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ESTIMATION OF SERUM ACTIVITIES OF ANTI-OXIDANT ENZYMES AND LEVELS OF INDICES OF RENAL DAMAGE IN FEMALE WISTAR RATS DOSED WITH ACETAMINOPHEN/METHIONINE

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ABSTRACT

Acetaminophen abuse is common in many parts of the world. At overdose levels it causes hepatic and renal damage and because its treatment is expensive and prognosis is poor in some cases, calls for inclusion of an antidote have been made. Results of a past study showed that inclusion of methionine with acetaminophen in the ratio of 1: 9 did not confer protection on the renal cells at very high level of exposure; 40% and 100% mortality occurred at 3000 & 5000 mg/kg (BW) levels of exposure respectively. The aim of this study is to exclude insufficient methionine in that combination as cause of death in those animals, by using a higher combination ratio of 1: 5 (methionine: acetaminophen). Female Wistar rats were divided into 17 groups (n = 8), 16 groups were administered with different doses of acetaminophen or acetaminophen/methionine ranging from 350 – 5000 mg/kg. The 17th group which served as the control received physiologic saline. Route of administration was by gastric gavage. Whole blood was obtained by retro-orbital bleeding; serum samples were utilized for the estimation of renal indices, using assay kits. Kidney sections were stained with hematoxylin & eosin. The combination ratio of 1: 5 (methionine: acetaminophen) conferred protection on renal cells even at doses as high as 3000 & 5000 mg/kg using biochemical and histological markers as indices of study. Urea, creatinine and uric acid were not significantly different in acetaminophen/methionine groups compared with controls while histology result showed non-visible lesion at all levels of exposure. The results suggested that the effectiveness of methionine as an antidote in preventing renal damage after exposure to toxic doses depends on the use of right combination ratio which in the case of Wistar rats was found to be 1: 5 (methionine: acetaminophen).

Keywords: Nephrotoxicity, acetaminophen, methionine, female rats.

INTRODUCTION

Acetaminophen (paracetamol) a widely used analgesic and antipyretic agent is considered a safe drug at therapeutic level but in overdose states it produces renal necrosis in both humans and experimental animals. [1] In experimental animals, it causes hepatic depletion of glutathione at overdose level. [2] Glutathione is a naturally occurring tripeptide, consisting of glycine, glutamic acid and cysteine. Mitchell and his colleagues were the first to elucidate the mechanisms of acetaminophen-induced toxicity. They showed that a minor route of acetaminophen metabolism involved its conversion by monooxygenases (cytochrome P450s) to a reactive arylating metabolite, known as N-acetyl- p-benzoquinone imine (NAPQI), which may cause acute tissue necrosis with toxic doses of this agent. [3] NAPQI causes a depletion of both the mitochondrial and cytosolic pools of reduced glutathione (GSH), thus cellular proteins are directly oxidized by the reactive metabolite. [4] NAPQI also causes inhibition of the antioxidant enzyme- glutathione peroxidase. [5] The inhibitory effect of acetaminophen on this

enzyme as well as on thiol transferase further renders the cell vulnerable to endogenous activated oxygen species which increases oxidation of protein thiols. In an earlier study [6] it was observed that at toxic doses of 3000 & 5000 mg\kg levels of exposure rats administered with acetaminophen: methionine in the ratio of 9:1 were hepatoprotected but still suffered 40% and 100% mortality, with renal damage identified as a cause of death using both biochemical and histological markers as indices of study.

Our submission from the results of study was that generation of a reactive species that could not be detoxified by glutathione or inadequate glutathione synthesis in the renal cells was a probable cause of death. This study sets out to rule out inadequate methionine in the earlier preparation as cause of death by using a higher combination ratio of 5:1 such that renal cells can have available to them higher levels of methionine for GSH synthesis. This study also sets out to identify if tissue protection occurs with unaltered activity of glutathione peroxidase, catalase and superoxide dismutase, enzymes known to prevent the damaging effects of oxidants generated not only through the normal physiologic processes but environmental exposure as well.

