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Evaluation of Nephroprotective Activity of *Psidium Guajava* Linn, Leaves Extract in Paracetamol Induced Nephrotoxicity in Rats

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ABSTRACT

Paracetamol is generally accepted as a safe drug for analgesic, an antipyretic when administered within the therapeutic range but the overdose can cause nephrotoxicity with oxidative stress as one of the possible mechanisms mediating the event. In this study Nephroprotective activity of hydroalcoholic extract (70% ethanol extract) of leaves of *Pisidium guajava* Linn. (HAPG) was assessed in paracetamol induced renal damage in rats. The protective property of 70% ethanol extract was assessed by measuring the levels of Physical parameters: body weight, urine volume, kidney weight; Urinary parameters: urinary sodium, potassium, glucose and creatinine; Blood parameters: blood urea, blood creatinine and blood total protein; tissue glutathione and lipid peroxidation in administered doses. The extract exhibited nephroprotective activity in dose dependant manner. Also, it significantly reduced the renal damage caused by paracetamol, as observed in histopathological studies. In conclusion, these data suggest that the ethanol extract of leaves of *Pisidium guajava* Linn. can prevent renal damage from paracetamol-induced nephrotoxicity in rats.

Key words: Paracetamol; Nephrotoxicity; *Psidium guajava* Linn.

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INTRODUCTION

Our body is always exposed to toxic organic compounds from both intentional and unintentional sources. Many hormones, neurotransmitters, and waste products of cellular metabolism as well as a wide variety of drugs are classified as organic anions and cations. To limit both systemic exposure and the duration of their pharmacological or toxicological effects, rapid and efficient elimination of these substances is fundamental defence system to our body. For organic anions and cations, active transport across the real proximal tubule followed by elimination via the urine is a major pathway in this detoxification process [1]. The incidence of drug-induced nephrotoxicity has been increasing with the ever increasing number of drugs and with easy availability of over-the-counter medication viz. nonasteroidal anti-inflammatory drugs (NSAIDs). Antibiotics, NSAIDs, angiotensin converting enzyme inhibitors (ACEI) and contrast agents are the major culprit drugs contributory to kidney damage. Drug-induced acute renal failure (ARF) accounted for 20% of all ARF in an Indian study [2]. Paracetamol/Acetaminophen was first discovered in 1889 and is widely used as over-the-counter analgesic and anti-pyretic agent. Paracetamol toxicity is one of the major causes of poisoning worldwide, and its overdose is commonly associated with hepatic and real damages. Paracetamol toxicity is mediated by the activity of its reactive metabolite known as *N*- acetyl-*p*-benzoquinoneimine(NAPQI), which is detoxified by intracellular glutathione (GSH). Therefore an overdose of paracetamol will saturate the conjugation pathways of GSH and cause depletion of cellular GSH. This subsequently led to a reduced capacity of GSH to detoxify NAPQI. Increased level of NAPQI mediates oxidative damage and thus enhances cellular injuries and organ dysfunction, including renal damage [3].

P. guajava Linn. (Myrtaceae) has a rich ethnobotanical history. In many parts of Africa, the leaf, stembark and roots are used traditionally for the management, control, and/or treatment of an array of human disorders [4]. In India it has been used ethnomedically for the diseases such as anorexia, cerebral ailments, child birth, cholera, convulsions, epilepsy, nephritis and jaundice [5].

MATERIALS AND METHODS

Plant material:

The leaves of *Pisidium guajava* Linn. used for the present study was collected from Bengaluru, Karnataka. The leaves were identified, confirmed and authenticated by Dr. K. Kempegowda, Professor and Head, Department of Horticulture, University of Agricultural Science, GKVK, Bengaluru.

Extraction:

The leaves were cleaned and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder. The powder was extracted directly with 70% v/v ethanol, which was used for biological investigations, after subjecting it to preliminary qualitative phytochemical studies [6]. The extract was concentrated under reduced pressure and stored in vacuum desiccators.

Determination of Acute Toxicity (LD_{50}):

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water *ad libitum.* Animals received a single dose of 2000 mg/kg, p.o. was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs [7].

Experimental animals:

Albino wistar rats weighing 150-250g was procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy. Animals were maintained under controlled condition of temperature at $27^{o} \pm 2^{o}$ C and 12-h light-dark cycles. They were housed in polypropylene cages and had a free access to standard pellets (Amruth) and water *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of Pharmacy, Bangalore (REF-IAEC/01/05/2011) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 491/01/c/CPCSEA), Govt. of India.

