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Biochemical and histologic presentations of female wistar rats administered with different doses of paracetamol/methionine

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Summary: This study was carried out to compare the hepatoprotective effect of methionine on paracetamol treated rats at both the peaks of toxicity and absorption. Female Wistar rats were divided into 17 groups consisting of eight rats per group and treated with different doses of paracetamol/methionine (5:1). Each control rat received 5 ml of physiologic saline. The study was terminated at two different end points –the 4th & 16th hours. Results show that rats administered with toxic doses (1000 mg/kg; 3000 mg/kg; 5000 mg/kg BW) of paracetamol exhibited significant increases ($p<0.05$) in the levels of ALT, AST, γ- GT compared with controls. These increases were much higher at the 16th than 4th hour but serum total protein, albumin and globulin were significantly decreased ($p<0.05$) by the end of the 16th hour. Histology results of rats in the 3000 & 5000 mg/kg (by the end of the 16th hour) confirmed hepatic damage; light microscopic evaluation of liver showed remarkable centrilobular necrosis. Moreover, the presence of mononuclear cells in liver section of rats intoxicated with APAP (5000 mg/kg) suggests a possible involvement of inflammatory process which resulted in regurgitation of bilirubin leading to its elevated level as well as increase activity of ALP. The hepatoprotective effect of methionine, on the other hand, was demonstrated in these rats at the 4th & 16th hours, and both results were comparable and therefore not significantly different ($p>0.05$) but elevation in GGT level still persisted. In conclusion, data obtained from this study suggest that these agents may be capable of inducing GGT, although further study is required to establish a possible relationship between methionine and this enzyme in some other animal species.

Keywords*:* Paracetamol, Toxicity, Absorption, Liver biochemistry, Liver histology

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INTRODUCTION

Paracetamol (acetaminophen), an over-the-counter drug, is widely used for the treatment of mild pain and pyrexia. Because of its over-the-counter status, cases of both accidental and intentional paracetamol (APAP) overdose are numerous, with as many as 26,000 subjects being hospitalized each year in the United States of America (Nourjah et al., 2006). As a result of this high rate of abuse, paracetamol has been described by Larson et al. (2005) as the most common cause of drug induced liver failure in the United States. At overdose level, paracetamol causes hepatic centrilobular necrosis (McJunkin et al., 1976) which has been linked to excessive generation of N-acetyl-p-benzoquinoneimine (NAPQI). Paracetamol (APAP) is oxidatively transformed to N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P450 (Mitchell et al., 1973; Jollow et al., 1973). Glutathione binds to and detoxifies NAPQI but at toxic level hepatic GSH depletion occurs when NAPQI formation exceeds the available supply of GSH. The undetoxified NAPQI eventually binds to critical cellular macromolecules (Park et al., 2005) e.g. cellular proteins (Nelson and Bruschi, 2003; Bulera et al., 1996), resulting in impairment in mitochondrial respiration (Meyers et al., 1988; Burcham and Harman, 1991), opening of the mitochondrial permeability transition pore (Masubuchi et al., 2005), elevation of the oxidative stress (Bajt et al., 2004) as well as hepatic necrosis.

Kon et al. (2004) have also observed that mitochondria may be the primary targets in acetaminophen (APAP) toxicity with particular

attention on the mitochondrial permeability transition. Involvement of other generated reactive oxygen species such as nitric oxide and superoxide anion cannot also be discounted in paracetamol-induced hepatocyte death (Hinson et al., 2004). Apart from the hepatocytes, Lawson et al. (2000); Gardner et al. (2003); Liu et al. (1994) have suggested the contribution of non- parenchymal cells such as Kupffer cells, Natural killer cells, and neutrophils that secrete cytokines and chemokines during acetaminophen-induced liver injury.

Results obtained from studies of Henderson et al. (2000) and Dahlin et al. (1984) have also indicated that liver toxicity is initiated by P450-mediated reactions that convert APAP to the reactive electrophile, N-acetyl-p-benzoquinone imine (NAPQI), leading to glutathione depletion and covalent binding (Henderson et al., 2000). This invariably results in mitochondria, cell membranes and nuclei damage. Disruption of cell death- and survival-related signalling pathways causes extensive necrosis and apoptosis (Lauterburg and Mitchell, 1982). Apart from CYP2E1, the major isoform in biotransformation of paracetamol, other P450s such as CYP1A2, CYP2A6 and CYP3A, have been identified as APAP-metabolizing enzymes. The involvement of generated Reactive Oxygen Species (ROS), such as superoxide anion (O_2-•), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO•), reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite (ONOO-), and peroxidation reaction products have been highlighted in mechanisms associated with APAP-induced hepatotoxicity (James et al., 2003a; James et al., 2003b; Reid et al., 2005; Bessems and Vermeulen, 2001).