MATERIALS AND METHODS

Experimental Animals: This study was carried out in conformity with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; especially as promulgated and adopted by United States Institutes of Health (1985). Twenty-four female Wistar rats of weighing between 250-350 g obtained from the animal house of the Department of Veterinary Physiology, University of Ibadan were used for the study. They were supplied with standard laboratory diet and drinking water without any form of restriction.

Chemicals and reagents: The following were used for the study; acetaminophen (Sigma-Aldrich, St. Louis, MO), methionine (Sigma-Aldrich®, St. Louis, MO) and normal saline (Unique Pharmaceuticals®, Sango- Otta, Nigeria). Kits for tests used to assess renal function were supplied by Dialab®, Holland. All the other reagents used for the determination of the antioxidant enzymes; glutathione peroxidase, catalase, superoxide dismutase were of analytical grade.

***APAP-induced nephrotoxicity*:** Rats were divided into groups, with each group consisting of 8 rats. Groups 1-4 consisted of rats administered with different doses of acetaminophen (APAP). Groups 1, 2, 3 and 4 received 350, 1000, 3000, 5000 mg/kg respectively while groups 5, 6, 7 and 8 received 350, 1000, 3000, 5000 mg/kg of acetaminophen and 70, 200, 600, 1000mg/kg of methionine respectively. Experimental studies of group 1-8 were terminated at the end of the 4th hour, which Zarro [7] have described as the peak of absorption of toxic doses. On the other hand, groups 9, 10, 11, 12 also received 350, 1000, 3000, 5000 mg/kg of APAP respectively while groups 13, 14, 15, 16 received 350, 1000, 3000, 5000 mg/kg of acetaminophen and 70, 200, 600, 1000mg/kg of methionine respectively, but the study was terminated at the end of the 16th hour which Trumper et al. [8] have described the peak of toxicity for the Wistar rats. Prior to administration, APAP or APAP/methionine combination was dissolved in 5 ml normal saline per rat. Rats in group 17 served as the controls and each received 5 ml of physiologic saline. Route of administration was by gastric gavage.

***Assay of antioxidant enzymes and biochemical markers of renal damage*:** Whole blood was obtained by retro-orbital bleeding; this was left to clot and centrifuged for 15 minutes at 3000 revolution per minute using a table centrifuge. The serum samples obtained were used for the estimations of levels of urea, uric acid and creatinine; kits supplied by Dialab®, Holland were utilized for this purpose. These estimations were carried out using Hitachi 902 Automated machines (Roche Diagnostic®, Germany). Estimation of the serum activities of superoxide dismutase, glutathione peroxidase and catalase were the methods of Kakkar et al. [9]; Rotruck et al., [10]; Sinha, 1972. [11]

***Histopathological studies of rat kidneys*:** The kidney of each rat was carefully removed, fixed in 10% buffered formalin and embedded in paraffin. Sections of five µm thickness were stained with hematoxylin-eosin (H & E) and observed under a microscope. Magnification was x 400.

***Statistical analysis*:** Data obtained were expressed as mean ± SD. (standard deviation) of eight observations. Using SPSS version 15, data were statistically assessed by Student t test to test the degree of difference between each of the treated group and the control. $P \leq 0.05$was considered significant.

RESULTS

Administration of female Wistar rats with acetaminophen resulted in significant increases ($p<0.05$) in the serum concentrations of urea, creatinine and uric acid at toxic doses of 1000, 3000

and 5000 mg/kg by the end of the 4th hour but the increases observed for the sub-toxic dose (350 mg/kg) were not significant (p>0.05) as shown in **Table 1.**

The serum concentrations of urea, creatinine and uric acid in acetaminophen/methionine-exposed rats were not significantly different (p>0.05) as **Table 1 also** reveals. In **Table 2** concentrations of urea, creatinine and uric acid were significantly increased (p<0.05) in acetaminophen-exposed rats at toxic levels by the end of the 16th hour but were not significantly different (p>0.05) at sub-toxic dose as well as in acetaminophen/methionine-exposed rats at all levels of exposure. In **Table 3**, serum activities of glutathione peroxidase and superoxide dismutase were significantly decreased (p<0.05) at both sub-toxic and toxic levels in not only acetaminophen-exposed rats but also in acetaminophen/methionine-exposed rats by the end of the 4th hour. Catalase though was not significantly different in acetaminophen/methionine treated rats at 350, 1000, 3000 mg/kg levels. Moreover, significant decreases (p<0.05) in the serum activities of glutathione peroxidase, catalase, and superoxide dismutase persisted to the end of the 16th hour in both acetaminophen & acetaminophen/methionine treated rats at all levels of exposure as shown in **Table 4**.

