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Original article

Biochemical and histologic evaluation following multiple dose administration of paracetamol alone and along with polyherbal extract mixture in rats

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Abstract

The present experiment was carried out to investigate the amelioration of paracetamol (500 mg/kg, PO) induced biochemical and pathological alterations by polyherbal extract mixture (PHEM) at 100, 200 and 300 mg/kg, PO and silymarin (10 mg/kg, PO) in rats. Symptoms of paracetamol toxicity were not observed in rats of any groups. No significant alterations in Hb, PCV, TEC, TLC, MCV, MCHC, MCH and DLC have been observed in rats of any group. Administration of paracetamol damaged the liver as shown with increased level of ALT, AST and total bilirubin, which were significantly lowered when rats treated with either PHEM or silymarin at 200 and 300 mg/kg orally for 21 days. The LDH level was also altered by paracetamol treatment which was observed unaltered in rats, treated with PHEM at all doses used in the study. The levels of blood urea nitrogen were also lowered in rats treated with PHEM at 200 mg/kg and 300 mg/kg compared to paracetamol control rats. The creatinine level in paracetamol was not altered in animals of different groups. The levels of total protein, albumin, globulin and uric acid were not altered significantly by paracetamol administration. The PHEM more than 200 mg/kg produced a protective effect against the damage of liver and kidney caused by paracetamol in rats.

Key words: Paracetamol, biochemical alterations, pathological changes, polyherbal extract mixture, rats

1. Introduction

In chronic disease conditions, drugs like NSAIDS, steroids, antibiotics and antiviral are commonly used for therapeutic purpose. NSAIDs drugs like nimesulide, meloxicam, ibuprofen and paracetamol, *etc*., are employed to reduce inflammatory changes and pain. Long term use of these drugs may cause side effects pertaining to liver and kidney. Liver damage is a common side effect following long term administration of NSAIDS. Paracetamol (acetaminophen, APAP) is an extensively used as inhibitor of the cyclooxygenase for relieving inflammation, pain and fever. Liver and kidney failure occur in human sometimes due to overdose of paracetamol or long term administration (Eguia and Materson, 1997). N-acetyl-P-Benzoquinoneimine, a metabolite of paracetamol is responsible for such toxicity. This metabolite of paracetamol causes marked hepatic damage with a lesser effect on kidney. The damage of the liver is mainly reversed with N-acetyl cysteine and silymarin, which may not be cost effective in animals (Payasi *et al*., 2010). The second most affected organ by paracetamol is kidney (Gulnaz *et al*., 2010). Administration of paracetamol for longer time may cause renal damage, followed by renal failure sometimes. Decreased glomerular filtration rate (GFR) and capacity of the kidney in excretion of metabolites of drugs and other products in the body are observed in renal damage condition. High dose or longer duration of administration of paracetamol leads to production of toxic metabolite which may lead to liver or kidney damage. Thus, homeostasis in the body is compromised and there will be apoptosis or programmed cell death, cause to tissue necrosis and finally to organ dysfunction (Gopi *et al*., 2010).

The use of herbal medicine in the last decade is increased and it is getting popularity in developing and developed countries due to its efficacy with lesser side effects (Padh and Patel, 2001; Pushpangadan *et al.*, 2015). Drugs of herbal origin provide a rational means for the treatment of several ailments in human and animals (Thaibinh, 1998; Manoharachary and Nagaraju, 2016; Udupa Nayanabhirama, 2016). The good therapeutic effect with patient compliance, less side effects and cost effectiveness are the reasons for choosing drugs from natural origin (Chandira and Jayakar, 2010). There is a growing demand of herbal medicine in most of the countries of the world. Therapeutic values of medicinal plants are due to the presence of various complex phytochemical compounds. The various experiments in the area of hepatoprotective and nephroprotective effects of drugs of herbal origin have been carried out by many researchers. Important hepatoprotective plants are *Capparis decidua* (Forssk.) Edgew. (ver. name: Karira, Family: Capparaceae), *Gymnosporia montana* (Roth.) Benth. (ver. name:

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Vikro, Vikankala, Family: Celastraceae), *Luffa echinata* Roxb. (Vern. name.: Bindaal; Family: Cucurbitaceae), *Allium cepa* L. (Vern. name: Pyaz; Family: Amaryllidaceae), *Sphaeranthus indicus* L. (Vern. name: Chhagul-nudi; Family: Asteraceae), *Tamarindus indica* L. (Vern. name: Emli; Family: Fabaceae), *etc*. Plants having nephroprotective properties are *Boerhavia diffusa* L. (Vern. name: Punarnava; Family: Nyctaginacea), *Moringa oleifera* Lam. (Vern. name: Sahjan; Family: Moringaceae), *Andrographis paniculata* Wall (Vern. name: Kirayat, Kalmegh; Family Acanthaceae), *Aerva lanata* (L.) A. L. Juss. ex Schultes. (Vern. name: Bui; Amaranthaceae) locally known as 'bui', *Crataeva uvula, etc.* (Adewusi and Afolayan, 2010).

With the objective to formulate and evaluate the effect of the herbal extract mixture having an ameliorating effect in hepatic and renal damage in rats; six medicinal plants (*Luffa echinata* Roxb., *Allium cepa* L., *Capparis decidua*, *Gymnosporia montana* (Roth) Benth., *Andrographis paniculata* (Burm. f.) Wall. ex Nees, *Boerhavia diffusa* L.) have been selected and used to make polyherbal extract mixture (PHEM) and employed in the study to evaluate its effect against paracetamol induced alterations in rats.

2. Materials and Methods

2.1 Experimental animals

Forty two rats of 10-12 weeks of age were used in the study, which were procured from Zydus Research Center, Ahmedabad, Gujarat. All animals were maintained as per standard husbandry practices (CPCSEA, 2003). The experimental protocol No. JAU/JVC/IAEC/SA/17 /2017 was approved by the Institutional Animal Ethics Committee (IAEC) of the college.

2.2 Plant materials, drugs and chemicals

Plant material (Leaves of *Andrographis paniculata*, *Gymnosporia montana*, *Boerhavia diffusa*, fruit of *Luffa echinata*, stem of *Capparis deciduas* and peels of *Allium cepa*) were collected from nearby areas of Junagadh and scientifically identified by Mr. Punit Bhatt (Pharmacognosist, Department of Pharmacology and Toxicology, Veterinary College, JAU, Junagadh). Shade dried method was used to dry the plant material and used further to make fine powder using electric grinder. Powder material of each plant was used to prepare hydro-alcoholic extracts using double distilled water and methanol (50:50). The mixture was filtered through a Whatman filter paper No. 1. Rotary evaporator was used to reduce the extracts. All extracts were completely dried and stored in refrigerator for further use in equal proportion to prepare PHEM. Silymarin (Lot No.: BCBT9170) and paracetamol (Lot No.: SLBR2060V) were purchased from Sigma Aldrich, USA. All other chemicals and solvents of analytical grade were purchased from Merck Ltd., Mumbai and S.D. Fine Chemicals, Mumbai.

