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Short Term and Long Term Histopathological and Biochemical Effects of Acetaminophen Toxicity on Mice Kidney

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ABSTRACT

Background: Acetaminophen is certainly a drug of choice globally and in all age groups for fever, and aches. With a narrow therapeutic window any individual taking the drug is a vulnerable to its toxic effects. This study was designed to determine the short term and long term renal effects of one single toxic dose.

Methods:27 male albino mice were procured and housed in appropriate environment in the animal house. Animals were randomly divided into three equal groups. Group A mice served as a control and was dissected on day ten. Group B and C mice were injected acetaminophen (600mg/kg) intraperitoneally and were sacrificed after 48 hours and on tenth day respectively. Both the kidneys were collected afterwards for microscopic examination and blood samples were drawn for biochemical analysis.

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Results: Renal tubular features (brush border loss, necrosis, luminal cast and vacuolations) were significantly affected among the experimental groups B & C as compared to the control group (p-value ≤ 0.05). Renal glomerular features (Basement membrane & necrosis) were unaffected in all the groups. Among the renal interstitial features, mesangeal hyper cellularity and vascular congestion were significant among the experimental groups (p-value ≤ 0.05) while interstitial inflammation was non-significantly higher in the experimental groups (p-value = 0.071). These pathological features were milder in group C animals.

Conclusions: Acetaminophen overdose causes acute tubular necrosis and abrupt impairment in serum urea and creatinine levels. The intensity of both of these parameters reduces with time suggestive of spontaneous self-recovery.

Keywords: Acetaminophen toxicity, Glomerular necrosis, Renal tubular necrosis, Serum Creatinine, Serum urea.

INTRODUCTION

Acetaminophen (N-Acetyl para-aminophenol - APAP) marketed as paracetamol, is the most preferred drug for mild to moderate body aches and fever.[1] It has analgesic and antipyretic effects but it lacks anti-inflammatory activity which distinguish it from all other NSAIDs. This difference has been explained by its discrete mechanism of action as it doesn't inhibit peripheral functioning of cyclooxygenases (like NSAIDs) instead it halts the production of cyclooxygenases in the central nervous system which leads to antipyretic effect.It moderates endogenous cannabinoid system in the brain which explains its analgesic and mood stabilizing effects.[2,3]

APAP is primarily metabolized in liver predominantly through glucuronidation and sulfation pathways while the end products are excreted through urine. Only about ten percent of drug is metabolized by cytochrome P450 system especially CYP2E1 leading to production of highly toxic chemical, N-acetyl P-benzoquinone imine (NAPQI), which is detoxified through glutathione conjugation. NAPQI production surpasses either by over-activation of cytochrome P450 system by certain drugs and alcohol consumption or if glutathione is depleted due to overconsumption (by NAPQI) or poor synthesis (e.g. malnutrition).[4,5]

Being most common over the counter globally used orally administered drug and having narrow therapeutic window render this drug potentially harmful. More than 1500 persons died due to APAP toxicity during first decade of 21st century in United States and in 2009 Food and Drug Administration (FDA) revised the drug advisory reducing its maximum recommended dose of 1000mg four times a day to 625mg four times a day, banned its combination with opioids and to strictly "use only as directed."[6] The triggering factors for APAP toxicity include compromised

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hepatic & renal status, diabetes mellitus, alcohol consumption, dieting and chronic use of nephrotoxic drugs like NSAIDs.[7,8]

The incidence of dose dependent APAP induced renal failure is much lower than the fulminant hepatic failure (FHF) and it can be acute with single overdose as well as chronic with long term use.[9,10] It has been investigated more comprehensively and the disease course has been divided into four distinct stages. Moreover the hepatic impairment can be overturned by its antidote N-acetyl cysteine (NAC).[5]

Less than 2% cases of APAP toxicity suffer from renal impairment which is rarely an absolute effect and is reported during second stage of FHF.[11,12]Histological findings reported so far suggest that APAP toxicity leads of acute tubular necrosis (ATN) and the glomerular damage is variable.(ref) About the pathogenesis, one group of researchers claim that cytochrome P450 system activation produces excess of NAPQI which binds to renal macromolecules, generates reactive oxygen & nitrogen species and suppress mitochondrial dehydrogenase resulting in cell necrosis.[13,14] This is similar to the hepatic injury but other claim cytochrome P450 system is not dominant in the renal tissue and APAP modulates prostaglandin synthetase leading to production of NAPQI[11].