A number of antidotes have been suggested for the treatment of APAP-induced liver damage, one of which is methionine. The aim of this study is to determine the hepatoprotective effect of methionine on female Wistar rats concurrently administered with toxic doses of paracetamol and methionine in the ratio of 5: 1, using liver enzymes (AST, ALT, ALP, GGT); total protein, albumin and liver histology as indices of study and to compare the effect at the peak of absorption with that of peak of toxicity.

MATERIALS AND METHODS

Animals/Experimental design

Female Wistar rats weighing between 250-350 g obtained from the animal house of the Department of Veterinary Physiology, University of Ibadan were utilized for this study. They were kept in cages and maintained on standard diet and supplied water *ad libitum*. The rats were divided into 17 groups consisting of eight rats per group. A two-week acclimation period was allowed before initiation of the experiment. On the day of the experiment, paracetamol (APAP) obtained from Sigma-Aldrich Chemicals (St. Louis, MO) was dissolved in physiologic saline obtained from Unique Pharmaceuticals, Sango-Ota, Ogun State. Each control rat received 5 ml of saline while each APAP-treated rat or APAP/methionine treated-rat received appropriate amount of the drug dissolved in saline. The APAP or APAP/methionine (ratio of 5:1, according to the study of Neuvonen et al., 1985) doses employed for the study were 350mg\kg (subtoxic dose, Abraham, 2004), 1000, 3000, 5000 mg\kg (toxic doses, Abraham, 2004; Trumper et al., 1992, Grypioti, 2005) body weight paracetamol.

The experiment was terminated at two-end periods, the 4th hour, the peak of absorption (Lewis & Paloucek, 1991; Albert et al., 1974; Zarro, 1987) and the 16th hour, the peak of toxicity (Trumper et al., 1992). The peak of paracetamol toxicity has been reported to be between the 16th and the 24th hours. Route of administration was by gastric gavage. At the end of both the 4th and the 16th hours, blood was collected by retro-orbital bleeding. The liver from each animal was promptly removed and preserved in formalin. Serum was separated immediately through centrifugation at 3000 r.p.m. for the determination of liver enzymes (alanine amino transferase, aspartate amino transferase, alkaline phosphatase, γ-glutamyl transferase), bilirubin, total protein and albumin. All experimental protocols complied with Institutional Ethical Committee guidelines as well as internationally accepted principles for laboratory animal use and care as found in US guidelines (NIH publication\85-23, revised in 1985).

Analytical Methods

Creatinine was estimated by the Jaffé reaction while the level of urea was also measured by the diacetyl monoxime oxidase method. Activities of alanine aminotransferase & aspartate aminotransferase (AST & ALT) were obtained by the method of Bergmeyer et al; 1978, alkaline phosphatase (ALP) was measured by method of Mc Comb and Bowers (1972). On the other hand, bilirubin and albumin were determined using modified Jendrassik-Groff (Koch and Doumas, 1982) and standard bromocresol method respectively. Total proteins were measured using Biuret's method (Kingsley, 1982). Hitachi® 902 Automated machine supplied by Roche Diagnostic, Germany was used for these estimations.

Tissue histology

The hepatic tissues were processed using paraffin embedding method; sections of five micron thickness were processed in a microtome. Slides were examined under the microscope to detect histologic

changes subsequent to staining with haematoxylin and eosin (H & E).

Statistical Analysis

Results are expressed as mean ± standard deviation (SD). Level of significant difference between each of APAP or APAP/methionine treated group and control was determined using Student 's T test, Analysis Of Variance (ANOVA) was used to determine significant difference between the three groups (APAP, APAP/methionine, control) at each exposure level. SPSS package version 15 was used for this purpose. The 0.05 level of probability was used as the criterion of significance for experimental groups.

RESULTS

Results of this study are shown in Tables 1-4 and Figures 1-5 below. These results revealed that rats administered with paracetamol showed significant increases ($p<0.05$) in the serum levels of bilirubin compared with controls, especially at toxic levels of exposure at the two end -points i.e. 4th & 16th hours except for 1000mg/kg when bilirubin was not significantly different ($p>0.05$) at the 4th hour. Total protein, albumin and globulin are significantly decreased ($p<0.05$) at 3000 mg/kg and 5000 mg/kg by the end of the 4th and 16th hours, although total protein was also significantly decreased ($p<0.05$) at 1000 mg/kg by the end of the 16th hour as shown in Table 1. In Table 2 significant increases in the activities of hepatic enzymes were noted in paracetamol administered rats at both end -points compared with control especially at toxic levels of exposure of 1000 mg/kg; 3000 mg/kg & 5000 mg/kg BW levels, although at sub-toxic level, such increases were also noted at the end of the 16th hour.