Figures 1-4 show the photomicrographs of kidney sections of rats administered with different doses of acetaminophen & acetaminophen/methionine, while **figure 5** is that of the control rats. F**igure 1** is for rats administered with different doses of acetaminophen and shows non-visible lesion at 350 mg/kg and presence of protein casts in the tubular lumen at 1000, 3000, 5000 mg/kg at the end of the 4th hour of exposure. Also after the same duration of exposure, rats administered with acetaminophen/methionine featured non-visible lesion at all levels of exposure namely; 350, 1000, 3000, 5000 mg/kg BW as shown in **figure 2.**

In **figure 3** the photomicrographs of rats exposed to acetaminophen showed tubular necrosis, with the presence of protein casts at toxic doses of 1000, 3000, 5000 mg/kg while that of the sub-toxic dose is non-visible lesion by the end of the 16th hour. **Figures 4 & 5** represent the photomicrographs of rats administered with acetaminophen/methionine by the end of the 16th hour and control rats respectively and both figures featured showed non-visible vision.

Although both biochemical and histological indices indicated renal protection in acetaminophen /methionine exposed rats and no mortality was recorded, 25% mortality rate was recorded for rats exposed to 5000 mg/kg (BW) acetaminophen by the end of the 16th hour.

DISCUSSION

Renal dysfunction was assessed using urea, creatinine, uric acid as well as kidney histology in acetaminophen-exposed rats, and results showed significant increases (p<0.05) in the levels of the biochemical indices at toxic doses at the end of the 16th hour compared with the controls. Although acetaminophen induced nephrotoxicity has been described to occur as a result of either overproduction of high level of N-acetyl-p- benzoquinone imine; C-S-Iyase-mediated metabolism of acetaminophen (paracetamol)-S conjugates in the kidney [12] or possible involvement of paracetamol-glutathione or paracetamol-cysteine conjugates. [13]

Other studies have identified the involvement of some other mechanisms in acetaminophen-induced toxicity, and which might have been a probable cause of death of the animals in the 5000mg\kg acetaminophen administered group, in which 25% mortality was recorded by end of the 16th hour. For example Trumper et al. [8], have reported that GSH-derived APAP metabolites formed in the liver, as well as renal γGT-dependent transport of these conjugates as perquisite for APAP-induced renal damage.

Apart from these, the possibility of some other mechanisms participating in the renal incorporation of the glutathione (GSH) conjugates to induce paracetamol-induced toxicity has been raised by Trumper et al. [14] ; because even with a significant inhibition of renal γGT activity by acivicin pretreatment, only a partial protection of APAP renal effects was observed. This may also be buttressed by an earlier observation by Lash and Jones [15] who characterized a probenecid-sensitive transport system for GSH and GSH conjugates in renal basolateral vesicles. Moreover, the direct effects of APAP on APAP-induced nephrotoxicity have been confirmed with the use of IPK model [16], as well as the effects of GSH-derived APAP metabolites. [13] In this regard, S-conjugates in systemic circulation have been identified to accumulate in the kidney via several carrier-mediated mechanisms, including the organic anion transporter and degradation by membrane bound γGT. [17]

Results of the studies of Palani et al. [1] and Ilbey et al. [18] have identified the nephrotoxic effects of APAP at both subtoxic (500 mg\kg) and toxic (1000

mg\kg) levels of exposure as revealed by significantly increases ($p<0.05$) in the levels of serum urea & creatinine. And according to Isik et al. [19] APAP treated rats had increased levels of blood urea nitrogen and creatinine as well as tubular epithelial degeneration. Vacuolization and cell desquamation were also clearly observed in the APAP treated rats. The cellular debris in the proximal tubules and cortical interstitial congestions were prominent in the kidneys of their APAP treated rats. Renal histology of our study also showed manifestations ranging from mild congestion of the cortical vessels; tubular epithelial degeneration (mild) to tubular necrosis, with protein casts in the tubular lumen.