As investigating the hepatic effects of APAP intoxication were researcher's priority,the pathogenesis and the pathological features of APAP induced renal damage are still under consideration. Present study is designed accordingly to investigate the short term and long term histological and biochemical effects of single dose APAP on mice renal status.

MATERIALS AND METHODS

This randomized controlled trial was conducted at Anatomy Department, University of Health Sciences, Lahore. APAP was obtained from Merck Pharmaceuticals in the form of white crystalline powder. Nephrotoxic dose of acetaminophen for mice was determined by previously published data and it was 600mg/kg body weight intraperitoneally single dose.[15] 600mg of APAP was dissolved in 16.6ml of 0.9% normal saline thus achieving drug concentration of 36mg/ml.

Twenty-seven male albino mice, 6-8 weeks old, weighing 25 to 35gm were procured from Foot and Mouth Disease Research Centre, Lahore. By using random number table the animals were divided into three groups, each consisting of nine mice. They were kept under controlled environment having optimal temperature, humidity and alternating light and dark cycles of twelve hours apart. Moreover they were acclimatized for a week before conducting the experiment.

The control group A animals were given 16.6 ml/kg of 0.9% normal saline solution intraperitoneally on first Day of experiment and were sacrificed on tenth day. Group B & Group C animals were injected APAP solution intraperitoneally, after dose calculation. Group B animals were sacrificed after 48 hours while the Group C animals were sacrificed on tenth day. All the animals were sacrificed under chloroform anesthesia and then dissected. Both the kidneys were removed, fixed in 10% formalin, dehydrated in rising grades of alcohol, washed in xylene

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and embedded in molten paraffin wax. Paraffin blocks were solidified, refrigerated and fixed in the chuck of rotator microtome. 5µm thick sections were obtained and stained with hematoxylin and eosin (H&E) and with periodic acid Schiff (PAS) according to standard guidelines and examined under light microscope.

The histopathological changes related to renal glomeruli, renal tubules, interstitium and blood vessels were graded as mild (+), moderate (++) and severe (+++) for a damage involving 10-25%, 25-50% &>50% of a visual field respectively.[16] For collecting blood sample, cardiac puncture was performed just before sacrificing the animals. Through centrifugation clear serum was extracted and preserved in sterilized labelled eppendorf tubes at -20^{o}C. Serum urea and serum creatinine were estimated by autoanalyzer (Humalyzer 3000) through reagent kits.

Data had been analyzed by SPSS version 22.0. Mean ± SD (Standard deviation) is given for quantitative variables. Frequencies and percentages are given for qualitative variables. Group mean differences for quantitative variables were estimated through One way ANOVA followed by Post-Hoc Tukey test to identify which group mean differs. Chi-Square or Fisher's exact test was applied to compare the qualitative parameters among the three groups. p-value of less than 0.05 will determine a statistically significant difference.

RESULTS

Qualitative parameters are the histological findings related to the renal glomeruli, renal tubules and interstitium and are listed in terms of frequency and percentage (Table 1 & Table 2). All the features listed in the table depicted significant renal damage in the toxic group B & C as compared to control group A except the interstitial inflammation which was non-significant. The percentage of tubular cast and interstitial inflammation significantly reduced in group C as compared to group B.

Table 1: Comparison of Frequency and Percentages of histological findings among the groups

Extent	Brush Border Loss			Tubular Necrosis			Tubular Casts			Tubular Vacuolations		
	Gr A n = 9	Gr B n = 9	Gr C n = 9	Gr A n = 9	Gr B n = 9	Gr C n = 9	Gr A n = 9	Gr B n = 9	Gr C n = 9	Gr A n = 9	Gr B n = 9	Gr C n = 9
Absent (-)	9 100 %	Nil	Nil	9 100 %	Nil	1 11.1 %	9 100 %	Nil	4 44.4 %	9 100 %	Nil	Nil
Mild (+)	Nil	3 33.3 %	4 44.4 %	Nil	2 22.2 %	4 44.4 %	Nil	Nil	5 55.6 %	Nil	6 66.7 %	1 11.1 %

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Moderate (++)	Nil	6 66.7 %	5 55.6 %	Nil	7 77.8 %	4 44.4 %	Nil	7 77.8 %	Nil	Nil	3 33.3 %	6 66.7 %
Severe (+++)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	2 22.2 %	Nil	Nil	Nil	2 22.2 %
	Fisher exact test = 25.67 p ≤0.001			Fisher exact test = 23.53 p = 0.001			Fisher exact test = 30.98 p = 0.049			Fisher exact test = 30.93 p ≤0.001		

p value ≤ 0.05 is considered statistically significant.