Effect of HAPG on in Paracetamol Induced Nephrotoxicity [3]:

The albino rats were divided in to 4 groups and each group contains 6 rats and treatment would be as follows.

Group I: Animals of this group served as untreated control and is fed p.o. with normal saline 5 ml/kg body weight daily for 8 days.

Group II: The animals of this group were treated as similarly as first group; Daily acetaminophen suspension was given by p.o., in a dose of 750 mg/kg for 7 days.

Group III: The animals of this group were given with 200 mg/kg, p.o. HAPG and acetaminophen suspension by p.o, at a dose of 750 mg/kg after one hour the HAPG was given to the animals for 7 days.

Group IV: The animals of this group were given with 400 mg/kg, p.o. HAPG and acetaminophen suspension by p.o, at a dose of 750 mg/kg after one hour the HAPG was given to the animals for 7 days.

After 24 hours all group of animals were kept in metabolic cages for urine collection, the urine was collected for next 24 hours and on 8^{th} day, sacrificed with mild ether anesthesia and the kidney tissues, urine and blood samples were collected and assessed.

Physical Parameters:

Body Weight [8]:

The weight of the animals before starting and at the end of the treatment was measured and percentage change in body weight was calculated in paracetamol induced nephrotoxicity.

Kidney Weight [9]:

The weight of the kidneys of the animals at the end of the treatment was measured in paracetamol induced nephrotoxicity.

Urine Volume [9]:

The urine volume of the animals was measured in paracetamol induced nephrotoxicity.

Estimation of biochemical parameters:

The following parameters are estimated by using standard procedures of Excel, Beacon and Transasia diagnostics estimating kits: Urinary parameters: sodium, potassium, creatinine, glucose and Blood parameters: urea, creatinine, total protein.

Estimation of antioxidant activity:

Glutathione estimation [8]

Tissue samples were homogenized in ice cold Trichloroacetic acid (1 gm tissue plus 10 ml 10% TCA) in a tissue homogenizer. Glutathione measurements were performed using a modification of the Ellamn procedure (Aykae, et.al.) Briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. % increase in OD is directly proportional to the increase in the levels of Glutathione. Hence, % increase in OD is calculated.

Lipid peroxidation [8]

Stock solution of TCA-TBA-HCl reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid. Combine 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-0.2 μmol of lipid phosphate) with 2.0 ml of TCA-TBA-HCl and mix thoroughly. The solution is heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the sample is determined at 535 nm against a blank that contains all the

reagents minus the lipid. % decrease in OD is directly proportional to the decrease in the levels of lipid peroxidation. Hence, % decrease in OD is calculated.

Statistical analysis:

The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS

Effect of HAPG on Change in Body Weight, Urine Volume and Kidney Weight:

There was found to be decrease of body weight and urine volume in paracetamol treated group (II). However, there was dose dependent increase of body weights and urine volume significantly ($p<0.001$) in animals treated with HAPG 200 mg/kg and 400 mg/kg, p.o ($p<0.001$) (III and IV). (Table no. 1)

There was found to be increase of kidney weight in paracetamol treated group (II). However, there was dose dependent decrease of kidney weight significantly in animals treated with HAPG 200 mg/kg, p.o ($p<0.001$) and 400 mg/kg, p.o ($p<0.001$) (III and IV). (Table no. 1)

Effect of HAPG on Urinary Sodium, Potassium, Glucose and Creatinine:

There was a decrease of sodium levels in paracetamol treated group (II) when compared to control (I). However 200 mg/kg, p.o (III) HAPG and 400 mg/kg, p.o (IV) HAPG slightly decreased the levels of sodium but not significantly in urine samples were observed when compared group (II). (Table no. 2)

Potassium levels in paracetamol treated group (II) were increased when compared to control group (I). However 200 mg/kg, p.o (III) HAPG decreased the levels of potassium significantly ($p<0.05$) and in the case of 400 mg/kg, p.o HAPG (IV) decreased the levels of potassium significantly ($p<0.001$) in urine samples were observed when compared with group (II). (Table no. 2)