Significant decreases ($p<0.05$) in activities of glutathione peroxidase, catalase and superoxide dismutase (SOD) occurred not only in acetaminophen treated rats but in those which received combination treatment (especially by the end of the 16th hour), an indication that it may be involved in the oxidative process which yields the reactive species- NAPQI as well as the protective process. Tirmenstein & Nelson[5] have reported of the inhibitory effect of NAPQI on another enzyme; glutathione peroxidase, and according to Isik et al.[19] APAP treated rats had decreased activities of catalase (CAT) and glutathione peroxidase (GSH-Px), but not SOD. Unlike our study Ronsein et al.[20] observed significant increase in activity of SOD four hours after male Wistar rats were treated with an overdose of acetaminophen (800 mg/kg, p.o.), whereas, Balamurugan et al. [21] observed a reduction in liver antioxidants e.g. superoxide dismutase (SOD).

These alterations are not limited to the serum, Kuvandik et al. [22] have also identified that in adult female Wistar Albino rats, APAP administration produced a decrease in hepatic SOD which was also accompanied by remarkable centrilobular (zone III) hepatic necrosis and mild to moderate sinusoidal congestion in the APAP group. Moreover, Demirbag et al. [23] have revealed that APAP administration deteriorated renal functions and significantly elevated renal malondialdehyde (MDA) levels and depleted SOD activities 48 hours post- administration. Evidence that SOD plays a role in tissue injury has been revealed by Jaeschke et al. [24] ; they indicated that in acetaminophen toxicity, nitric oxide scavenges superoxide to produce peroxynitrite, which then causes protein nitration and tissue injury. In inducible nitric oxide synthase knockout mice, nitration is prevented but unscavenged superoxide production then causes toxic lipid peroxidation. Muriel et al. [25] , have also identified lipid peroxidation as a destructive process in liver injury due to acetaminophen administration. Knight et al. [26] though have indicated that lipid peroxidation does not appear to be a critical event in APAP-induced hepatotoxicity.

Superoxide dismutase, CAT and GPx are enzymatic antioxidant defense systems which are natural protective barriers against lipid peroxidation and important scavengers of superoxide ion and hydrogen peroxide. Superoxide dismutase in association with CAT and GPx, according to Halliwell and Gutteridge [27] abolishes the generation of hydroxyl radicals and protect the cellular constituents from oxidative damage. A number of agents used in the treatment of acetaminophen-induced toxicity have been reported to increase the activity of SOD, e.g. methanolic extract of stem bark of Bauhinia racemosa Lam. [28] The increases in the activity of these enzymes have been linked to changes in the expression of genes that code for them. Crawford [29] has identified such genes, whose steady-state mRNA levels are modulated by oxidant stress agents e.g. such as superoxide, hydroperoxide, nitric oxide, redox active quinines, hyperbaric oxygen, singlet oxygen, diethyl maleate and glutathione depletion. And a study in bacterial system has identified many of the modulated genes as those of SOD, CAT, alkyl hydroperoxidase reductase and glutathione reductase as well as manganese superoxide dismutase.

Both methionine and N-acetylcysteine have been identified as suitable antidotes in alleviating the clinical as well as biochemical features of acetaminophen toxicity since GSH itself cannot cross the plasma membrane. Methionine is a glutathione precursor and replenishes glutathione stores depleted as a result of acetaminophen overdose. Glutathione occurs naturally as a tripeptide, consisting of glycine, glutamic acid and cysteine; it detoxifies the reactive intermediate metabolite of acetaminophen, NAPQI. Methionine is first demethylated and then transulfurated to generate cysteine before it can act as glutathione precursor. [30] Like N-acetylcysteine it may also reduce the severity of renal necrosis by directly conjugating with and\or reducing the reactive metabolite NAPQI. [31]

