachloride but this is likely due to decreased hepatic synthesis and or secretion of clotting factors. However it is contrary to the finds of Hayes et al., (1986). Oral exposure of mice to carbon tetrachloride did not result in any consistently significant hematological change. Moreover, the work done by Bruckner et al., (1986) stated that; severity of hepatic lesions as evidenced by centrilobular fatty vacuolization sometimes with single cell necrosis was correlated with the level of increase in serum enzyme levels.

CONCLUSION

Combined administration of Zn and Essentiale forte may be considered as more potentially and synergistically therapeutic through deterrence of enzyme leakage mechanism and thereby inhibiting liver toxicity induced by CCl4. Zn and or Essentiale forte® has hepatoprotective activity against carbon tetrachloride-induced liver damage, this activity could be due to the presence of flavinoids in EPL and membrane stabilization ability in both, thereby preventing cellular leakage. However, further studies are needed to expound the possible prophylactic and or protective effect of the zinc sulphate and essential forte combination in managing chronic liver disease clinically.

RECOMMENDATION

Further studies are recommended to give details about the histomorphometric correlation of hepatocytes and kupffer cells in liver of rats treated with CCl_4 vis a vis hepatic damage, possible relationship between the zinc level and the degree of liver damage via Zinc bioassay and possible prophylactic effect of the Zinc Sulphate and Essential forte® combination in managing chemical-induced injury in liver disease.

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