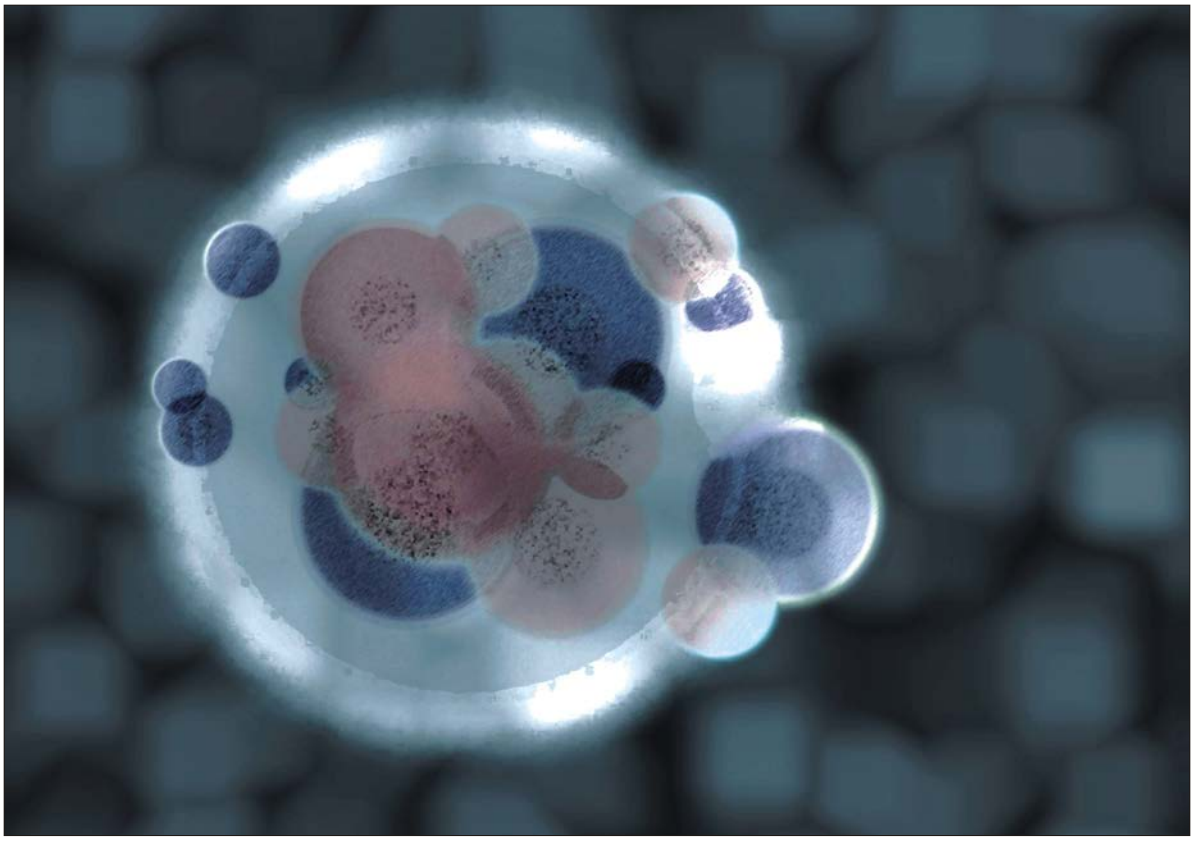

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 15 (4): 187-193, 2015
ISSN 1727-3048

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/jbs.2015.187.193

Acetaminophen Induced Kidney Failure in Rats: A Dose Response Study

[1]Suchismita Roy, [1]Shrabani Pradhan, [1]Koushik Das, [1]Arpita Mandal, [1]Shreya Mandal, [1]Arpita Patra, [1]Animesh Samanta, [2]Banadeb Sinha and [1]Dilip Kumar Nandi

[1]Research Unit, Department of Nutrition, Physiology and Microbiology, Raja N. L. Khan Women's College, Midnapore, West Bengal, India

[2]Department of Biochemistry, Midnapore Medical College and Hospital, Midnapore, West Bengal, India

ARTICLE INFO

Article History:
Received: March 13, 2015
Accepted: October 14, 2015

Corresponding Author:
Dilip Kumar Nandi
Department of Human Physiology, Nutrition and Microbiology,
Raja N.L. Khan Women's College,
Midnapore, 721102, West Bengal, India
Tel : +91-9434229882