Although, through an earlier study [6] we observed a significant decrease ($p<0.05$) in the levels of urea and creatinine of rats administered with acetaminophen: methionine at 100mg\kg and 350 mg\kg as well as 1000 mg\kg levels of exposure, rather non significant differences were observed in the results of present study for the four different levels of exposure at the end of the 4th and 16th hours, this may be ascribed to differences in composition of agents administered; instead of the acetaminophen:

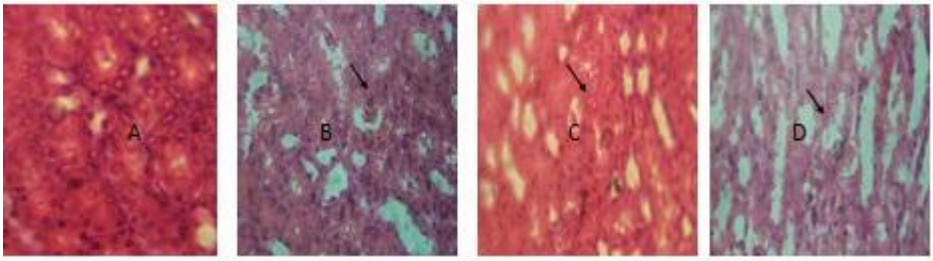
methionine of ratio 9:1 in the earlier study, a ratio of 5:1 was employed for this study, moreover, this study was terminated at the 16th hour instead of the 24th hour employed for the earlier study. Though the earlier combination could not confer protection to the nephrons at large doses of 3000 & 5000 mg\kg, as 40% and 100% mortality rates were recorded for 3000 & 5000 mg\kg respectively, with renal necrosis as a cause of death, complete nephroprotection was conferred on all the rats exposed to acetaminophen: methionine of ratio 5:1 at all levels of exposure. The histology results confirmed the nephroprotective effects of methionine at this combination level by the end of the 4th and 16th hours, non visible lesion was observed as the renal histology results even at dose level of 5000 mg\kg APAP. The nephroprotective effects of methionine also reflected in results of biochemical indices used to assess renal damage. They were not significantly different ($p>0.05$) in the APAP\methionine exposed group compared with controls. The mild congestion of the cortical vessels; tubular epithelial degeneration (mild) and tubular necrosis, with protein casts in the tubular lumen which we observed for the APAP treated rats at one level of exposure or the other was replaced by non-visible lesions of renal histology at all levels of exposure in APAP\methionine treated rats.

Although, the precise mode of action by which methionine confers protection has been linked to enhancement in the level of glutathione but increase in the quantity of unchanged drug excreted through the urine can also not be ruled out. Pretreatment with another nephroprotetive agent, probenecid caused APAP plasma levels 16 hr after APAP administration to be significantly lower in rats compared with the level attained in rats treated with APAP alone.

CONCLUSION

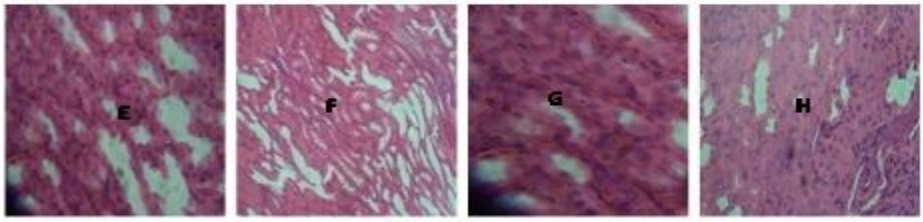
The results of this study suggest that incorporation of methionine with acetaminophen in the ratio of 1: 5 is capable of protecting the kidney even at very high levels of exposure since both biochemical and histological indices did not indicate any form of derangement not only at the peak of absorption but also at the peak of toxicity, but whether such tissue protection will not be accompanied by oxidative stress mediated by trace elements alteration may require further investigation. Especially as superoxide dismutase was found to be significantly decreased at all levels of exposure in not only acetaminophen dosed rats but in acetaminophen/methionine treated rats as well. Therefore, it may be essential to evaluate the impact of this combination on elements which are cofactors for superoxide dismutase as well as other antioxidant enzymes; elements which earlier studies have shown that their levels are altered by acetaminophen (only) administration.