Glucose levels in paracetamol treated group (II) were increased when compared to control group (I). However 200 mg/kg, p.o HAPG (III) reduced the glucose levels significantly ($p<0.001$) and 400 mg/kg, p.o HAPG (IV) reduced the glucose levels significantly ($p<0.001$) in the urine samples when compared with group (II). (Table no. 2)

Urinary creatinine levels in paracetamol treated group (II) were decreased when compared to control group (I). However 200 mg/kg, p.o HAPG (III) increased the levels of creatinine significantly ($p<0.05$) and in the case of 400 mg/kg, p.o HAPG (IV) increased the levels of creatinine significantly ($p<0.001$) in urine samples were observed when compared with group (II). (Table no. 2)

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Effect of HAPG on Blood Urea, Blood Creatinine and Blood Total Protein:

Blood urea level increased in paracetamol treated group (II) when compared with control group (I). However 200 mg/kg and 400 mg/kg, p.o HAPG (III and IV) decreased urea levels significantly ($p<0.001$) in the blood samples when compared with group (II). (Table no. 3). Blood creatinine level increased in paracetamol treated group (II) when compared with control group (I). However 200 mg/kg p.o. HAPG (III) slightly decreased blood creatinine levels but not significantly and 400 mg/kg, p.o HAPG (IV) decreased creatinine levels significantly ($p<0.05$) in the blood samples when compared with group (II). (Table no. 3). Blood total protein level decreased in paracetamol treated group (II) when compared with control group (I). However 200 mg/kg, p.o HAPG (III) increased total protein levels significantly ($p<0.01$) and 400 mg/kg, p.o HAPG (IV) increased total protein levels significantly ($p<0.001$) in the blood samples when compared with group (II). (Table no. 3)

Table No. 1: Effect of HAPG on Change in Body Weight, Urine Volume and Kidney Weight in Paracetamol Induced Nephrotoxic Rats.

Group	Treatment	Change in Body Weight (g)	Urine Volume (ml)	Kidney Weight (g)
I	Vehicle	8.820 ± 0.7562	5.533 ± 0.2108	0.6150 ± 0.0240
II	Paracetamol 750 mg/kg p.o	-8.810 ± 0.2802	3.233 ± 0.3159	0.9467 ± 0.0247
III	Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HAPG	-4.765 ± 0.2662***	5.300 ± 0.3215***	0.7100 ± 0.0236***
IV	Paracetamol 750 mg/kg p.o + 400 mg/kg p.o HAPG	-3.043 ± 0.2953***	6.317 ± 0.4175***	0.6217 ± 0.0177***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01, *P<0.05 and ns represents Not significant. All values are compared with Toxicant control. HAPG- Hydro alcoholic extract of *Psidium guajava* Linn

Effect of HAPG on Tissue Lipid Peroxidation (LP) and Glutathione (GSH):

There was dose dependent inhibition of *in-vivo* LP by both the doses of HAPG. 200 mg/kg p.o HAPG showed 24.575% inhibition, whereas 400 mg/kg, p.o HAPG showed 64.964% inhibition. There was a marked depletion of GSH level in paracetamol treated groups. HAPG showed a dose dependent increase in the level of GSH. However at 200 mg/kg, p.o HAPG showed 52.831% increase in GSH level and 400 mg/kg p.o HAPG showed 27.123% increase in GSH levels.

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Table No. 2: Effect of HAPG on Urinary Sodium, Potassium, Glucose and Creatinine Levels in Paracetamol Induced Nephrotoxic Rats.

Group	Treatment	Urinary sodium levels (mmol/l)	Urinary potassium levels (mmol/l)	Urinary glucose levels (mg/dl)	Urinary creatinine levels (g/L)
I	Vehicle	197.1 ± 3.385	4.369 ± 0.2520	1.353 ± 0.1390	3.283 ± 0.2554
II	Paracetamol 750 mg/kg p.o	130.4 ± 2.432	7.278 ± 0.4885	33.72 ± 2.039	0.9625 ± 0.1772
III	Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HAPG	128.7 ± 1.462^{ns}	5.524 ± 0.3843*	13.50 ± 1.091***	1.893 ± 0.3104*
IV	Paracetamol 750 mg/kg p.o + 400 mg/kg p.o HAPG	125.3 ± 1.263^{ns}	3.880 ± 0.3804***	6.474 ± 0.9443***	3.158 ± 0.2723***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01, *P<0.05 and ns represents Not significant. All values are compared with Toxicant control. HAPG- Hydro alcoholic extract of *Psidium guajava* Linn.