All the hepatic indices used to assess hepatic damage in rats administered with paracetamol/methionine show non-significant difference ($p>0.05$) at all levels of exposure and at both end-points compared with control as shown in Table 3 except for 1000 mg/kg. Table 4 also shows non-significant difference ($p>0.05$) between serum activities of hepatic enzymes in rats administered with paracetamol/methionine compared with control. The histologic presentations are shown in Figures 1-5 below. Figure 1 shows the photomicrographs of liver sections of rats exposed to paracetamol at the end of the 4th hour, some of the presentations include periportal fatty infiltration, extensive vacuolar degeneration and central portal congestion. Figure 2 on the other hand shows histologic presentations of paracetamol/methionine-administered rats also at the 4th hour; with nonvisible lesion at 350 mg/kg and diffuse hepatic degeneration at toxic levels. Figure 3 shows among other features hepatic necrosis and cellular infiltration by mononuclear cells for rats exposed to toxic doses of paracetamol whereas Figure 4 shows that even at the end of the 16th hour rats exposed to paracetamol/methionine manifested absence of necrosis but featured diffuse hepatic vacuolar degeneration. The control rats on the other hand, manifested non-visible lesion as shown in Figure 5.

Using ANOVA, inter-group comparisons between the controls, 4th & 16th hour-groups show a marked difference between the degrees of hepatic damage in paracetamol-exposed group, with the 16th hour group revealing a greater degree of hepatic damage than the 4th hour. The hepatic enzymes are significantly higher ($p<0.05$) in the 16th hour group compared with the 4th hour group and control, an observation which was supported by the histology results.

Table 1:

Serum levels of hepatic indices in paracetamol - exposed Wistar rats- 4 & 16 hours post dosing.

	Bilirubin (µmol\L)	Total protein (g\dl)	Albumin (g\dl)	Globulin (g\dl)
Controls	6.75±2.37	6.03±0.54	3.26±0.34	2.68±0.21
350mg\kg				
4 hours	7.25±1.49	5.88±0.57	3.18±0.28	2.58±0.65
16 hours	8.13±1.81	5.58±0.42	3.01±0.09	2.21±0.61
1000mg\kg	(F=8.94)ǂ			
4 hours	8.36±2.20	5.68±0.41	3.24±0.37	2.48±0.43
16 hours	11.11±1.90*	5.53±0.36*	3.01±0.57	2.52±0.48
3000mg\kg	(F=10.58)ǂ	(F = 8.63)ǂ	(F = 3.31)ǂ	(F = 3.16)ǂ
4 hours	10.25±2.43*	5.50±0.39*	3.08±0.35	2.41±0.31
16 hours	13.75±4.03*	5.08±0.44*	2.83±0.34*	2.35±0.29*
5000mg\kg	(F = 23.21)ǂ	(F = 19.31)ǂ	(F = 11.15)ǂ	(F = 6.48)ǂ
4 hours	11.75±4.23*	5.31±0.24*	2.93±0.27	2.36±0.34*
16 hours	18.33±2.16*	4.73±0.29*	2.47±0.33*	2.10±0.34*

Results are expressed as mean ± standard deviation. *$p<0.05$ is significant when each exposure group is compared with control. ǂ $p<0.05$ is significant at the dose level, when control, 4th and 16th hours groups are compared using ANOVA.

Table 2:
Serum activity of hepatic enzymes in paracetamol- exposed Wistar rats- 4 & 16 hours post dosing.

	AST (IU\L)	ALT (IU\L)	GGT (IU\L)	ALP (IU\L)
Controls	36.36±10.76	33.75±12.14	40.00±5.78	52.25±15 .21
350mg\kg	(F = 48.62)ǂ	(F = 28.98)ǂ	(F = 67.53)ǂ	(F = 41.44)ǂ
4 hours	49.00±9.24	37.75±7.55	42.38±9.29	71.25±1.49*
16 hours	92.38±16.34*	68.63±12.31*	68.0±12.31*	119.88±18.86*
1000mg\kg	(F = 215.17)ǂ	(F = 36.71)ǂ	(F = 10.32)ǂ	(F = 52.82)ǂ
4 hours	99.75±12.37 *	61.0±1.81*	83.75±13.29*	115.75±37.59*
16 hours	487.33±80.39*	379.78±153.92*	242.11±33.41*	358.33±101.34*
3000mg\kg	(F = 277.34)ǂ	(F = 234.75)ǂ	(F=168.88)ǂ	(F = 204.92)ǂ
4 hours	498.75±155.40*	315.88±87.83*	211.38±32.47*	198.00±27.12*
16 hours	1872±233.68*	1506±233.86*	432.63±201.15*	743.38±120.74*
5000mg\kg	(F =482.99)ǂ	(F = 188.42)ǂ	(F = 680 98)ǂ	(F = 663.43)ǂ
4 hours	695.0±105*	581.0±121.10*	286.25±62.78*	338.13±66.89*
16 hours	3882±450.95*	2916±539.38*	707.50±199.66*	890.50±19.77*