ABSTRACT

Acetaminophen is a commonly used analgesic and antipyretic drug, high doses of which cause hepatic and renal injury. In development and progression of kidney disease research, it is necessary to have a suitable common drug to induce uremia and renal failure of rats. It is also required to select the threshold doses for the said drug; a therapeutic dose and toxic dose for kidney failure using standard guidelines. An acute toxicity of acetaminophen was conducted by the limit test at a dose of 2000 mg kg^{-1} b.wt., on either sex rats (n = 5) and a main test was conducted by a dose progression factor of 3.2 times as per Organization of Economic Co-Operation and Development guidelines 425. Eighteen male albino rats (n = 18) were divided into three groups, group I served as control, groups II and III rats were administered 175 mg and 550 mg kg^{-1} b.wt., acetaminophen intraperitoneally for 14 days, respectively. Different parameters were considered to analyze renal failure. Urea, creatinine, GOT, GPT and MDA levels were increased significantly ($p<0.05$) in group III, compared to groups I and II. Antioxidant enzymes like SOD, catalase and GSH level were decreased significantly ($p<0.05$) in group III rats, compared to group I and II rats. Increase in blood uremia profile indicated that the higher dose of acetaminophen causes uremia. Increase in the toxicity markers and lipid peroxidation marker enzymes indicate the nephrotoxicity. Histological structures of kidney of group III animals showed a severe disorganization of glomerulus and dilation of renal tubules. These results indicate that intraperitoneal injection of acetaminophen at high dose causes nephrotoxicity and renal cellular damage to experimental rats.

Key words: Acetaminophen, nephrotoxicity, histology, toxicity, catalase

INTRODUCTION

Now-a-days, heart disease and diabetes provide the first and second leading cause of death in the world, respectively and people are suffering from these diseases are likely to be more proned to kidney disease. There is great urgency for a nonconventional, affordable therapy for patients who cannot afford expensive dialysis or kidney transplant to keep them alive. With this background our research work aimed to find out the different anti-uremic and nephroprotective phytocompounds from different plant extracts such as hydro-methanolic root extract of *Asparagus racemosus* (Roy *et al*., 2013), methanolic bark extract of *Terminalia arjuna* (Das *et al*., 2010a), methanolic root extract of *Withania somnifera* (Das *et al*., 2010b) which had been effective in the reduction of uremic toxins from acetaminophen induced chronic renal failure rats. Recent research work of this same laboratory undertaken on nutraceuticals like alpha lipoic acid (Pradhan *et al*., 2013) and probiotic (Mandal *et al*., 2013) therapy have shown excellent nephroprotective activity against acetaminophen induced renal failed male rats. Acetaminophen is used to induce uremia and

renal failure in our laboratory. Acetaminophen is a commonly used antipyretic agent which, in high doses, causes renal tubular damage and uremia. An acute acetaminophen (paracetamol, N-acetyl p-aminophenol; APAP) overdose may result in a potentially fatal hepatic and renal necrosis in humans and experimental animals (Jones and Vale, 1999). Etiopathological basis of acetaminophen nephrotoxicity has recently been used for kidney disease research (Henrich *et al*., 1996). It is necessary to establish proper doses and durations of acetaminophen for inducing nephrotoxicity. Therefore, objectives of this study was to establish the standardization of the threshold dose of acetaminophen induced kidney disease following OECD guidelines.

MATERIALS AND METHODS

Collection of drugs and chemicals: Acetaminophen (paracetamol, N-acetyl p-aminophenol; APAP) was purchased from AshChemie, India. It was administered intraperitoneally with saline water. All the chemicals used for preparation of extracts and at the time of bio chemical tests including urea, creatinine, Na, K kit and methanol, K_2HPO_4, KH_2PO_4, pyragallol, tris, TCA, TBA and other chemicals were collected from Merck Specialities Private Limited Worli. Mumbai, HiMedia Laboratories Pvt. Ltd. Mumbai, India and Crest Biosystems Goa, India.

Selection of animals and care: The study was conducted on 23 healthy, adult, male (n = 20) and female (n = 3) albino rats of Wistar strain (Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata 54) having a body weight of 100±15 g. They were acclimatized to laboratory conditions for 2 weeks prior to experimentation. Animals were housed at three rats/cage in a temperature-controlled room (22±2°C) with 12-12 h dark-light cycles (8.00-20.00 h light, 20.00-8.00 h dark) at a humidity of 50±10%. They were provided with standard food and water *ad libitum* throughout the experimental period. Animal care was provided according to the Guiding Principles for the Care and Use of Animals (Olfert *et al*., 1993). This experiment was approved by our Institutional Animal Ethical Committee (IAEC), guidelines followed by CPCSEA.