FIG 1: Photomicrographs of kidneys of rats exposed to different doses of acetaminophen (4 hours post-dosing). H&E × 400



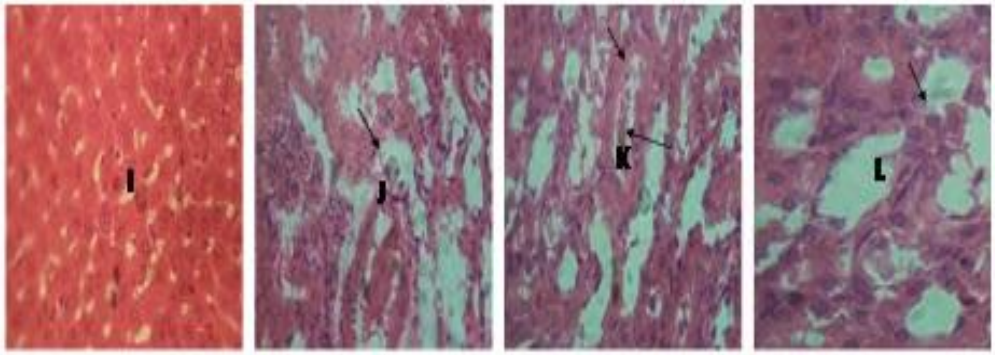
A (350mg/kg) shows no visible lesion; B (1000mg/kg); C (3000mg/kg) & D (5000mg/kg) showing protein casts in the tubular lumen.

FIG 2: Photomicrographs of kidney exposed to different doses of acetaminophen & methionine (4 hour post-dosing). H&E × 400



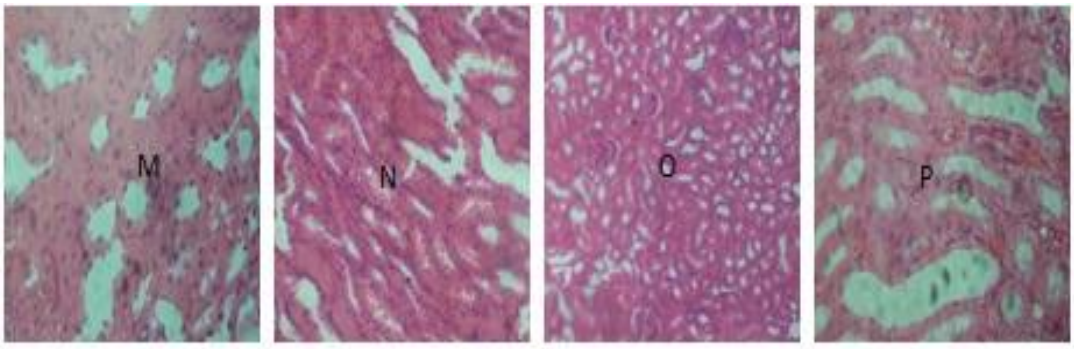
E (350mg/kg); F (1000mg/kg); G (3000mg/kg) & H (5000mg/kg) showing no visible lesion.

FIG 3: Photomicrographs of kidneys of rats exposed to different doses of acetaminophen (16 hours post-dosing). H&E × 400



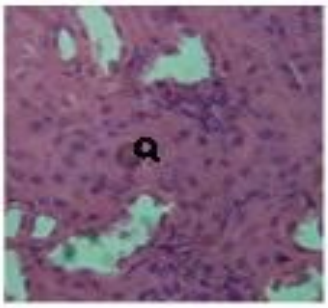
I (350mg/kg); J (1000mg/kg); K (3000mg/kg) & L (5000mg/kg) showing no visible lesion; perivascular tubular necrosis and protein casts in the tubular lumen; tubular necrosis with protein casts in the tubular lumen; tubular necrosis with protein casts in the tubular lumen respectively.

FIG 4: Photomicrographs of kidneys of rats exposed to different doses of acetaminophen & methionine (16 hours post-dosing). H&E × 400



M (350mg/kg); N (1000mg/kg); O (3000mg/kg) & P (5000mg/kg) showing no visible lesion.