2.3 Experimental design

As mentioned by Eliwa *et al.* (2014), paracetamol was employed to damage the liver and kidney. Thirty ml sunflower was used to dissolve 6 g of paracetamol and administered orally (500 mg/kg) to all animals except normal control and vehicle control groups. Completely Randomized Design (CRD) has been employed with seven different treatments. Each treatment was given to six rats randomly divided based on body weight in seven groups. Groups of rats treated with different treatments were normal control (C1), vehicle control (C2), toxicity control (C3), standard drug control (C4), treatment 1 (T1), treatment 2 (T2), treatment 3 (T3) for 21 days. Silymarin as a standard drug was given at the dose rate of 10 mg/kg body weight orally for 21 days. Silymarin (30 mg) was dissolved in 3 ml of distilled water. PHEM (1200 mg) was dissolved in 12 ml of distilled water and given by oral route at the dose rate of 100, 200 and 300 mg/kg daily for 21 days to animals of group T1, T2 and T3, respectively. Paracetamol, silymarin, PHEM and vehicle in different groups were administered daily using oral gavage needle as per treatment protocol. Before administration of test substances, live body weight of animals was recorded daily.

2.4 Acute toxicity study

Acute toxicity study of polyherbal mixture was conducted in rats as per OECD guideline 423. Polyherbal mixture was administered at the dose rate of 2000 mg/kg orally to 3 rats.

2.5 Collection of samples

Blood samples were collected on day fifteen and twenty two of experiment for evaluation of hematological and biochemical parameters. The tissues of major organs like liver, kidney, spleen, heart, lungs, stomach and intestine were collected in 10% formalin for histopathological examination.

2.6 Parameters studied

2.6.1 Body weight, feed consumption and organ: Body weight ratio

The body weight of all rats was recorded daily and feed consumption (g/rat/day) was calculated based on records of feed offered and leftover feed to each group. Weights of major organs were recorded using analytical balance (Sartorius, BSA-423SCW) which was used to calculate the relative organ body weight ratio.

2.6.2 Hematological parameters

Hematological parameters like hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), differential leukocyte count (DLC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were estimated using automated hematology analyzer (Abacus Junior Vet 5, Diatron, Hungary).

2.6.3 Biochemical parameters

Blood glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine, total protein (TP), albumin, and total bilirubin were estimated by using standard kits (Diatek Health Care Pvt. Ltd.) with fully automatic biochemistry analyzer (Dia-chem 240 plus, Diatek, China).

2.6.4 Gross and histopathological observations

All animals were humanely sacrificed at the end of experiment to observe pathological changes in organs. To evaluate histopathological changes in major organs, formalin fixed tissues were subjected to paraffin wax embedding, followed by tissue sectioning. Fine sections of each tissue (6-8 m) were cut using semi-automated rotary microtome (Leica Biosystems, Germany) and were stained with haematoxyline and eosin (H and E) stain to observe under microscope for histopathological lesions.

2.7 Statistical analysis

All the data obtained were presented as Mean ± standard error (SE). Data were analyzed statistically by one way ANOVA and different treatment group means were compared by Duncan's Multiple Range Tests to observe differences among the treatments (Snedecor and Cochran, 1982).

3. Results and Discussion

During the experiment, noticeable clinical signs of toxicity or side effects were not observed in rats of any groups. Paracetamol treatment did not produce significant alteration on feed consumption (Figure 1) and changes in body weight during the experiment (Figure 2). PHEM as well as silymarin treatment also did not affect the body weight of animals. The results of the present study are in agreement with a previous report by Soha (2017) that paracetamol treatment had no effect on body weight in mice. However, significant ($p > 0.05$) increased in liver body weight ratio was observed in paracetamol treated rats as compared to other groups (Table 1). The mean values of liver body weight ratio in other treatment groups were not significantly differ from paracetamol treated groups.

Table 1: Effects of daily oral administration of silymarin (10 mg/kg, p.o.) and PHEM (100, 200 and 300 mg/kg, p.o.) for 21 days on mean values of organ body weight ratio (liver, kidneys, heart and lung) against paracetamol induced toxicity in rats.

Organ	Organ: bodyweight ratio						
	C1	C2	C3	C4	T1	T2	T3
Liver	0.0298 ± 0.0019^{a}	0.0353 ± 0.0014^{ab}	0.0379 ± 0.0012^{bc}	0.0353 ± 0.0004^{ab}	0.0369 ± 0.0006^{bc}	0.0419 ± 0.0020^{c}	0.0362 ± 0.0010^{bc}
Kidney (Left)	0.0032 ± 0.0001^{a}	0.0033 ± 0.0002^{a}	0.0033 ± 0.0002^{a}	0.0031 ± 0.0001^{a}	0.0029 ± 0.0001^{a}	0.0031 ± 0.0002^{a}	0.0033 ± 0.0001^{a}
Kidney (Right)	0.0031 ± 0.0001^{a}	0.0034 ± 0.0001^{a}	0.0032 ± 0.0001^{a}	0.0029 ± 0.0004^{a}	0.0031 ± 0.0001^{a}	0.0033 ± 0.0001^{a}	0.0032 ± 0.0001^{a}
Heart	0.0031 ± 0.0001^{a}	0.0033 ± 0.0002^{a}	0.0033 ± 0.0002^{a}	0.0031 ± 0.0001^{a}	0.0034 ± 0.0002^{a}	0.0038 ± 0.0004^{a}	0.0035 ± 0.0002^{a}
Lung	0.0065 ± 0.0004^{a}	0.0062 ± 0.0002^{a}	0.0059 ± 0.0003^{a}	0.0058 ± 0.0003^{a}	0.0056 ± 0.0002^{a}	0.0060 ± 0.0004^{a}	0.0097 ± 0.0026^{a}

Values with different superscript in a raw were significantly different ($p > 0.05$).