Table 2: Comparison of Frequency and Percentages of histological findings among the groups

Extent	**Mesangeal Hyper cellularity**			**Interstitial Inflammation**			**Vascular Congestion**		
	Gr A n = 9	**Gr B n = 9**	**Gr C n = 9**	**Gr A n = 9**	**Gr B n = 9**	**Gr C n = 9**	**Gr A n = 9**	**Gr B n = 9**	**Gr C n = 9**
Absent (-)	9 100%	Nil	3 33.3 %	9 100%	1 11.1 %	7 77.8 %	8 88.9 %	Nil	Nil
Mild (+)	Nil	4 44.4 %	3 33.3 %	Nil	1 11.1 %	1 11.1 %	1 11.1 %	2 22.2 %	3 33.3%
Moderate (++)	Nil	5 55.6 %	3 33.3 %	Nil	5 55.6 %	1 11.1 %	Nil	5 55.6 %	5 55.6%
Severe (+++)	Nil	Nil	Nil	Nil	2 22.2 %	Nil	Nil	2 22.2 %	1 11.1%
	Fisher exact test = 19.16 p = 0.05			Fisher exact test = 16.40 p = 0.071			Fisher exact test = 21.76 p ≤0.001		

p value ≤ 0.05 is considered statistically significant.

Glomerular deposits and glomerular sclerosis indicative of glomerular damage were not observed in any histological section of experimental group B & C. Glomerular capsule and space b/w parietal and visceral layers were intact.

Mean and standard deviation was calculated for estimation of biochemical parameters including serum urea and serum creatinine. For group A, B & C, mean serum urea levels were 44.49±7.52, 83.15±6.25 & 72.14±5.51 mg/dl respectively and mean serum creatinine levels were

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0.46±0.11, 1.98±0.26 & 1.83±0.23 mg/dl respectively. According to Post hoc tukey test, serum urea and serum creatinine of group B & C was significantly higher than that of control group A (P-value ≤0.001). The mean serum urea level in group C mice was less than that of group B and the difference was statistically significant (P-value=0.003). Mean serum creatinine level in group C mice was also less than that of Group B but it was not significant.

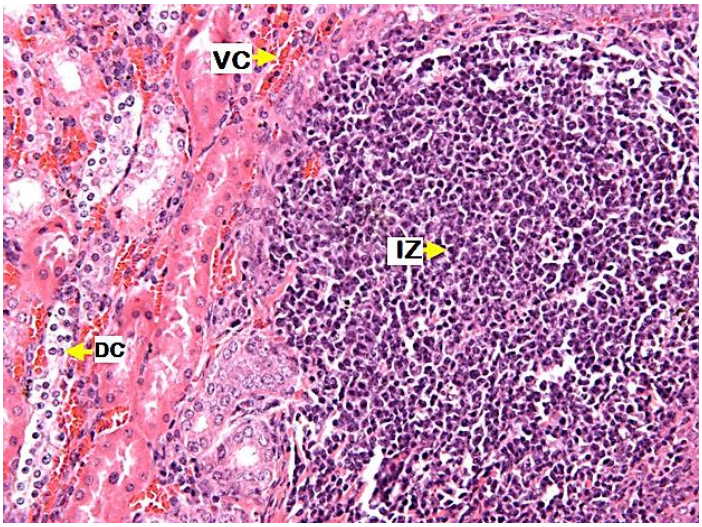

Figure 1: Histological section of kidney of group B, showing inflammatory zone (IZ) and degenerating cells' (DC) inside tubule with marked Vascular Congestion (VC) into the interstitium. H&E stain X200.