FIG 5: A photomicrograph of kidney of control at 0 hour. H&E × 400



Q (control) showing no visible lesion.

Table 1: Levels of renal indices in acetaminophen- exposed Wistar rats- 4 hours post dosing.

	UREA (mmol\l)	CREATININE (µmol\l)	URIC ACID (nmol\l)
X ± SD controls	3.40±0.70	56.50±15.50	226.13±119.18
350mg\kg			
X ± SD (P)	3.98±1.14 (0.245)	64.88±5.06 (0.168)	228.25±112.73 (0.971)
X ± SD (P&M)	3.63±0.62 (0.507)	58.58±9.67 (0.753)	234.75±127.34 (0.891)
F-value	0.928 (0.412)	1.273 (0.301)	0.017 (0.989)
1000mg\kg			
X ± SD (P)	10.61±1.81 (0.008)*	208±23.13 (0.007)*	240.00±103.23 (0.807)
X ± SD (P&M)	3.64±0.65 (0.494)	61.56±14.54 (0.511)	221.25±116.89 (0.935)
F-value	95.842 (0.003)*	180.120 (0.005) *	0.059 (0.943)
3000mg\kg			
X ± SD (P)	15.85±3.64 (0.005)*	239..13±32.29 (0.005)*	496.88±30.45 (0.000)*
X ± SD (P&M)	3.81±1.54 (0.501)	58.55±11.03 (0.765)	237.50±90.79 (0.833)
F-value	74.491 (0.004)*	187.849 (0.003)*	24 080 (0.006)*
5000mg\kg			
X ± SD (P)	19.25±3.22 (0.005)*	306.88±32.19 (0.003)*	577.75±20.21 (0.000)*
X ± SD (P&M)	3.95±1.93 (0.460)	59.26±11.28 (0.690)	239.75±116.71 (0.821)
F-value	133.282 (0.004)*	353.354 (0.002)*	33.736 (0.005)*

*Results are expressed as mean (X) ± standard deviation (SD); *p <0.05 is significant. p values are in parentheses.*

Table 2: Levels of renal indices in acetaminophen-exposed Wistar rats-16 hours post dosing.

	UREA (mmol\L)	CREATININE (µmol\L)	URIC ACID (nmol\L)
X ± SD controls	3.40±0.70	56.50±15.50	226.13±119.18
350mg\kg			
X ± SD (P)	4.41±1.90 (0.188)	56.99±14.85 (0.950)	236.63±96.57 (0.849)
X ± SD (P&M)	3.14±1.10 (0.577)	61.44±16.90 (0.552)	213.13±104.16 (0.820)
F-value	1.982 (0.163)	0.238 (0.790)	0.097 (0.908)
1000mg\kg			
X ± SD (P)	15.0±2.41 (0.006)*	394.44±88.48 (0.005)*	573.00±37.11 (0.006)*
X ± SD (P&M)	3.66±1.13 (0.558)	59.01±11.04 (0.714)	227.38±72.69 (0.965)
F-value	95.841 (0.003) *	180.120 (0.002) *	0.059 (0.943)
3000mg\kg			
X ± SD (P)	23.81±2.46 (0.004)*	583.25±40.21 (0.002)*	221.25±87.41 (0.010)
X ± SD (P&M)	2.91±0.88 (0.239)	64.04±21.69 (0.437)	221.25±87.41 (0.927)
F-value	466.900 (0.001)*	940.266 (0.001)*	26.915 (0.004)*
5000mg\kg			
X ± SD (P)	37.58±4.08 (0.004)*	809.67±128.81 (0.001)*	683.17±117.96 (0.004)*
X ± SD (P&M)	4.26±2.07 (0.282)	54.74±15.86 (0.825)	209.38±56.34 (0.726)
F-value	405.24 7(0.002)*	272.812 (0.002)*	47.018 (0.009)*

Results are expressed as mean (X) ± standard deviation (SD); p <0.05 is significant. p values are in parentheses.*

Table 3: Serum activities of glutathione peroxidase, catalase and superoxide dismutase and levels of renal indices in acetaminophen- exposed Wistar rats- 4 hours post dosing.