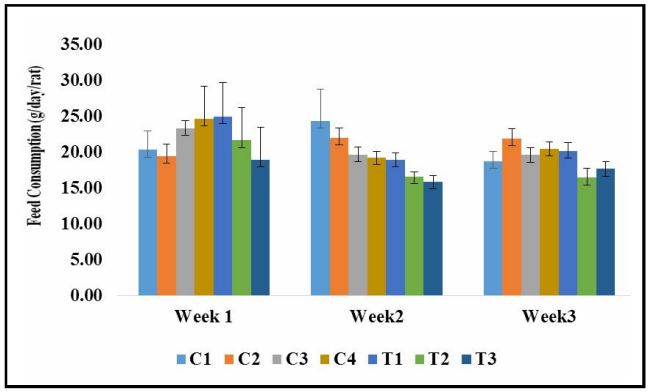

Figure 1: The average feed consumption (g/day/rat) of experimental animals of different groups.

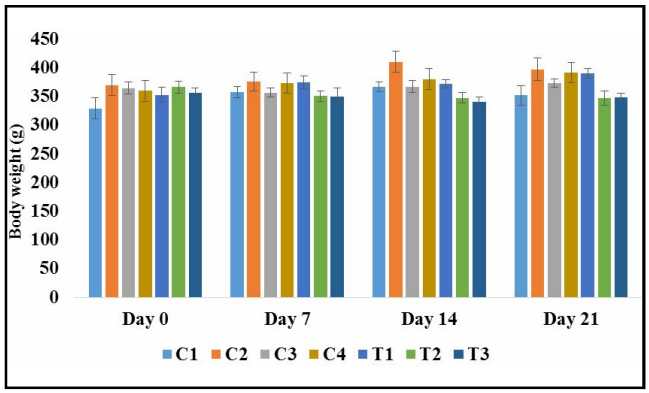

Figure 2: Body weight (g) in experimental animals of different groups.

Haematological parameters in rats under different treatments at day 15 and 22 of experiment are presented in Tables 2 and 3, respectively. Whereas, biochemical parameters in rats under different treatments at day 15 and 22 of experiment are presented in Tables 4 and 5, respectively. In the study, no significant alterations in the values of Hb, PCV, TEC, TLC, MCV, MCHC, MCH and DLC have been observed in rats of different groups. Similar to our findings, acetaminophen administration at 800 mg/kg did not affect hematological parameters in rats (Adeneye *et al.*, 2008). However, long term (42 days) administration of paracetamol at lower dose was reported to cause non-significant reductions in PCV, Hb and RBC values, but not in MCV, MCHC, MCH, platelets, neutrophil, lymphocyte, eosinophil and monocyte values relative to their controls in rats (Oyedeji *et al.*, 2013). It is indication of effect of paracetamol on hematological parameters relies on duration of treatment rather than dose.

Sensitive cytosolic enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) generally alters in liver affections when hepatocellular plasma membrane is damaged. These enzymes are categorized as a marker for evaluation of liver injury (Sallie *et al.*, 1991). In the present study, level of ALT was significantly increased in paracetamol treated rats while level of AST was non-significantly increased at day 15. The levels of these both enzymes were found significantly higher on day 22 in animals treated with paracetamol only. Increased levels of respective enzymes were also reported after paracetamol treatment in rats (Payasi *et al.*, 2010). The animals treated with PHEM (T2 and T3 groups) showed a significant lower values of ALT at day 15 as compared to the respective value in rats treated with only paracetamol (C3) and were also comparable to the mean value of control rats. While, mean level of ALT at 22nd day of study in rats of silymarin (C4) and PHEM groups (T2 and T3) were significantly

lower than value observed in rats treated with only paracetamol (C3). The mean level of AST at day 15 of study in rats of PHEM groups (T2 and T3) were at par with the mean value in control rats and non-significantly lower than the mean value of rats treated with only paracetamol (C3). The mean level of AST at day 22 of study in rats of silymarin and PHEM groups (T1, T2 and T3) was significantly lower than mean value of rats treated with only paracetamol (C3). The rats when treated with PHEM, the values of above parameters were restored to normal levels. *Andrographis paniculata* (Vetriselvan *et al.*, 2012), *Gymnosporia montana* (Patel *et al.*, 2010), *Allium cepa* (Kumar *et al.*, 2013), *Boerhavia diffusa* (Shameela *et al*., 2015), *Capparis decidua* (Ali *et al*., 2010) and *Luffa echinata* (Ahmed *et al*., 2000) individually in animals have been reported to cause reduction in increased level of ALT and AST due to tested toxicants or paracetamol. The protective effect of oxidative damage caused by paracetamol might be the reason for the restoration of AST and ALT enzymes when rats treated with PHEM along with paracetamol.

Table 2: Effects of daily oral administration of silymarin (10 mg/kg, p.o.) and PHEM (100, 200 and 300 mg/kg, p.o.) on day 15th on mean values of hematological parameters against paracetamol induced toxicity in Wistar rats

Parameters	Treatment groups						
	C1	C2	C3	C4	T1	T2	T3
HB (g/dl)	16.40 ± 0.18^a	15.30 ± 1.11^a	16.77 ± 0.25^a	16.13 ± 0.51^a	16.95 ± 0.22^a	16.85 ± 0.41^a	16.48 ± 0.26^a
PCV (%)	44.43 ± 0.80^a	42.76 ± 3.17^a	45.28 ± 0.69^a	43.55 ± 1.38^a	46.21 ± 0.68^a	45.00 ± 0.97^a	44.11± 0.71^a
TEC (10^6/µl)	8.69 ± 0.12^a	8.49 ± 0.63^a	9.06 ± 0.06^a	8.83 ± 0.25^a	9.14 ± 0.10^a	9.16 ± 0.23^a	8.76 ± 0.15^a
TLC (10^3/cmm)	13.63 ± 0.74^a	14.63 ± 0.85^a	16.98 ± 0.64^a	15.89 ± 0.53^a	17.95 ± 1.29^a	19.61 ± 1.76^a	21.70 ± 1.12^a
MCV (fl)	51.17 ± 0.54^a	50.00 ± 0.37^a	50.17 ± 0.60^a	49.33 ± 0.67^a	50.33 ± 0.33^a	49.33 ± 1.05^a	50.33 ± 0.33^a
MCHC (%)	36.97 ± 0.35^a	35.80 ± 0.34^a	37.00 ± 0.30^a	37.07 ± 0.38^a	36.62 ± 0.30^a	37.47 ± 0.36^a	37.07 ± 0.38^a
MCH (pg)	18.87 ± 0.11^a	17.92 ± 0.12^a	18.47 ± 0.22^a	18.25 ± 0.16^a	18.52 ± 0.16^a	18.43 ± 0.26^a	18.82 ± 0.16^a
Lymphocyte (%)	75.78 ± 5.44^a	68.85 ± 14.69^a	72.77 ± 2.30^a	73.35 ± 2.81^a	72.17 ± 3.83^a	66.03 ± 5.83^a	55.03 ± 7.95^a
Monocytes (%)	5.45 ± 1.97^a	3.80 ± 1.45^a	3.10 ± 0.96^a	3.68 ± 1.23^a	3.77 ± 1.42^a	5.92 ± 1.93^a	6.25 ± 1.17^a
Eosinophil (%)	0.17 ± 0.17^a	0.17 ± 0.17^a	0.17 ± 0.17^a	0.00 ± 0.00^a	0.17 ± 0.17^a	0.17 ± 0.17^a	0.00 ± 0.00^a
Neutrophils (%)	18.73 ± 2.78^a	26.45 ± 6.02^a	24.35 ± 2.35^a	22.98 ± 2.21^a	24.05 ± 3.27^a	28.25 ± 4.98^a	38.73 ± 8.04^a
Basophill(%)	0.00 ± 0.00^a	0.17 ± 0.17^a	0.00 ± 0.00^a	0.17 ± 0.17^a	0.00 ± 0.00^a	0.17 ± 0.17^a	0.00 ± 0.00^a