Data are presented as Mean ± SD, ALT-alanine aminotransferase; AST- aspartate aminotransferase; ALP- alkaline phosphatase; GGT- γ-glutamyl transferase.*p <0.05 is significant when each exposure group is compared with control. ǂ p <0.05 significant at the dose level, when control, 4th and 16th hours groups are compared using ANOVA.

Table 3:
Serum levels of hepatic indices in paracetamol/methionine exposed Wistar rats- 4 & 16 hours post dosing.

	Bilirubin (µmol\L)	Total protein (g\dl)	Albumin (g\dl)	Globulin (g\dl)
Controls	6.75±2.37	6.03±0.54	3.26±0.34	2.68±0.21
350mg\kg				
4 hours	6.63±3.25	6.36±0.86	3.39±0.43	2.98±1.01
16 hours	6.25±2.38	5.85±0.58	3.14±0.29	2.58±0.33
1000mg\kg				
4 hours	7.25±3.45	5.90±0.48	3.19±0.36	2.71±0.55
16 hours	7.50±4.07	5.25±0.35*	2.80±0.40*	2.44±0.33
3000mg\kg				
4 hours	6.86±2.75	5.95±0.67	3.31±0.53	2.64±0.46
16 hours	7.75±3.06	5.85±0.42	3.31±0.37	2.63±0.72
5000mg\kg				
4 hours	7.25±3.45	6.12±0.63	3.36±0.54	2.76±0.97
16 hours	6.88±3.94	5.89±0.58	3.10±0.28	2.69±0.46

Results are expressed as mean ± standard deviation; *p <0.05 is significant when each exposure group is compared with control.

Table 4:
Serum levels of hepatic enzymes in paracetamol/methionine - exposed Wistar rats- 4 hours post dosing.

	AST (IU\L)	ALT (IU\L)	GGT (IU\L)	ALP (IU\L)
Controls	36.36±10.76	33.75±12.14	40.00±5.78	52.25±15 .21
350mg\kg			(F = 31.31) ǂ	
4 hours	32.38±17.97	29.38±13.54	40.75±8.43	52.38±14.46
6 hours	38.75±9.36	31.25±11.59	51.50±8.50*	55.63±23.14
1000mg\kg			(F = 28.51) ǂ	
4 hours	32.00±8.94	31.50±12.42	43.13±9.89	51.50±20.12
16 hours	33.88±11.87	29.88±17.45	59.00±14.10*	51.00±13.06
3000mg\kg			(F = 22.81) ǂ	
4 hours	34.25±17.52	30.25±11.79	49.13±3.40*	48.75±16.61
16 hours	32.75±13.63	30.25±10.95	67.25±14.54*	57.25±18.92
5000mg\kg			(F = 18.86) ǂ	
4 hours	34.25±17.52	26.13±11.96	54.75±15.52*	54.25±22.00
16 hours	37.88±12.64	29.50±12.56	71.00±14.31*	55.63±13.91

Results are expressed as mean ± standard deviation, ALT-alanine aminotransferase; AST- aspartate aminotransferase; ALP- alkaline phosphatase; GGT- γ-glutamyl transferase. *p <0.05 is significant when each exposure group is compared

with control. ǂ p <0.05 is significant at the dose level, when control, 4th and 16th hours groups are compared using ANOVA.