Experimental design

Acute toxicity study by limit test: An acute toxicity of acetaminophen was conducted using acute toxic class method as per Organization of Economic Co-operation and Development (OECD) guidelines 425 (OECD., 2001) where the limit dose of 2000 mg kg^{-1} b.wt., was used. Healthy wistar strain rats (n = 5) of either sex selected by random sampling technique were employed in this study. Wellness parameters of animals were made and recorded systematically 30 min, 4, 24 and 48 h after dose administration for skin and fur, eyes, mucus membrane, behavioral pattern changes, tremor, convulsions, salivations, diarrhea, lethargy, sleep and mortality.

Main test: The main test was conducted on eighteen healthy male albino rats (n = 18). Here a dose progression factor of 3.2 times was chosen as per OECD guidelines 425. The doses were selected 175 mg and 550 mg kg^{-1} b.wt., for 14 days continuously. The rats were divided into three equal groups as follows: Group I rats that served as untreated control were administered single daily dose of 5 mL kg^{-1} b.wt., de-ionized water intraperitoneally, where group II and III animals were administered 175 and 550 mg kg^{-1} b.wt., with de-ionized water 5 mL kg^{-1} b.wt., intraperitoneally, one at a time usually at 24 h intervals for 14 days.

Animals sacrificed, plasma and organ collection: This experimental design was continued for 14 days. At 15th day, the animals were sacrificed and blood was collected from the aorta after which the kidneys were collected for different biochemical and histopathological analysis.

Blood uremia profile

Biochemical estimation of blood urea: The collected blood was centrifuged and plasma fraction was separated. Urea level of plasma was measured by commercially available standard Blood Urea Kit (Merck, Japan) using Semiautoanalyzer (Merck, Japan) by standard protocol for photometric determination of urea, according to the Urease GLDH method (kinetic UV test) (Burtis and Edward, 1999).

Biochemical estimation of blood creatinine: The collected blood was centrifuged and plasma fraction was separated. The plasma creatinine level was measured by commercially available standard Blood Urea Kit (Merck, Japan) using Semiautoanalyzer (Merck, Japan) by standard protocol for phtotometric determination of creatinine based on Jaffe kinetic method without de-proteinization (Sabbagh *et al*., 1988).

Anti-oxidant enzyme profile

Biochemical assay of catalase (CAT) activity: For the evaluation of CAT activity, kidney homogenized separately in 0.05 M Tris hydrochloric acid (HCl) buffer solution (pH-7.0) at a tissue concentration of 50 mg mL^{-1}. These homogenates were centrifuged separately at 10,000 g at 4°C for 10 min. Following this, 0.5 mL of hydrogen peroxide (H_2O_2) and 2.5 mL of distilled water were mixed and reading of absorbance was noted using a spectrophotometric cuvette at 240 nm. Forty microliters of tissue supernatant was added separately and six subsequent readings were noted at 30 sec intervals (Beers and Sizer, 1952).

Biochemical assay of Superoxide dismutase (SOD): The kidneys were homogenized in ice-cold 100 mM Tris-cocodylate buffer to give a concentration of 50 mg mL^{-1} and centrifuged at 10,000x g for 20 min at 4°C. From the supernatant, SOD activity was estimated by measuring the percentage of inhibition of the pyragallol autooxidation by SOD. The buffer was 50 mM Tris (pH 8.2) containing 50 mM cocodylic acid (pH 8.2), 1 mM Ethylene Diamine Tetra Acetic

acid (EDTA) and 10 mM hydrochloric acid (HCl). In a spectrophotometric cuvette, 2 mL of buffer, 100 µL of 2 mM pyragallol and 10 µL of supernatant were poured and the absorbance was noted in spectrophotometer at 420 nm for 3 min. One unit of SOD was defined as the enzyme activity that inhibited the autooxidation of pyragallol by 50% (Marklund and Marklund, 1974).

GSH content: Renal glutathione reductase (GSH) was determined from the kidney homogenate (Moron *et al.*, 1979). Estimation of lipid peroxidation from the levels of malondialdehyde (MDA).