Values with different superscript in a raw were significantly different (p>0.05).

Table 3: Effects of daily oral administration of silymarin (10 mg/kg, p.o.) and PHEM (100, 200 and 300 mg/kg, p.o.) on 22nd day on mean values of hematological parameters against paracetamol induced toxicity in Wistar rats

Parameters	Treatment groups						
	C1	C2	C3	C4	T1	T2	T3
HB (g/dl)	17.22 ± 0.60^a	16.30 ± 0.15^a	16.67 ± 0.67^a	17.43 ± 0.23^a	16.70 ± 0.25^a	16.63 ± 0.23^a	16.87 ± 0.70^a
PCV(%)	44.56 ± 1.63^a	43.86 ± 0.68^a	43.57 ± 2.19^a	45.69 ± 0.91^a	43.64 ± 0.69^a	43.78 ± 0.64^a	45.05 ± 1.53^a
TEC (10^6/µl)	8.82 ± 0.26^a	8.93 ± 0.17^a	8.60 ± 0.41^a	9.13 ± 0.21^a	8.58 ± 0.21^a	8.80 ± 0.21^a	8.81 ± 0.28^a
TLC (10^3/cmm)	13.51 ± 1.39^a	13.34 ± 0.59^a	14.10 ± 1.03^a	11.50 ± 0.40^a	14.05 ± 0.78^a	18.84 ± 1.22^a	15.47 ± 1.35^a
MCV (fl)	50.67 ± 0.61^a	49.17 ± 0.17^a	50.67 ± 0.33^a	50.00 ± 0.86^a	50.17 ± 0.83^a	49.83 ± 1.14^a	51.17 ± 0.70^a
MCHC (%)	38.53 ± 0.42^a	37.17 ± 0.32^a	38.40 ± 0.75^a	38.20 ± 0.31^a	38.18 ± 0.40^a	38.02 ± 0.36^a	37.43 ± 0.42^a
MCH (pg)	19.52 ± 0.30^a	18.28 ± 0.21^a	19.45 ± 0.39^a	19.13 ± 0.36^a	19.28 ± 0.39^a	18.95 ± 0.44^a	19.13 ± 0.23^a
Lymphocyte(%)	50.20 ±12.34^a	56.60 ± 9.77^a	75.30 ± 4.46^a	82.30 ± 2.23^a	74.30 ± 3.72^a	83.70 ± 4.25^a	70.40 ± 5.65^a
Monocytes (%)	4.75 ± 1.87^a	2.42 ± 0.54^a	4.35 ± 1.22^a	4.43 ± 1.78^a	7.92 ± 1.72^a	4.35 ± 1.90^a	4.17 ± 0.40^a
Eosinophil (%)	0.17 ± 0.17^a	0.00 ± 0.00^a	0.17 ± 0.17^a	0.17 ± 0.17^a	0.00 ± 0.00^a	0.17 ± 0.17^a	0.17 ± 0.17^a
Neutrophils (%)	18.28 ± 1.45^a	17.63 ± 1.24^a	24.03 ± 3.97^a	21.90 ± 1.48^a	28.79 ± 4.00^a	26.15 ± 3.21^a	25.43 ± 5.36^a
Basophill(%)	0.17 ± 0.17^a	0.17 ± 0.17^a	0.17 ± 0.17^a	0.00 ± 0.00^a	0.17 ± 0.17^a	0.00 ± 0.00^a	0.17 ± 0.17^a

Values with different superscript in a raw were significantly different (p > 0.05).

Table 4: Effects of daily oral administration of silymarin (10 mg/kg, p.o.) and PHEM (100, 200 and 300 mg/kg, p.o.) on day 15th on mean values of biochemical parameters against paracetamol induced toxicity in Wistar rats

Parameters	Treatment groups						
	C1	C2	C3	C4	T1	T2	T3
ALT (IU/l)	41.38 ± 1.31^{ab}	43.43 ± 2.39^{ab}	54.56 ± 3.91^{b}	50.49 ± 1.64^{b}	51.85 ± 4.05^{b}	36.16 ± 3.67^{a}	46.02 ± 7.62^{ab}
AST (IU/l)	73.75 ± 3.36^{a}	80.06 ± 7.69^{a}	91.47 ± 6.54^{a}	82.05 ± 4.27^{a}	83.18 ± 0.88^{a}	73.52 ± 5.63^{a}	78.45 ± 6.64^{a}
LDH (IU/l)	220.58 ± 18.60^{a}	202.59 ± 28.95^{a}	380.89 ± 88.97^{ab}	262.02 ± 39.48^{a}	587.39 ± 42.44^{b}	301.90 ± 40.52^{a}	293.86 ± 83.83^{a}
BUN (mg/dl)	17.40 ± 0.73^{a}	18.21 ± 0.78^{ab}	20.85 ± 1.17^{b}	17.71 ± 0.56^{ab}	19.07 ± 0.43^{ab}	17.35 ± 0.66^{a}	16.08 ± 1.38^{a}
Creatinine (mg/dl)	0.37 ± 0.02^{a}	0.33 ± 0.03^{a}	0.40 ± 0.01^{a}	0.30 ± 0.03^{a}	0.36 ± 0.01^{a}	0.33 ± 0.05^{a}	0.34 ± 0.03^{a}
Urea (mg/dl)	37.23 ± 1.57^{a}	38.96 ± 1.67^{ab}	44.61 ± 2.51^{b}	37.90 ± 1.12^{ab}	40.82 ± 0.91^{ab}	37.13 ± 1.40^{a}	34.42 ± 2.94^{a}
Uric acid (mg/dl)	0.91 ± 0.05^{a}	0.87 ± 0.11^{a}	1.01 ± 0.10^{a}	0.90 ± 0.08^{a}	1.14 ± 0.11^{a}	1.01 ± 0.151^{a}	0.87 ± 0.17^{a}
Total protein (g/dl)	6.52 ± 0.09^{a}	6.23 ± 0.09^{a}	6.51 ± 0.08^{a}	6.44 ± 0.15^{a}	6.60 ± 0.10^{a}	6.82 ± 0.07^{a}	6.55 ± 0.13^{a}
Albumin (g/dl)	2.99 ± 0.02^{a}	2.95 ± 0.04^{a}	3.08 ± 0.05^{a}	3.08 ± 0.04^{a}	3.15 ± 0.03^{a}	3.01 ± 0.07^{a}	2.97 ± 0.06^{a}
Globulin (g/dl)	3.53 ± 0.10^{a}	3.28 ± 0.08^{a}	3.44 ± 0.07^{a}	3.36 ± 0.11^{a}	3.45 ± 0.09^{a}	3.82 ± 0.11^{a}	3.59 ± 0.15^{a}
Total bilirubin (mg/dl)	0.25 ± 0.02^{a}	0.26 ± 0.03^{a}	0.36 ± 0.01^{b}	0.32 ± 0.01^{ab}	0.34 ± 0.01^{b}	0.32 ± 0.03^{ab}	0.30 ± 0.02^{ab}