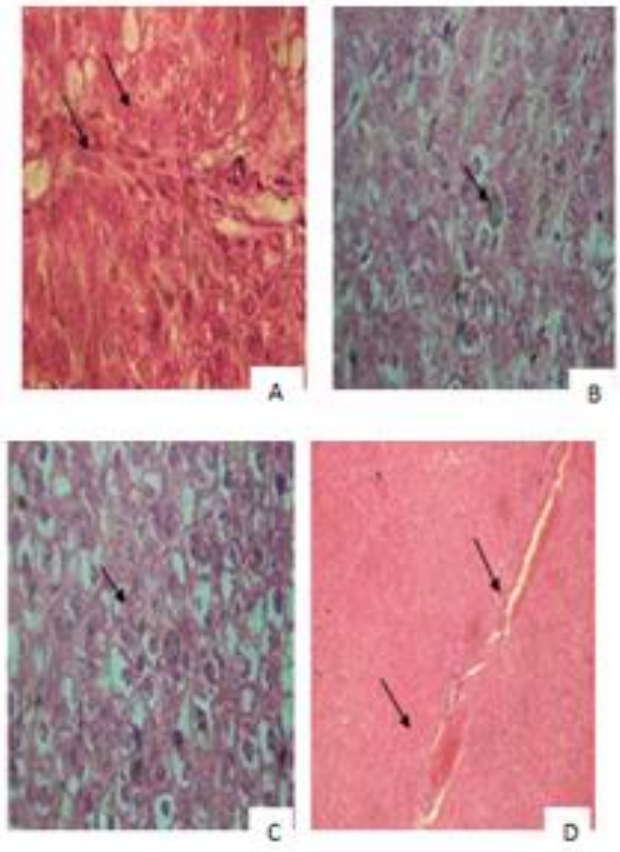

Fig.1.
Photomicrograph of liver of rats exposed to different doses of paracetamol (4 hours post dosing), Mag. X 400 (A) 350mg/kg, showing periportal fatty infiltration, extensive vacuolar degeneration and central venous congestion; (B) 1000mg/kg, showing severe extensive hepatic vacuolation, (C) 3000mg/kg, showing severe diffuse hepatic vacuolar degeneration; (D) 5000mg/kg, showing portal congestion and severe diffuse hepatic vacuolar degeneration.

The inter-group comparisons between the control, 4th & 16th hour-groups of paracetamol/methionine treated rats on the other hand, show non-significant difference (p>0.05) in the levels of the hepatic enzymes.

DISCUSSION

Serum levels of hepatic enzymes e.g. ALT, AST are very sensitive markers usually employed in the diagnosis of liver diseases. When the hepatocellular plasma membrane is damaged, the enzymes normally present in the cytosol are released into the blood stream. They are usually quantified to assess the type and extent of liver injury (Sallie et al., 1991). All the indices used to assess hepatic damage were significantly increased (p<0.05) in paracetamol exposed rats compared with controls; with these increases being more prominent at the peak of toxicity, the 16th hour post-administration than at the 4th hour, the peak of absorption.

Specifically, results show that administration of paracetamol (APAP) at toxic doses of 1000, 3000, 5000 mg\kg caused significant increases (p<0.05) in

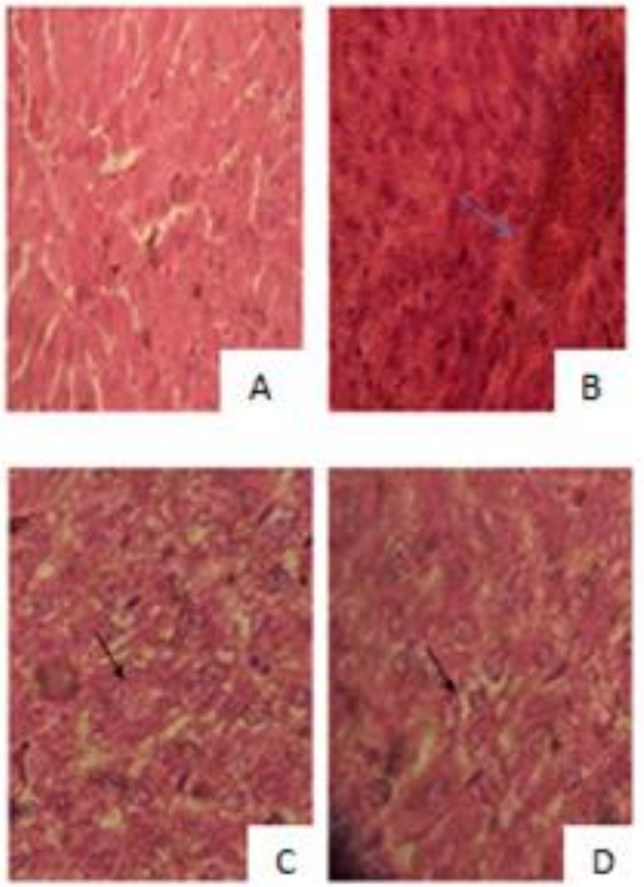

Fig.2.
Photomicrograph of liver of rats exposed to different doses of paracetamol and methionine formulation (4 hours post dosing), Mag. X 400 (A) 350mg/kg, showing no visible lesion; (B) 1000mg/kg, showing mild portal congestion, (C) 3000mg/kg, showing diffuse hepatic degeneration; (D) 5000mg/kg, showing diffuse hepatic degeneration.