	GPX (µmol GSH consumed/(min·mg protein)	CAT (µmol H_2O_2 consumed/(min·mg protein))	SOD (U/mg protein)
X ± SD controls	9.59±0.35	2.06±0.23	12.82±0.31
350mg\kg			
X ± SD (P)	8.78±0.40 (0.007)*	1.93±0.11 (0.167)	12.04±0.25 (0.004)*
X ± SD (P&M)	8.99±0.28 (0.022) *	2.07±0.22 (0.914)	12.09±0.35 (0.004)*
F-value	11.950 (0.004)*	1.410 (0.267)	16.523 (0.007)*
1000mg\kg			
X ± SD (P)	8.50±0.55 (0.040)*	1.86±0.11 (0.040)*	11.81±0.88 (0.003)*
X ± SD (P&M)	8.30±0.48 (0.009)*	1.97±0.15 (0.350)	12.24±0.31 (0.001)*
F-value	0.902 (0.421)	2.853 (0.080)	6.363 (0.007)*
3000mg\kg			
X ± SD (P)	6.24±1.10 (0.004)*	1.37±0.12 (0.001)*	10.34±0.31 (0.001)*
X ± SD (P&M)	7.16±0.15 (0.006)*	1.92±0.18 (0.191)	12.08±0.81 (0.030)*
F-value	53.476 (0.003)*	32.075 (0.004)*	46.190 (0.008)*
5000mg\kg			
X ± SD (P)	5.08±0.07 (0.004)*	1.15±0.10 (0.001)*	10.15±0.13 (0.001)*
X ± SD (P&M)	7.07±0.16 (0.006)*	1.70±0.13 (0.002)*	10.83±0.18 (0.002)*
F-value	819.779 (0.005)*	63.368 (0.002)*	369.992 (0.001)*

*Results are expressed as mean (X) ± standard deviation (SD); GPX- glutathione peroxidase, CAT- catalase, SOD- superoxide dismutase. p values are in parentheses. *p <0.05 is significant.*

Table 4: Serum activities of glutathione peroxidase, catalase and superoxide dismutase and levels of renal indices in acetaminophen- exposed Wistar rats- 4 hours post dosing.

	GPX (µmol GSH consumed/(min·mg protein)	CAT (µmol H_2O_2 consumed/(min·mg protein))	SOD (U/mg protein)
X ± SD Controls	9.59±0.35	2.06±0.23	12.82±0.31
350mg\kg			
X ± SD (P)	6.82±0.13 (0.001)*	1.69±0.05 (0.003)*	11.95±0.18 (0.001)*
X ± SD (P&M)	8.53±0.14 (0.007)*	1.97±0.08 (0.318)	12.05±0.16 (0.001)*
F-value	305.542 (0.012)*	14.122 (0.006)*	35.718 (0.005)*
1000mg\kg			
X ± SD (P)	5.34±0.81 (0.001)*	1.11±0.10 (0.001)*	10.63±0.29 (0.009)*
X ± SD (P&M)	8.03±0.17 (0.007)*	1.85±0.13 (0.046)*	11.87±0.19 (0.001)*
F-value	650.765 (0.018)*	79.893 (0.006)*	138.536 (0.009)*
3000mg\kg			
X ± SD (P)	3.44±0.14 (0.001)*	0.85±0.05 (0.001)*	9.11±0.19 (0.0011*
X ± SD (P&M)	7.96±0.15 (0.001)*	1.52±0.06 (0.002)*	11.39±0.16 (0.000)*
F-value	1489.260 (0.001)*	147.652 (0.004)*	545.237 (0.006)*
5000mg\kg			
X ± SD (P)	1.17±0.02 (0.001)*	0.53±0.07 (0.001)*	9.14±0.57 (0.001)*
X ± SD (P&M)	8.23±0.48 (0.004)*	1.79±0.15 (0.034)*	10.83±0.18 (0.001)*
F-value	2640.635 (0.002)*	161.931 (0.009)*	179.836 (0.006)*

*Results are expressed as mean (X) ± standard deviation (SD); GPX- glutathione peroxidase, CAT- catalase, SOD- superoxide dismutase. p values are in parentheses. *p <0.05 is significant.*

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