Values with different superscript in a raw were significantly different (p<0.05)

Table 5: Effects of daily oral administration of silymarin (10 mg/kg, p.o.) and PHEM (100, 200 and 300 mg/kg, p.o.) on 22nd day on mean values of biochemical parameters against paracetamol induced toxicity in Wistar rats

Parameters	Treatment groups						
	C1	C2	C3	C4	T1	T2	T3
ALT (IU/l)	44.21 ± 3.11^{bc}	42.48 ± 1.56^{bc}	52.95 ± 1.18^{c}	45.27 ± 1.71^{bc}	36.36 ± 2.75^{ab}	36.20 ± 1.83^{ab}	30.08 ± 1.66^{a}
AST (IU/l)	73.19 ± 5.04^{a}	73.38 ± 5.42^{c}	112.79 ± 11.98^{bc}	83.54 ± 5.76^{abc}	75.15 ± 4.15^{ab}	75.04 ± 1.91^{abc}	80.03 ± 3.01^{abc}
LDH (IU/l)	467.03 ± 49.54^{a}	448.46 ± 115.06^{a}	1169.84 ± 276.18^{b}	454.73 ± 60.97^{a}	433.15 ± 71.20^{a}	553.15 ± 233.22^{a}	467.27 ± 136.45^{a}
BUN (mg/dl)	16.44 ± 1.27a	18.20 ± 1.24^{a}	19.46 ± 1.16^{a}	17.40 ± 0.60^{a}	17.50 ± 0.67^{a}	16.39 ± 0.49^{a}	15.35 ± 0.71^{a}
Creatinine (mg/dl)	0.28 ± 0.02^{a}	0.28 ± 0.03^{a}	0.31 ± 0.02^{a}	0.24 ± 0.03^{a}	0.28 ± 0.02^{a}	0.26 ± 0.03^{a}	0.28 ± 0.03^{a}
Urea (mg/dl)	35.19 ± 2.27^{a}	38.94 ± 2.65^{a}	41.64 ± 2.49^{a}	37.25 ± 1.28^{a}	37.46 ± 1.43^{a}	35.08 ± 1.04^{a}	32.85 ± 1.53^{a}
Uric acid (mg/dl)	1.18 ± 0.10^{a}	1.19 ± 0.10^{a}	1.31 ± 0.12^{a}	0.98 ± 0.09^{a}	1.21 ± 0.18^{a}	1.19 ± 0.05^{a}	1.28 ± 0.05^{a}
Total protein (g/dl)	6.47 ± 0.12^{a}	6.55 ± 0.07^{ab}	7.05± 0.08^{c}	6.69 ± 0.11^{abc}	6.59± 0.10^{abc}	6.95 ± 0.21^{bc}	6.98 ± 0.12^{bc}
Albumin (g/dl)	3.04 ± 0.09^{a}	3.05 ± 0.06^{a}	3.29 ± 0.06^{a}	3.19 ± 0.06^{a}	3.21 ± 0.06^{a}	3.07 ± 0.10^{a}	3.11 ± 0.11^{a}
Globulin (g/dl)	3.44 ± 0.09^{a}	3.50 ± 0.04^{a}	3.77 ± 0.08^{a}	3.51 ± 0.08^{a}	3.38 ± 0.08^{a}	3.88 ± 0.24^{a}	3.88 ± 0.11^{a}
Total bilirubin (mg/dl)	0.20 ± 0.02^{ab}	0.18 ± 0.02^{a}	0.28 ± 0.02^{c}	0.22 ± 0.02^{abc}	0.28 ± 0.01^{c}	0.25 ± 0.01^{abc}	0.25 ± 0.01^{bc}

Values with different superscript in a raw were significantly different (p < 0.05)

LDH is an organ specific sensitive intracellular enzyme located in cytoplasm of cell, which catalyses conversion of lactate to pyruvate using NAD+ as coenzyme of NAD (Burtis *et al*., 1986). It gets expelled during hepatocellular injury and increase level of LDH indicates hepatocellular damage or acute liver necrosis (Kim *et al*., 2001). In the present study, the mean level of LDH in animals treated with paracetamol was significantly increased. Dwivedi *et al*. (2015) also reported increased levels of LDH after paracetamol administration in rats. The mean level of LDH at day 15 of the study in rats of PHEM groups (T2 and T3) were non-significantly lower as compared to the respective value of rats treated with only paracetamol. The mean levels of LDH at day 22 of study in rats of silymarin (C4) and PHEM groups (T1, T2 and T3) were significantly lower than the mean value of rats treated with only paracetamol. When the rats treated with PHEM, the values of above parameter were restored to normal level at all doses used in the study. The finding is an indication of the protective effect of PHEM against increased LDH level by paracetamol. *Allium cepa* (Ozougwu and Eyo, 2014), *Capparis deciduas* (Deepak *et al*., 2014), *Andrographis paniculata* (Ojha *et al.*, 2009) and *Boerhavia diffusa* (Devaki *et al.*, 2004) individually produced LDH lowering effect. Restoration of LDH level compare to paracetamol treated group (C3) in liver and kidney damage in the study might be due to the presence of different bioactive components like punarnavoside, flavonol, andrograpanin, and capparisterol in *Boerhavia diffusa, Allium cepa, Andrographis paniculata*, and *Capparis decidua*, respectively. Bilirubin is produced when haemoglobin breaks and it is transported from the spleen to the liver and excreted through bile. Concentration of billirubin in serum increases due to increased haemolysis, genetic errors, neonatal jaundice, ineffective erythropoiesis and drugs. The spewing of bile in obstructive or inflamed liver caused by paracetamol leads to increase in serum bilirubin (Stocker *et al*., 1987). The level of total bilirubin in the present study was also significantly increased along with alterations in levels of ALT, AST in paracetamol treated animals at day 15 and 22 of the study. The mean levels of total billirubin at day 15 and 22 of study in rats of silymarin and PHEM