Table No. 3: Effect of HAPG on Blood Urea and Blood Creatinine Levels in Paracetamol Induced Nephrotoxic Rats.

Group	Treatment	Blood Urea (mg/dl)	Blood Creatinine (mg/dl)	Blood Total protein (g/dl)
I	Vehicle	50.29 ± 2.022	1.587 ± 0.2424	6.442 ± 0.0748
II	Paracetamol 750 mg/kg p.o	80.73 ± 2.192	2.601 ± 0.3986	2.931 ± 0.3581
III	Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HAPG	42.29 ± 1.277***	1.919 ± 0.2499^{ns}	4.696 ± 0.3908**
IV	Paracetamol 750 mg/kg p.o + 200 mg/kg p.o HAPG	31.14 ± 1.987***	1.380 ± 0.2538*	5.696 ± 0.2553***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01 and *P<0.05. All values are compared with Toxicant control. HAPG- Hydro alcoholic extract of *Psidium guajava* Linn

Table No. 4: Effect of HAPG on Tissue LP and GSH Levels in Paracetamol Induced Nephrotoxicity Model.

Group	Treatment	Absorbance (LP)	%Inhibition (LP)	Absorbance (GSH)	% Increase (GSH)
I	Vehicle	0.2647 ± 0.0081	-	1.625 ± 0.1157	-
II	Paracetamol 750 mg/kg, p.o	0.8220 ± 0.0219	-	0.8487±0.0461	-
III	Paracetamol 750 mg/kg, p.o + 200 mg/kg, p.o HAPG	0.6207± 0.0106***	24.575	1.248± 0.0476**	52.831
IV	Paracetamol 750 mg/kg, p.o + 400 mg/kg, p.o HAPG	0.2880± 0.0032***	64.964	1.466± 0.004***	27.123

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001, **P<0.01, *P<0.05 and ns represents Not significant. All values are compared with Toxicant control. HAPG- Hydro alcoholic extract of *Psidium guajava* Linn

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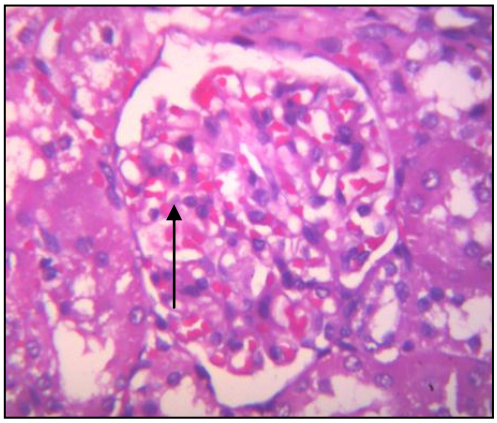
Histopathological Study of Kidneys in Paracetamol Induced Nephrotoxicity:

Group I: Negative control showed intact architecture of renal parenchyma. In glomerulus [Fig. A, Arrow] bowman's space and mesangial cells appeared intact. Intact renal tubules [Fig. B, Arrow] blood vessels and Interstitium were unremarkable.

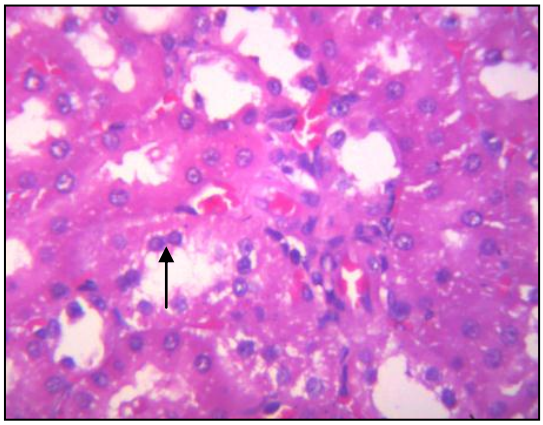
Group II: Positive control i.e. paracetamol treated showed focally distorted renal parenchyma architecture [mainly tubules]. In glomerulus [Fig. A, Arrow]: intact bowman's space, extravasation of erythrocytes seen mesangial cells appear increased. Most of the renal tubules showed degenerative changes [Fig. B, Arrow]. Blood vessels and Interstitium were unremarkable.