aminotransferase (AST), aspartate aminotransferase (ALT), γ- glutamyl transferase (GGT), and alkaline phosphatase (ALP) at both the 4th and the 16th hour, with the 16th hour results being more significantly (p<0.05) higher than the 4th hour. Both AST and ALT are sensitive indicators of hepatic function with the serum activity being raised as early as the 4th hour; Ilic et al. (2010) have even revealed that elevation in activity of AST & ALT can occur as early as 25 min post-paracetamol administration. These increases are an indication of the hepatotoxic effect of paracetamol (Sallie et al., 1991).

The higher level of AST compared to ALT is a confirmation of the involvement of not only the cytosol but also of the mitochondria; AST occurs in both compartments. Decreased plasma membrane Ca^{2+}-ATPase activity and impaired mitochondrial sequestration of Ca^{2+} which lead to influx of extracellular Ca^{2+} has been suggested by Tsokos-Kuhn et al. (1988); and Tirmenstein & Nelson, (1989). In addition, large-scale calcium cycling by mitochondria resulting in oxidative stress and cell death has been reported as some other complications

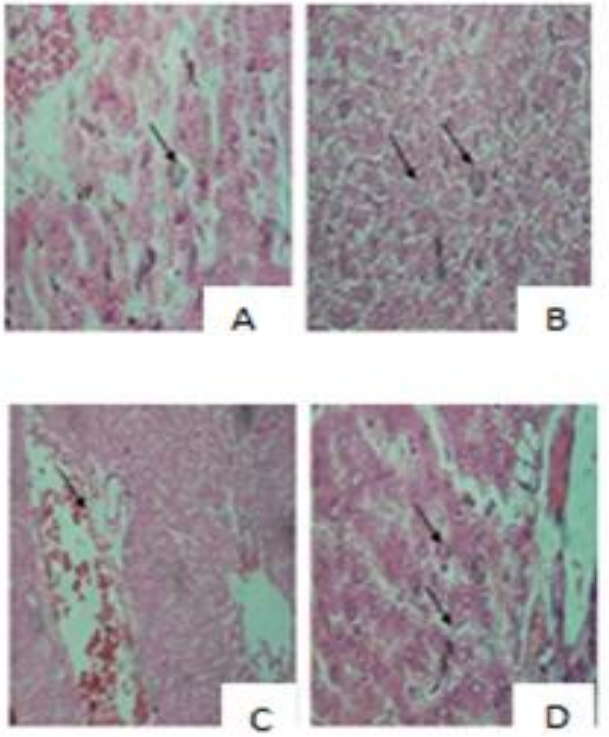

Fig.3.
Photomicrograph of liver of rats exposed to different doses of paracetamol (16 hours post dosing). Mag. X 400 (A) 350mg/kg, showing focally extensive areas of hepatic degeneration and necrosis, few islands of regenerative hepatocyte; (B) 1000mg/kg, showing severe diffuse hepatic degeneration and necrosis, with diffuse mononuclear cellular infiltration, (C) 3000mg/kg, showing diffuse vacuolar degeneration and hepatic necrosis, severe portal congestion; (D) 5000mg/kg, showing severe portal congestion, focally extensive periportal hepatic necrosis and fatty infiltration. There is also a mild cellular infiltration by mononuclear cells

of toxicity (Thomas & Reed, 1988). Disturbed Ca^{2+} homeostasis is likely to activate Ca^{2+}-dependent catabolic processes such as phospholipid degradation, protein degradation, disruption of the cytoskeleton and DNA fragmentation (Ray et al., 1990; Orrenius et al., 1991), thereby resulting in, among other things, hepatocellular membrane damage. Hepatocellular plasma membrane damage causes leakage and subsequent release of these enzymes into the extracellular compartment. Although ALP is not a sensitive indicator of hepatic damage, studies (Ekam & Ebong, 2007; Balamurugan et al., 2008) have indicated that at huge doses of APAP and after many hours of exposure, increase in ALP serum activity may occur. Rajesh & Latha (2004) have also identified that ALP increase may be the result of defective excretion of bile by the liver cells due to hepatic injury.