groups (C4, T2 and T3) were non-significantly and significantly lower, respectively than the mean value of total billirubin in rats treated with only paracetamol. Similar to our findings, significantly increased level of bilirubin in rats treated with paracetamol has also been noticed (Kumar *et al.,* 2013). The reduction in increased level of bilirubin due to tested chemicals or drug like paracetamol has been reported in experimental animals when they were treated with extracts of *Andrographis paniculata* (Nasir *et al.,* 2013), *Capparis decidua* (Ali *et al.,* 2010; Dogan *et al.,* 2016), *Allium cepa* (Kumar *et al.,* 2013; Ozougwu and Eyo, 2014), *Boerhavia diffusa* (Jayavelu *et al.,* 2013, Beedimani and Jeevangi, 2015) and *Luffa echinata* (Bapat and Chandra, 1968) individually in animals.

The PHEM used in the present study contained extracts of six different plants as mentioned earlier and have shown the protective effect on liver damage induced by paracetamol. Hepatoprotective effect of extract of *Andrographis paniculata* (Vetriselvan *et al.,* 2012), *Boerhavia diffusa* (Jayavelu *et al.,* 2013), *Capparis decidua* (Ali *et al.,* 2010), *Gymnosporia montana* (Pathan *et al.,* 2014), *Allium cepa* (Kumar *et al.,* 2013) and *Luffa echinata* (Ahmed *et al.,* 2000) have been previously reported in various models of chemical induced hepatotoxicity. Antioxidant and anti-inflammatory effect of andrographolide from *Andrographis paniculata,* antioxidant effect of quercetin and kaemferol from *Boerhavia diffusa,* anti-inflammatory effect of beta-sitosterol from *Capparis deciduas* (Ajay Kumar and Azm, 2014), antioxidant effect of kaemferol and beta-sitosterol from *Gymnosporia montana,* antioxidant and anti-inflammatory effect of quercetin from *Allium cepa* and anti-inflammatory effect of curcubitacin from *Luffa echinata* might be responsible for hepatoprotective effects as the PHEM used in the study contains extracts of these plants.

Blood urea nitrogen (BUN) is used to evaluate the ability of the kidneys to remove nitrogenous waste from the blood. Paracetamol overdose produces less damage in the kidney than liver (Boutis and Shannon, 2001). In the present study, level of BUN was significantly increased at day 15 and non-significantly altered at day 22 in rats treated with paracetamol only. The mean value of BUN at day 15 of study in rats of silymarin and PHEM groups (C4, T2 and T3) were slightly lowered than that of paracetamol treated rats. Level of BUN was little higher on day 22 in rats treated with paracetamol only but the data was non-significant compared to the value of control group. The result indicates the less effect of paracetamol on kidney of rats when administered alone or co-administered with silymarin and PHEM at a dose rate of 200 and 300 mg/kg body weight. The mean values of BUN at day 22 of study in rats of silymarin and PHEM groups (C4, T1, T2 and T3) were slightly lower than that observed in paracetamol treated group and comparable to the mean value of BUN in control rats. However, the little increased in BUN was normalized by silymarin and PHEM. Creatinin levels in animals of different groups were not significantly altered at day 15 and 22 of the study which indicates the less effect of paracetamol on kidney compared to the liver. Gross examination at the end of the experiment revealed enlarged and congested liver in rats treated with only paracetamol. Whereas, kidneys of rats treated with paracetamol only have shown mild to moderate enlargement and congestion. Appreciable lesions in other organs were not observed.

Microscopic examination of liver of rats of control and vehicle treated rats showed the normal architecture with hexagonal hepatic acini (Figure 3). Liver of rat treated with paracetamol only revealed centri-lobular necrosis, vacuolar degeneration, congestion and disturbed the architecture of hepatic lobules (Figure 4). However, histopathological changes in silymarin along with paracetamol (C4) treated rats shown central vein congestion and vacuolar degeneration along with the regeneration of hepatic cells (Figure 5). Histopathological changes in liver of rats treated with 100 mg/kg PHEM along with paracetamol (T1) rats showed congestion along with necrosis of hepatic cells and vacuolar degeneration of hepatic cells (Figure 6). Histopathological changes in rats treated with 200 mg/kg PHEM along with paracetamol (T2) revealed vein congestion along with mild hepatic necrosis and mild regenerative hepatic cells (Figure 7) and rats treated 300 mg/kg PHEM along with paracetamol (T3) show mild hepatic necrosis along with mild central vein congestion and marked regeneration of hepatic cells (Figure 8).

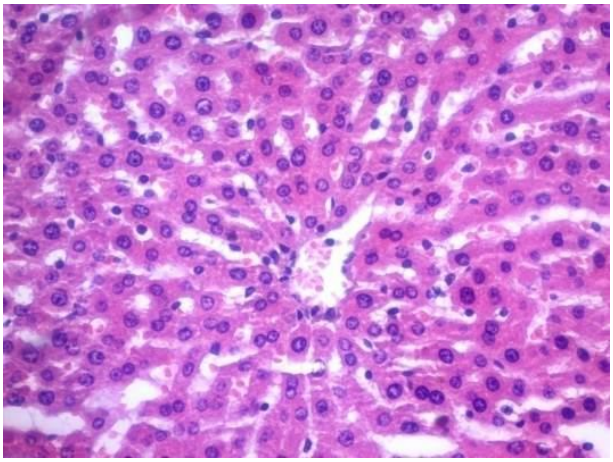

Figure 3: Section of liver from group C1 showing normal architecture with hexagonal hepatic acini.