Group III: Treatment done with HAPG 200 mg/kg, p.o showed intact architecture of renal parenchyma. In glomerulus [Fig.A, Arrow]: Intact Bowman's space, Extravasation of erythrocytes was seen; Mesangial cells appear increased. Few renal tubules show degenerative changes [Fig.B, Arrow]. Blood vessels and Interstitium were unremarkable.

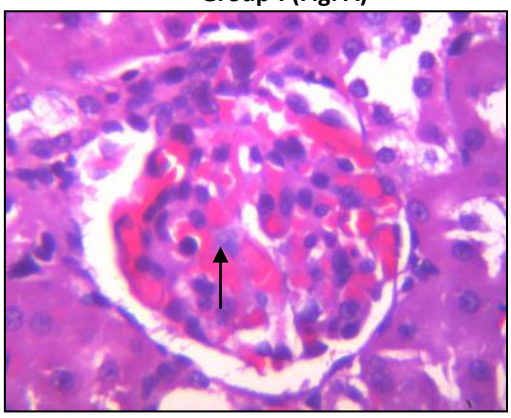
Group IV: Treatment done with HAPG 400 mg/kg, p.o showed intact architecture of renal parenchyma. In glomerulus [Fig.A, Arrow]: Hypercellular with increased extravasation of erythrocytes was observed. Renal tubules were unremarkable [Fig.B, Long - Arrow]. Blood Vessels and Interstitium remained unremarkable.



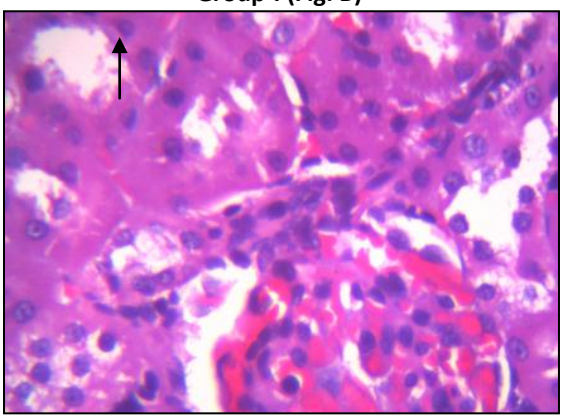
Group-I (Fig. A)



Group-I (Fig. B)



Group-II (Fig. A)



Group-II (Fig. B)

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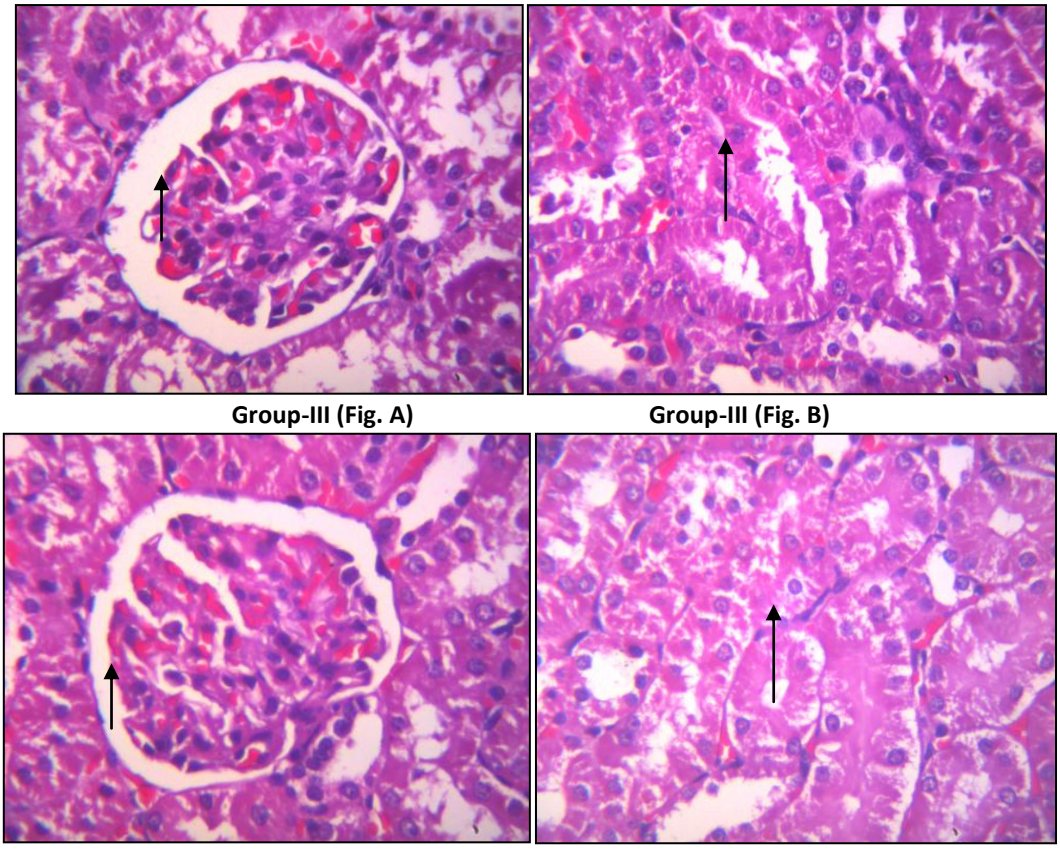