The kidneys were homogenized separately at a tissue concentration of 50 mg mL^{-1} in 0.1 M of ice-cold phosphate buffer (pH = 7.4) and centrifuged at 10,000 g at 4°C for 5 min. Supernatant was used for the estimation of MDA. For the measurement of MDA, 0.5 mL homogenate was mixed separately with 0.5 mL normal saline and 2 mL of TBA-TCA mixture (0.392 g of TBA in 75 mL of 0.25 N HCl with 15 g of TCA, with the final volume of the mixture being made up to 100 mL with ethanol) and, then boiled at 100°C for 10 min. The mixture was then cooled at room temperature and centrifuged at 4000x g for 10 min. The whole supernatant was transferred into a spectrophotometer cuvette and read at 535 nm. Calibration was performed by using the acid hydrolysis of 1, 1, 3, 3 tetramethoxy propane, as a standard. The MDA present within the sample was calculated by using the extinction coefficient of 1.56×105 M cm^{-1} and expressed as the unit of nM/mg of tissue (Ohkawa *et al*., 1979).

Toxicity study: For the assessment of toxicity plasma Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) were measured by commercially available standard Kit (Merck, Japan) using Semiautoanalyzer (Merck, Japan) by standard protocol for phtotometric determination (Goel, 1988).

Histopathological examination: Kidneys from the experimental rats were fixed in 10% buffered Bouins reagent and were processed for paraffin sectioning. Sections of about 5 mm thickness were stained with haematoxylin and eosin for photomicroscopic observations (Palani *et al*., 2009).

Statistical analysis: Analysis of variance (ANOVA) followed by a multiple two-tail 't' test with Bonferroni modification was used for statistical analysis of the collected data. Differences were considered significant when $p<0.05$.

RESULTS

Acute toxicity study by limit test: According to OECD guidelines 425, the limit test dose of 2000 mg kg^{-1} b.wt., was given. Mortality occurred in two rats within 24 h and three rats within 48 h out of five at the dose of 2000 mg kg^{-1} b.wt., Therefore, the approximate LD_{50} is less than 2000 mg kg^{-1}. The toxicity evaluation was further carried out by observing wellness parameters shown in Table 1.

Main test

On body weight: Effect of body weight and kidney somatic index of acetaminophen induced renal failure of male rats at the dose of 175 mg and 550 mg kg^{-1} b.wt., was observed in Table 2. The body weight of group I and II animals was increased significantly compared to group III rats. Weight of kidney of group III rats were low compared to group I and II.

On urea, creatinine, SOD, catalase, GSH, GOT and GPT levels: Percentage of plasma urea, creatinine, GOT and GPT levels were increased significantly in group III animals compared to groups I and II. The antioxidant enzymes like SOD, catalase, GSH content in the kidney tissues were decreased significantly ($p<0.05$) due to acetaminophen induced nephrotoxicity at the higher dose of 550 mg kg^{-1} (Table 3 and 4). But the low dose of acetaminophen i.e., 175 mg kg^{-1} does not significantly alter any biochemical or histological parameter.

Table 1: Observation by different wellness parameters of rats for the limit test of acetaminophen at the dosage rate of 2000 mg kg^{-1} body weight

	Observation for the test at 2000 mg kg^{-1} b.wt., of rats									
	30 min		4 h		12 h		24 h		48 h	
Observations	C	E	C	E	C	E	C	E	C	E
Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal	2X3L	Normal	3X2L
Eyes	Normal	Normal	Normal	Normal	Normal	Red	Normal	2X3L	Normal	3X2L
Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal	2X3L	Normal	3X2L
Salivation	No	No	No	Little	No	Little	No	2X3L	No	3X2L
Lethargy	No	Normal	No	Yes	No	Yes	No	2X3L	No	3X2L
Sleep	Normal	Normal	Normal	Normal	Normal	No	Normal	2X3L	Normal	3X2L
Convulsions	No	No	No	N	No	Yes	No	2X3L	No	3X2L
Tremors	No	No	No	N	No	Yes	No	2X3L	No	3X2L
Diarrhoea	No	No	No	No	No	Yes	No	2X3L	No	3X2L
Morbidity	No	No	No	Yes	No	Yes	No	2X3L	No	3X2L
Mortality	No	No	No	No	No	No	No	2X3L	No	3X2L

C: Control, E: Experimental, 2X3L: 2 Expired 3 live, 3X2L: 3 Expired 2 Live

Table 2: Changes in body weight and kidney somatic index on acetaminophen induced renal failure of male rats

Groups	Initial body weight (g)	Final body weight (g)	Increases or decreases in body weight (g%)	Kidney somatic index
I	100.2±2.53[a]	120.0±5.32[a]	+19.8	0.76[a]
II	102.5±2.34[a]	105.7±3.56[b]	+3.2	0.68[b]
III	100.1±1.29[a]	124.2±5.70[a]	+24.1	0.79[a]