The significant increase ($p<0.05$) in the activity of serum ALT, AST, ALP and GGT in paracetamol treated rats were also accompanied by significant

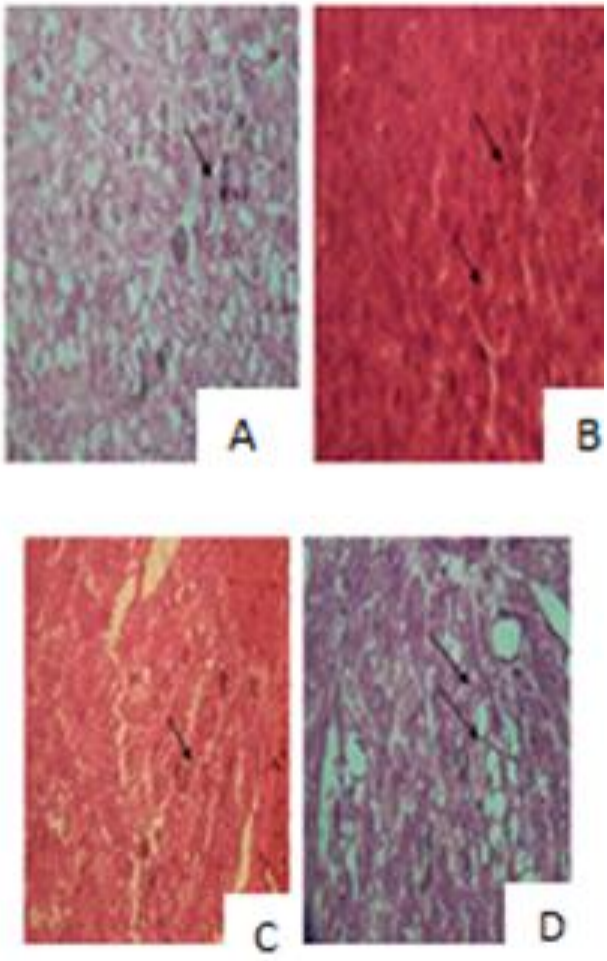

Fig.4.
Photomicrograph of liver of rats exposed to different doses of paracetamol and methionine formulation (16 hours post dosing). Mag. X 400 (A) 350mg/kg, showing severe, diffuse hepatic vacuolar degeneration; (B) 1000mg/kg, showing mild portal and central venous congestion, (C) 3000mg/kg, showing diffuse hepatic vacuolar degeneration and portal congestion; (D) 5000mg/kg, showing diffuse fatty infiltration more severe at the periportal area

increases ($p<0.05$) in the level of bilirubin in the 16 hour-exposure group; the liver cells are the main site of detoxification and excretion of bilirubin.

Furthermore, the hepatotoxic nature of paracetamol was confirmed by the results of total protein and albumin. Both were significantly decreased ($p<0.05$) especially at 1000, 3000, 5000 mg\kg levels, these decreases were more evident at

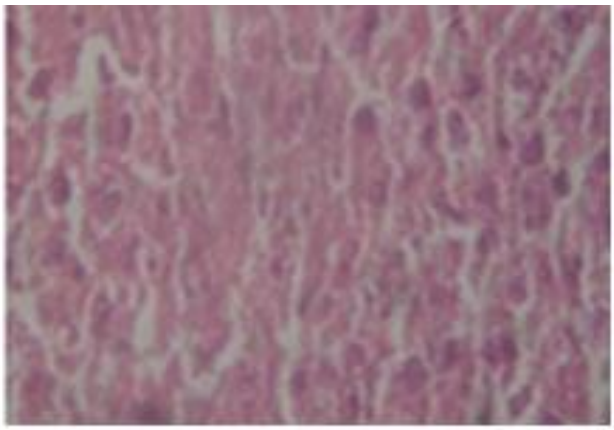

Fig.5.
Photomicrograph of liver of control rats Mag. X 400 showing no visible lesion

the 16th hour – which Trumper et al. (1992) have described as the peak of toxicity- compared with the 4th hour results as well as the controls. Most of the plasma proteins are synthesized by the hepatocytes, the abnormality of which resulted in lower serum level.

Stocker et al. (1987) have indicated that increased serum bilirubin in APAP treated groups can be linked to regurgitation of bile due to obstruction within the liver as a result of damage or inflammation caused by paracetamol whereas, Sallie et al. (1991) have identified that regurgitation of bile resulted in the increase of ALP activity in rats they administered with APAP but Moss and Butterworth (1974) have pointed out that elevated level of serum ALP may be due to the increased synthesis in presence of increasing biliary pressure. In the methionine treated paracetamol intoxicated rats, we observed that the levels of bilirubin and ALP were not significantly different ($p>0.05$) compared with controls, an indication that the liver normal activity and integrity was preserved by action of methionine.