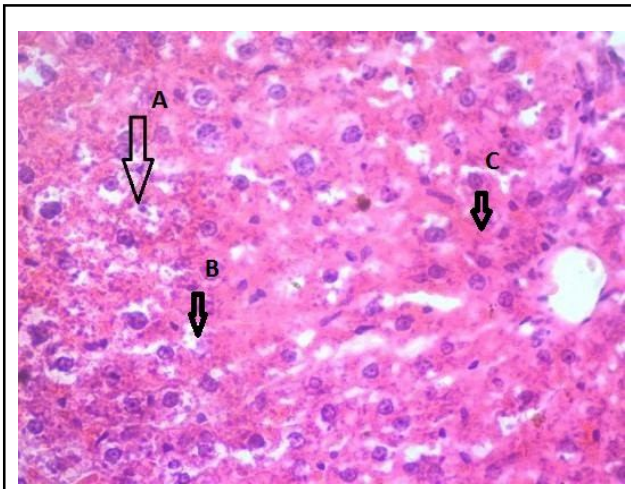

Figure 4: Section of liver from group C3 showing disturbed the architecture of hepatic lobules with (A) centri-lobular necrosis, (B) vacuolar degeneration and (C) congestion.

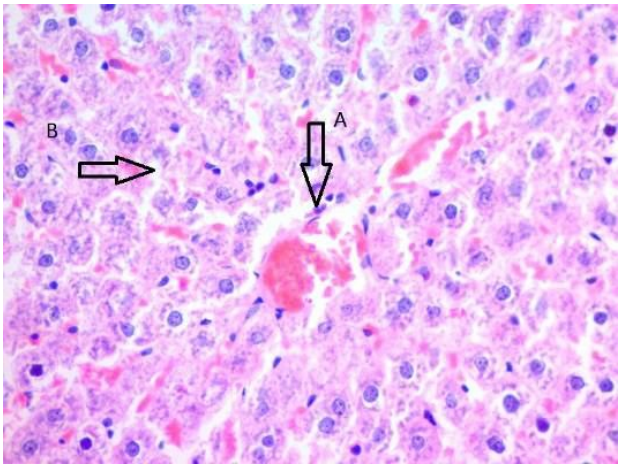

Figure 5: Section of liver from group C4 showing (A) central vein congestion and (B) vacuolar degeneration of hepatic cells.

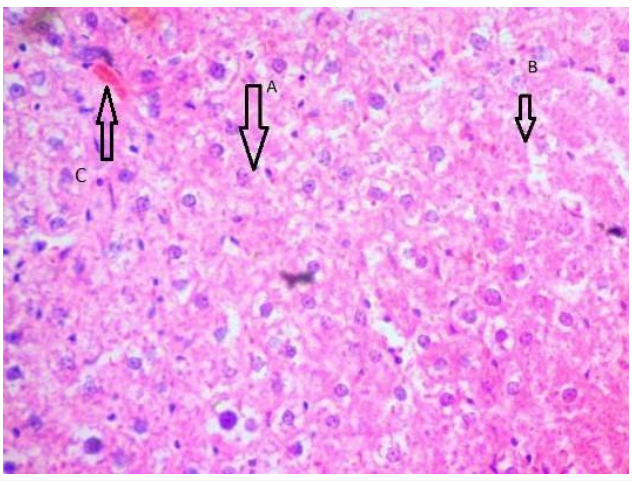

Figure 6: Section of liver from treatment group T1 showing (A) necrosis of hepatic cells, (B) vacuolar degeneration of some of the hepatic cells and (C) congestion.

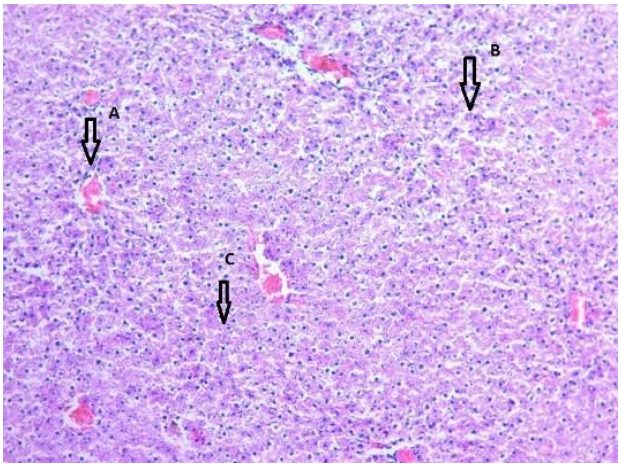

Figure 7: Section of liver from group T2 showing (A) vein congestion, (B) mild hepatic necrosis and (C) mild regenerative hepatic cells at end of experiment.

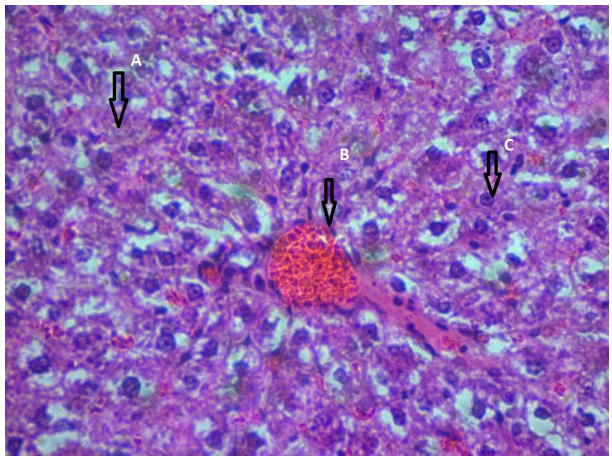

Figure 8: Section of liver from treatment group T3 showing (A) mild hepatic necrosis, (B) central vein congestion and (C) regeneration of hepatic cells.

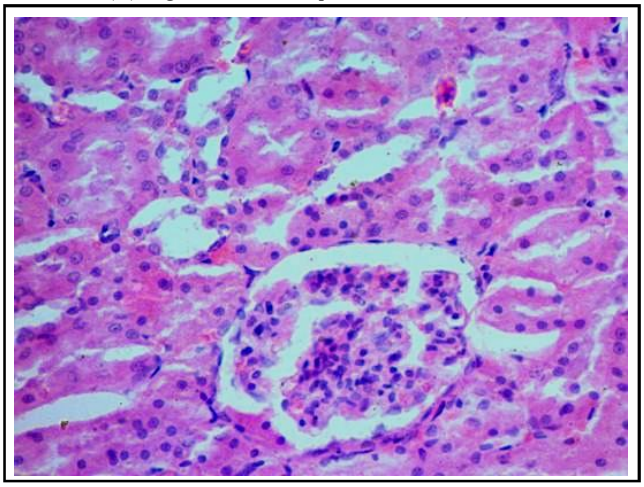

Figure 9: Section of kidney from treatment group C1 showing normal architecture of glomeruli and tubules.