Data is expressed as Mean±SE (n = 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b) in a specific vertical column differ from each other significantly ($p<0.05$). Group I: control, Group II: Acetaminophen 175 mg kg^{-1}, Group III: 550 mg kg^{-1} b.wt., for 14 days

Table 3: Biochemical changes in urea, creatinine, GOT, GPT, SOD, catalase, GSH and MDA level on acetaminophen induced renal failure in male rats

Groups	Urea (mg dL^{-1} of plasma)	Creatinine (mg dL^{-1} of plasma)	Plasma GOT(U L^{-1})	Plasma GPT(U L^{-1})	SOD (m mol of H_2O_2 consumption mg^{-1} of tissue min^{-1})	Catalase (m mol of H_2O_2 consumption mg^{-1} of tissue min^{-1})	Kidney GSH (mg dL^{-1})	Kidney MDA (n mol/ mg of tissue)
I	14.55±2.33[a]	0.62±0.04[a]	24.2±3.68[a]	21.52±2.31[a]	0.85±0.14[a]	1.23±0.13[a]	12.41±1.67[a]	35.62±2.33[a]
II	15.37±1.88[a]	0.68±0.03b[a]	21.5±2.35[a]	20.72±2.82[a]	0.76±0.03[b]	1.25±0.05[a]	11.32±2.31[a]	42.38±3.51[b]
III	68.57±3.27[b]	2.85±0.08[b]	69.2±3.68[b]	68.61±6.59[b]	0.18±0.11[c]	0.29±0.08[b]	6.33±1.53[b]	64.78±2.44[c]

GOT: Glutamate oxaloacetate transaminase, GPT: Glutamate pyruvate transaminase, SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde, Data is expressed as Mean±SE (n = 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c) in a specific vertical column differ from each other significantly ($p<0.05$). Group I: control, Group II: Acetaminophen 175 mg kg^{-1}, Group III: 550 mg kg^{-1} b.wt., for 14 days

Table 4: Percentage changes in urea, creatinine, GOT, GPT, SOD, catalase, GSH and MDA level between three groups of acetaminophen induced renal failure male rats

Groups	Urea	Creatinine	SGOT	SGPT	SOD	Catalase	Kidney GSH	Kidney MDA
II								
Percentage changes with group I	5.33↑	8.82↑	11.15↓	6.07↓	10.58↓	1.6↑	8.78↓	15.95↑
III								
Percentage changes with group I	78.78↑	77.5↑	78.24↑	76.14↑	65.02↑	68.93↑	68.63↑	69.8↑
Percentage changes with group II	78.82↓	76.31↓	76.42↓	76.8↓	48.9↓	44.08↓	45.01↑	34.57↑

SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde, ↑: Increases, ↓: Decreases

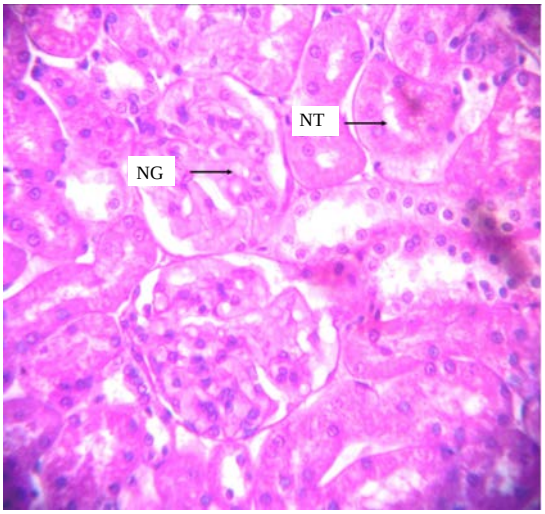

Fig. 1: Normal histology of kidney of control group of rats, NG: Normal glomerulus and NT: Normal renal tubules with intact well organized cellular boundary

Histopathological examinations: The histological architecture of kidney shows severe degenerative changes in the acetaminophen treated at higher dose of 550 mg kg^{-1} b.wt., where the control group shows a well organized glomerulus and renal tubules. But at the lower dose of acetaminophen at 175 mg kg^{-1} b.wt., does not alter the histological structure of kidney (Fig. 1-3).