The significant increases ($p<0.05$) in the activities of ALT and AST in this study are in agreement with a number of other studies; Kuvandik et al. (2008) who also used adult female Wistar Albino rats equally observed significant elevations in serum AST and ALT activities in the APAP treated group. Light microscopic evaluation of their rats' livers showed that there were remarkable centrilobular (zone III) hepatic necrosis and mild to moderate sinusoidal congestion in the APAP group, just like our study where histologic presentation varied from periportal fatty infiltration, extensive vacuolar degeneration and central venous congestion (at lower doses) to severe portal congestion, focally extensive, centrilobular hepatic necrosis and fatty infiltration. There was also mild cellular infiltration by mononuclear cells (at 5000 mg\kg). The involvement of macrophages known to play critical role as cellular effectors of nonspecific host defense and which are also potent in their role as secretory cells, producing an array of mediators (e.g. pro-inflammatory and cytotoxic cytokines and growth factors, bioactive lipids, hydrolytic enzymes, and reactive oxygen and nitrogen intermediates) can also not be discounted, most of these mediators have also been implicated in tissue injury, with a consequent rise in liver enzymes (Laskin & Laskin, 2001).

Such increases in the activities of these enzymes have been reported by Rajesh et al. (2009) to reflect also in urine as a spill-over effect. da Silva et al. (2006) equally observed such reflection; that urinary activity of GGT, ALP and LDH enzymes were significantly higher ($P<0.05$) 24 hours after drug administration compared to the activity of controls. Moreover, Kocaoğlu et al. (1997) indicated that twenty-four hours after a single dose of 900 mg/kg paracetamol (APAP) administered to rats intraperitoneally. Urinary GGT activity in the APAP administered group was significantly higher than in the control group, histological examination of the kidneys under light microscopy also showed tissue damage.

Our results of altered activity of liver enzymes as well as alteration in the levels of albumin and total protein in the serum of paracetamol (APAP) treated rats are in agreement with the observation of Balamurugan et al. (2008). These workers observed a reduction in serum total protein and an increase in serum ALP, AST, ALT, bilirubin and liver thiobarbituric acid reactive substances (TBARS) due to liver injury in the paracetamol-administered rats. The significant decrease ($p<0.05$) in the levels of total protein and albumin by the end of the 16th hour of exposure could not entirely have been as a result of decreased synthesis although the liver is the site of production of a number of these proteins; only 4 % of albumin (which constitute about 50% of all serum proteins) is turned over each day (Crook, 2006).

Depletion in levels of albumin and total protein is more of a common presentation of chronic liver diseases where albumin: globulin ratio is always decreased (Murray, 2006). Paracetamol (acetaminophen) is also nephrotoxic at overdose level and loss through the urine is another probable cause of low serum protein level. Being also a negative acute phase protein, the depletion in albumin can also be linked to acute phase response especially as liver histology confirmed infiltration by mononuclear cells.

The hepatoprotective effects of methionine also reflected in results of biochemical indices used to assess hepatic damage. They were not significantly different ($p>0.05$) in the APAP\methionine exposed

group compared with controls, except GGT which was slightly higher than controls at 3000 & 5000 mg\kg. The cause of mild increase in GGT activity without hepatic dysfunction is difficult to identify especially as APAP has not been identified as a GGT inducers, though the ability of APAP to induce some CYP enzymes has been recognized. Crook, (2006) has recognized that mild increases in GGT activity are sometimes difficult to interpret.

Liver histology revealed absence of hepatic necrosis but changes in liver architecture were observed only at the highest level of exposure i.e. 5000 mg/kg. These changes can be linked to the massive influx of methionine to the liver because earlier on Hardwick et al. (1970) on postmortem examination had discovered that liver of rats exposed to high doses of methionine was found to be fatty; lipid being concentrated in the periportal hepatocytes with a little present around the central veins. Just like this study hepatic lipid appeared as early as 16 h into their experiment, after a total dose of 584 mg/kg. No hepatic lipid was found in their control animals as well as ours.

Contrary to the previous reports that APAP causes damage to plasma membrane of liver cells leading to significantly higher levels of AST and ALT, the results of this study revealed the presence of mononuclear cells in liver histology of rats intoxicated with APAP suggests a possible involvement of inflammatory process which resulted in regurgitation of bilirubin leading to its elevated level as well as increased activity of alkaline phosphatase. In addition, the hepatoprotective ability of methionine was demonstrated in female Wistar rats administered even with toxic doses, not just few hours after exposure but at the 16th hour of exposure. GGT was also observed to be raised in hepatoprotected rats. Since the ability of methionine to induce GGT has not earlier been reported, further study may be necessary to determine a possible relationship between methionine and this enzyme in some other animal species.

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