Group-III (Fig. A) Group-III (Fig. B)

Group-IV (Fig. A) Group-IV (Fig. B)

Figure No 1: Histopathology of Kidney

DISCUSSION

In recent time, the safety of the chronic use of acetaminophen at therapeutic dose has generated a lot of hot debate. Acetaminophen overdose has been associated with significant glutathione depletion and consequent lipid peroxidation. As a consequence of lipid peroxidation, intracellular accumulation and covalent bonding of its highly reactive metabolite, N-acetyl-*para*-benzoquinone-imine (NAPQI), hepatocyte malfunction and death often result. Similar effect is often recorded for renal tissues. The selective renal accumulation of non-steroidal anti-inflammatory nephrotoxins including acetaminophen in animal and human is thought to result in a chain of biochemical reactions which culminate in acute or chronic nephropathies. In addition, acetaminophen has been reported to promote hepatocyte and renal apoptosis[10]. In paracetamol induced nephrotoxicity (750 mg/kg, p.o) reduced the body weight, urine volume, urinary creatinine, urinary sodium and increased kidney weight, urinary potassium, urinary glucose, blood urea, blood creatinine, blood total protein levels indicating that nephrotoxicity was induced. It also depleted the levels of GSH and increased the levels of LP. The treatment with 200 mg/kg and 400 mg/kg HAPG p.o normalized all the parameter of toxicity in a dose dependant manner. Even the histopathological showed reduction in renal damage occurred due to paracetamol toxicity.

CONCLUSION

The overall result suggests that the hydroalcoholic extract of leaf of *Psidium guajava* Linn. possesses nephroprotective potential and improves derangements associated with

repeated dose paracetamol nephrotoxicity. Although, the possible mechanism of action was not investigated in the present study, future studies will be required.

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REFERENCES

[1] Naohiko Anzai, Hitoshi Endou. Jpn Soc Alt Animal Exp 2007;14:447-452.
[2] Singh NP, Ganguli A, Prakash A. J Assoc Physicians India 2003;51:970-979.
[3] Zariyantey Abdul Hamid, Siti Balkis Budin, Ng Wen Jie, Asmah Hamid, Khairana Husain, Jamaludin Mohamed. J Zhejiang Univ Sci B 2012;13(3):176-185.
[4] Ezekwesili JO, Nkemdilim UU, Okeke CU. Biokemistri 2010;22(2):85-90.
[5] Baby Joseph, Mini Priya R. Int J Pharm Bio Sci 2011;2(1):53-69
[6] Amad Movaedian, Bezad Zolfaghari, Ebrahim Sajjadi, Reza Moknatjou S. Clinics 2010;65(6):629-33.
[7] OECD Guidelines for the Testing of Chemical. Acute Oral Toxicity – Up and Down Procedure (UDP) 2001. http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg425.pdf.
[8] Pramod Kumar, Devala Rao G, Lakshmayya, Ramachandra Setty S. Avicenna J Med Biotech 2011;3(2):87-93.
[9] Gaurav Vijay Harlalka, Chandragauda Raosaheb Patil, Mahesh Ramu Patil. Indian J Pharmacol 2007;39(4):201-205.
[10] Adeneye AA, Olagunju JA, Benebo AS, Elias SO, Adisa AO, Idowu BO, Oyedeji MO, Isioye EO, Braimoh OB, Oladejo OO, Alana EO. Int J Appl Res Natural Products 2008;1(1):6-14.