DISCUSSION

Nephrotoxicity occurs as a disturbance in renal function due to various drug interactions and chemicals (Watkins *et al*., 2006). The primary toxicity of APAP is the result of drug metabolism in both the liver and extra hepatic tissues (Gu *et al*., 2005). At therapeutic doses, APAP is metabolized via glucuronidation and sulfation reactions occurring primarily in the liver which result in the water-soluble metabolites that are excreted via the kidney. When large doses of APAP are ingested, there is more severe GSH depletion as well as massive production of metabolites, which compounds the toxicity, leaving large amounts of reactive metabolite unbound (Bessems and Vermeulen, 2001). This process disrupts homeostasis and initiates apoptosis or programmed cell death, leading to tissue necrosis and ultimately to organ dysfunction.

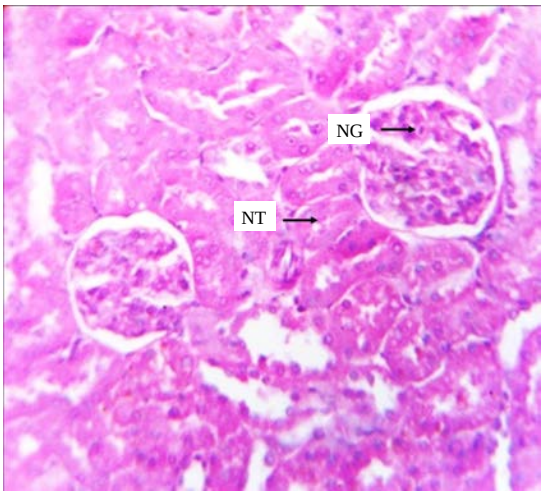

Fig. 2: Normal histology of kidney of acetaminophen treated at 175 mg kg^{-1} b.wt., NG: Normal glomerulus and NT: Normal renal tubules that are not affected due to the lower dose of acetaminophen

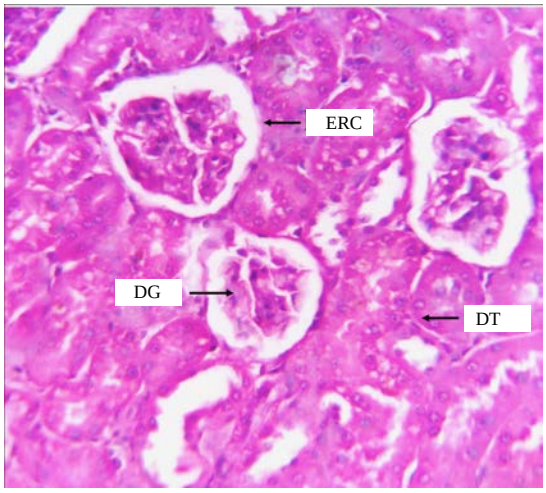

Fig. 3: Severe disorganization of rat kidney after acetaminophen injection of 550 mg kg^{-1} b.wt., ERC: Endothelial rupture in capsule, DG: Damaged glomeruli and DT: Tubules are dilated with loss of cellular boundary

Acetaminophen overdose deplete antioxidant enzymes in kidney tissues and increase lipid peroxidation (Cohen *et al*., 1998). Acetaminophen also promote increase of urea and creatinine (Adelman *et al*., 1981) leading to uremia. Toxicity study was confirmed by SGOT and SGPT. Acetaminophen overdose is often associated with urea and creatinine derangements, elevation in plasma concentration of these parameters are considered reliable for investigating drug-induced nephrotoxicity in animals (Adelman *et al*., 1981; Mandal *et al*., 2015). In kidney diseases, increased plasma urea is due to the higher rate of plasma urea production, which exceeds the rate of urea clearance (Mayne, 1994). Tissue creatinine breakdown increases plasma creatinine level when nephrotoxicity occurs. Several studies have indicated that APAP might induce oxidative injury, including tissue lipid peroxidation, enzyme inactivation and changes in cellular non-enzymatic and enzymatic antioxidant defense systems and glutathione (GSH) status (Tukel, 1995).

Studies on healthy rats of either sex were chosen for studying the acute toxicity of any drugs orally in single increasing doses to the rat. If the higher dose is well tolerated then lower doses at different amount will be chosen for further studies. But if the higher dose was not tolerated then a main test has to be conduct to choose the effective dose (Shenoy *et al*., 2012; Adeneye *et al*., 2008; Abraham, 2005). In the present study, results obtained in the main test showed that the dose of acetaminophen nephrotoxicity were established with the higher dose of 550 mg kg^{-1} day^{-1} but nephrotoxicity does not occur at the lower dose of acetaminophen at 175 mg kg^{-1} b.wt., as evidence by significant ($p<0.05$) elevation in plasma urea and creatinine in group III rats compared to group I and II (Table 2).