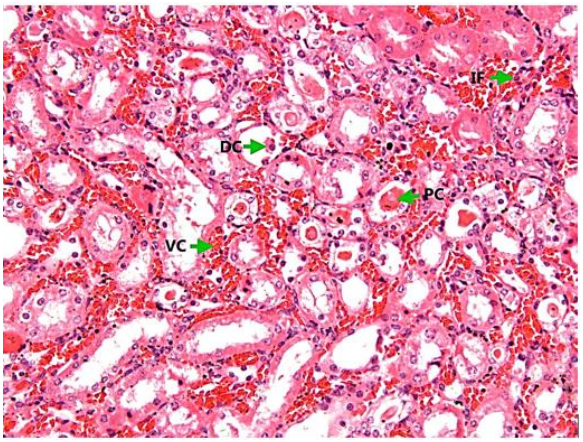

Figure 2: Histological section of kidney of group C, showing inflammatory cells (IF) and marked Vascular Congestion (VC) into the interstitium. Protein casts (PC) & Desquamating cells (DC) are seen in the lumen of degenerating tubules. H&E stain X200.

DISCUSSION

Our results showed that toxic dose of APAP in group B animals resulted in marked tubular damage in both the proximal and distal convoluted tubules including brush border loss,

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tubular necrosis, tubular vacuolations and tubular cast in the lumen. Mesangeal hyper cellularity, interstitial inflammation and vascular congestion was also significant among group B as demonstrated in figure 1 and indicates active inflammation. Microscopic structure of renal glomeruli was intact with no pathological impact in all the animals dissected. Our histological observations are supported by the compromised renal function in group B animals as serum urea and serum creatinine levels were markedly raised as compared to the control group A.

Our results are in accordance with the data published by Hussain et al which states that APAP treatment in mice resulted in cytoplasmic vacuolations and necrosis of renal tubules along with significantly raised serum urea and serum creatinine levels.[17] Similar findings were also reported by Hussam et al only the serum creatinine levels were not raised in their study because animals were sacrificed six hours after APAP dose and this much time may not be sufficient for alteration in serum creatinine levels.[18]However, Sohail et al demonstrated significant elevation of serum urea, serum creatinine and blood urea nitrogen with single toxic dose of APAP and concluded that APAP overdose is potentially nephrotoxic.[19]

Adil et al reported against our observations that APAP causes recognizable glomerular disruption in rat kidney.[20]Dallak et al also demonstrated APAP induced disruption of glomerular architecture in rats under tissue electron microscope.[21] This difference of observation is probably because of different research animal not suitable for exploring drug induced renal damage.[18] Our findings strongly suggest that APAP-intoxication induced renal impairment is primarily tubular and leads to ATN. Abdeen et al also investigated the effect of APAP overdose on renal morphology and concluded that proximal convoluted tubule (PCT) is the actual site for pathogenesis while renal glomeruli are unaffected.[22]

Bektur et al injected 500mg single dose of APAP in mice and observed similar histopathological features and biochemical disruption of renal function tests. They also reported locally raised nitrogen oxide level which is responsible for cascade of cellular events leading to necrosis and cell death.[23]Verbova et al conducted in vitro experiment on cells of PCT for determining APAP toxic effects and the cellular mechanisms involved. They concluded that tubular cell is the site where reactive oxygen species are generated due to APAP in a dose dependent manner and lead to necrotic changes. Glutathione levels are merely reduced which means renal tissue necrosis is not related to cytochrome P450 system overload responsible for APAP induced FHF.[13]

In group C animals dissected 10 days after single toxic dose of APAP, histopathological findings were significant as compared to that of control group A but when compared with that of group B, the intensity of these parameters was reduced. The reduction in the degree of tubular cast and interstitial inflammation was statistically significant indicating spontaneous recovery from toxicity (figure 2). The statistical analysis of biochemical parameters also inferred improvement of renal status in group C animals on tenth day of experiment.

Auto-recovery of renal tissue was also observed by Karaali et al in mice after two weeks of APAP overdose and was evident by decreased hydropic degeneration of tubules and decreased

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hyper-cellularity of glomeruli.[24] Impairment of renal function in human coincides with stage II of APAP acute toxicity and among the survivors renal function restores without any specific treatment as N-acetyl cysteine administration only heels hepatic damage.[7, 25]

CONCLUSION

Statistically significant observations concludes that APAP overdose adversely affects the renal architecture especially the tubules along with concomitant elevations in serum urea and serum creatinine levels. Kidneys examined from the animals sacrificed on tenth day had milder pathological features and reduction in biochemical parameters suggesting restoration of renal tissue to a certain extent.

FUTURE RECOMMENDATIONS

There is need of further investigation at cellular level how APAP affects the tubular cells and how the disease process can be countered. Moreover study duration can be prolonged to observe the long term effects and differentiate whether the acute effects are temporary or permanent.

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