Reduction in antioxidant enzymes in cells when there is disruption of cellular redox balance. Acetaminophen induced oxidative stress results in reduction in antioxidant enzymes including SOD, catalase and reduced glutathione (GSH). Significant ($p<0.05$) reduction in renal tissue concentration of SOD, catalase and reduced glutathione were evident in group III animals compared to group I and II (Table 2).

Acetaminophen increases the plasma level of GOT and GPT indicating chemical induced renal cellular toxicity. Plasma levels of these enzymes are very sensitive markers of toxicity. When the endothelial cell layer of renal tubules is damaged, the enzymes are released into the blood stream and increase in level (Mandal *et al*., 2015). In group III animals, plasma GOT and GPT levels were increased significantly ($p<0.05$), compared to other animals.

Lipid peroxidation is the oxidative degradation of lipids, which generates free radicals that cause cell damage. The end product of lipid peroxidation is malondialdehyde, which is known as second messenger of free radicals. High concentration of MDA in kidney tissue indicates renal toxicity (Mandal *et al*., 2015). The MDA concentration in kidney tissue of group III animals was increased significantly compared to groups I and II due to acetaminophen induced oxidative damage. The APAP-induced renal damage is consistent with acute tubular necrosis. In this study, the results of histopathological examination showed clear evidence of nephrotoxicity following the administration of APAP in an overdose. Acute tubular necrosis was the most relevant histopathological change. Kidney histology of control group shows a normal morphology of renal parenchyma with Normal Tubular (NT) brush-borders and intact Normal Glomerulus (NG) and surrounding Bowman's capsule (Fig. 1). Also the lower dose of acetaminophen does not evidently alter the (Fig. 2) histopathology of kidney. Glomerular damages were evident by glomerular bleeding and partial endothelial rupture in capsule. Proximal tubules were dilated with loss of cellular boundary and epithelial degeneration. Intraluminal cell debris and glassy pink cytoplasm were observed as indicators of the cell death (Fig. 3).

CONCLUSION

The biochemical and histopathological results obtained in this work confirmed that acetaminophen in high dose causes renal failure and oxidative stress. However, further investigations are needed to ascertain the mechanism of actions of acetaminophen in the different cell lines of kidney.

ACKNOWLEDGMENTS

The author thanks for the financial help provided by Department of Science and Technology (DST), New Delhi, in the form Of inspire fellowship to first author and also acknowledges UGC-CPE, UGC MAJOR, New Delhi, for providing research fund.

REFERENCES

Abraham, P., 2005. Vitamin C may be beneficial in the prevention of paracetamol-induced renal damage. Clin. Exp. Nephrol., 9: 24-30.

Adelman, R.D., W.L. Spangler, F. Beasom, G. Ishizaki and G.M. Conzelman, 1981. Frusemide enhancement of netilmicin nephrotoxity in dogs. J. Antimicrob. Chemother., 7: 431-440.

Adeneye, A.A., J.A. Olagunju, A.S. Benebo, S.O. Elias and A.O. Adisa *et al*., 2008. Nephroprotective effects of the aqueous root extract of *Harungana madagascariensis* (L.) in acute and repeated dose acetaminophen renal injured rats. Int. J. Appl. Res. Nat. Prod., 1: 6-14.

Beers, R.F.Jr. and I.W. Sizer, 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem., 195: 133-140.

Bessems, J.G.M. and N.P.E. Vermeulen, 2001. Paracetamol (acetaminophen)-induced toxicity: Molecular and biochemical mechanisms, analogues and protective approaches. Crit. Rev. Toxicol., 31: 55-138.

Burtis, C.A. and R.A. Edward, 1999. Tietz Textbook of Clinical Chemistry. 3rd Edn., W.B. Saunders, Philadelphia, PA., ISBN: 9780721656106, Pages: 1917.

Cohen, S.D., D.J. Hoivik and E.A. Khairallah, 1998. Acetaminophen-Induced Hepatotoxicity. In: Toxicology of the Liver, Plaa, G.L. and W.R. Hewitt (Eds.). Taylor and Francis, Philadelphia, PA., pp: 159-186.

Das, K., P.P. Chakraborty, D. Ghosh and D.K. Nandi, 2010a. Protective effect of aqueous extract of *Terminalia arjuna* against dehydrating induced oxidative stress and uremia in male rat. Iran. J. Pharm. Res., 9: 153-161.

Das, K., T.T. Samanta, P. Samanta and D.K. Nandi, 2010b. Effect of extract of Withania Somnifera on dehydration-induced oxidative stress-related uremia in male rats. Saudi J. Kidney Dis. Trans., 21: 75-80.

Goel, B.K., 1988. Routine Biochemical Test. In: Medical Laboratory Technology, Mukhejee, K.L. (Ed.). Vol. 3, McGraw-Hill, New Delhi, India, pp: 985-1097.

Gu, J., H. Cui, M. Behr, L. Zhang and Q.Y. Zhang *et al*., 2005. *In vivo* mechanisms of tissue-selective drug toxicity: Effects of liver-specific knockout of the NADPH-cytochrome P450 reductase gene on acetaminophen toxicity in kidney, lung and nasal mucosa. Mol. Pharmacol., 67: 623-630.

Henrich, W.L., L.E. Agodoa, B. Barrett, W.M. Bennett and R.C. Blantz *et al*., 1996. Analgesics and the kidney: Summary and recommendations to the Scientific Advisory Board of the National Kidney Foundation from an ad hoc Committe of the National Kidney Foundation. Am. J. Kidney Dis., 27: 162-165.

Jones, A.F. and J.A. Vale, 1999. Paracetamol poisoning and the kidney. J. Clin. Pharm. Ther., 18: 5-8.

Mandal, A., K. Das, S. Roy, K.C. Mondal and D.K. Nandi, 2013. *In vivo* assessment of bacteriotherapy on acetaminophen-induced uremic rats. J. Nephrol., 26: 228-236.

Mandal, A., A. Patra, S. Mandal, S. Roy and S.D. Mahapatra *et al*., 2015. Therapeutic potential of different commercially available synbiotic on acetaminophen-induced uremic rats. Clin. Exp. Nephrol., 19: 168-177.

Marklund, S. and G. Marklund, 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.

Mayne, P.D., 1994. The Kidneys and Renal Calculi. In: Clinical Chemistry in Diagnosis and Treatment, Mayne, P.D. (Ed.). 6th Edn., Edward Arnold Publications, London, UK., pp: 2-24.

Moron, M.S., J.W. Depierre and B. Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione *S*-transferase activities in rat lung and liver. Biochimica Biophysica Acta (BBA)-Gen. Subj., 582: 67-78.

OECD., 2001. Organization of Economic Co-Operation and Development Guidelines. OECD, Paris, France, Pages: 425.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

Olfert, E.D., B.M. Cross and A.A. McWilliams, 1993. Guide to the Care and Use of Experimental Animals. 2nd Edn., Canadian Council on Animal Care, Ottawa, Canada, pp: 82-93.

Palani, S., S. Raja, R.P. Kumar, S. Jayakumar and B.S. Kumar, 2009. Therapeutic efficacy of *Pimpinella tirupatiensis* (Apiaceae) on acetaminophen induced nephrotoxicity and oxidative stress in male albino rats. Int. J. PharmTech Res., 1: 925-934.

Pradhan, S., S. Mandal, S. Roy, A. Mandal and K. Das and D.K. Nandi, 2013. Attenuation of uremia by orally feeding alpha-lipoic acid on acetaminophen induced uremic rats. Saudi Pharm. J., 21: 187-192.

Roy, S., K. Das, S. Mandal, S. Pradhan, A. Patra and D.K. Nandi, 2013. Crude root extract of *Asparagus racemosus* ameliorates acetaminophen induced uremic rats. Int. J. Pharm. Sci. Res., 4: 3004-3012.

Sabbagh, M., W. Rick and S. Schneide, 1988. A kinetic method for the direct determination of creatinine in serum with 3,5-dinitrobenzoic acid without deproteinization. J. Clin. Chem. Clin. Biochem., 26: 15-24.

Shenoy, S., H. Kumar, N.V. Thashma, K. Prabhu and P. Pai, 2012. Hepatoprotective activity of *Plectranthus amboinicus* against paracetamol induced hepatotoxicity in rats. Int J. Pharmacol. Clin. Sci., 1: 32-38.

Tukel, S.S., 1995. Effects of acetaminophen on methemoglobin, superoxide dismutase and Na^+-K^+ ATPase activities of human erythrocytes. Biochem. Mol. Biol. Int., 35: 719-724.

Watkins, P.B., N. Kaplowitz, J.T. Slattery, C.R. Colonese, S.V. Colucci, P.W. Stewart and S.C. Harris, 2006. Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: A randomized controlled trial. J. Am. Med. Assoc., 296: 87-93.