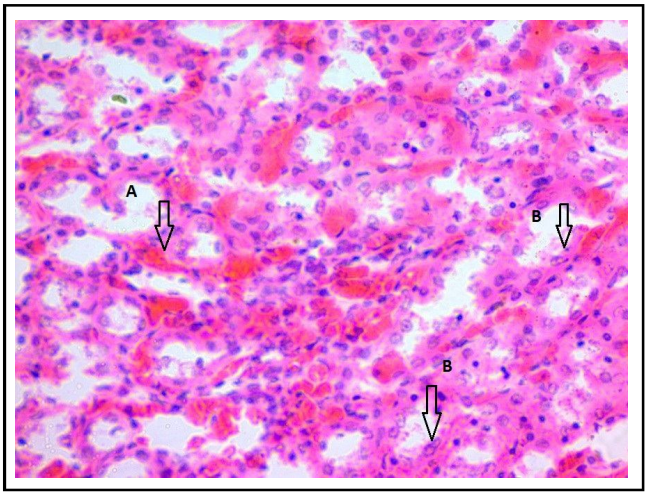

Figure 10: Section of kidney from treatment group C3 showing (A) congestion and (B) tubular degeneration.

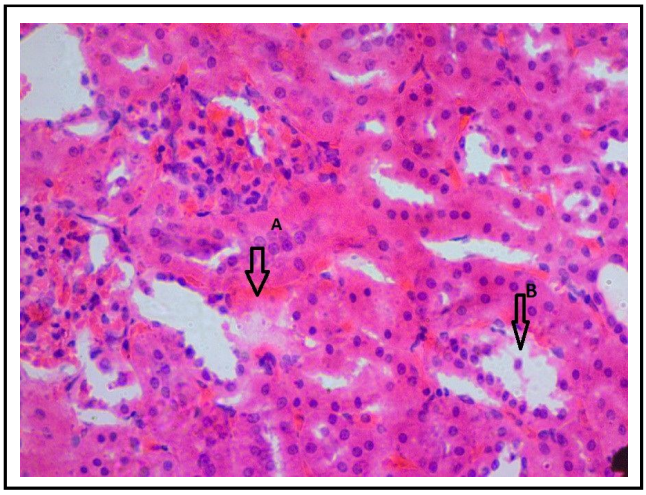

Figure 11: Section of kidney from treatment group C4 showing (A) mild congestion and (B) tubular degeneration.

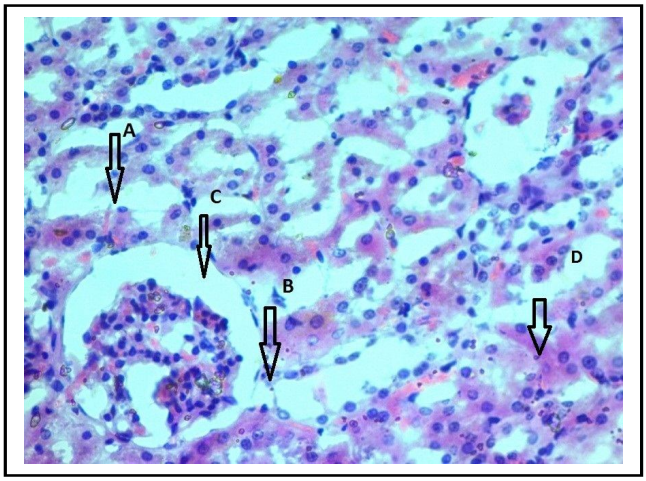

Figure 12: Section of kidney from treatment group T1 showing (A) tubular necrosis, (B) loss of parietal epithelium of Bowman's capsule, (C) increased Bowman's space with glumerular atrophy and (D) congestion.

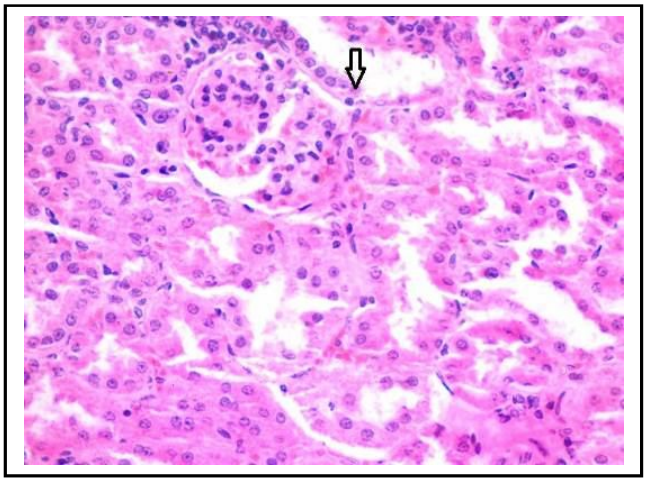

Figure 13: Section of kidney from treatment group T2 showing proximal tubular degeneration with necrosis compared to group T1.

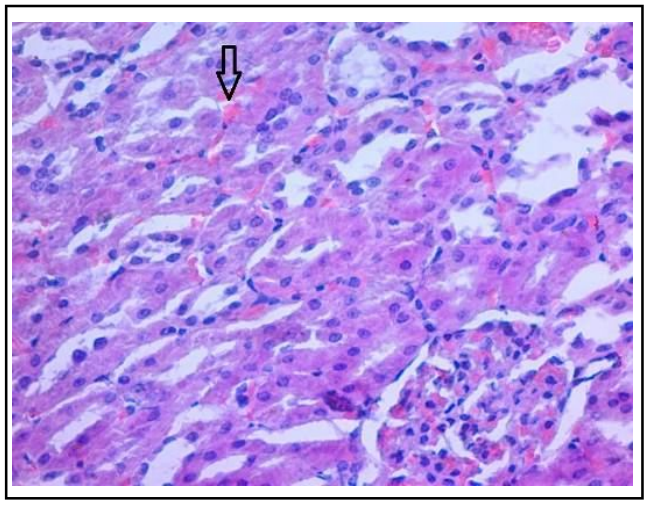

Figure 14: Section of kidney from treatment group T3 showing almost normal architecture of tubules along with mild congestion.

Upon microscopic examination, no pathological changes were observed in kidneys of rats of control (C1) and vehicle control group (Figure 9). The histopathological changes of kidney of rats of paracetamol control group (C3) revealed congestion and tubular degeneration (Figure 10). However, histopathological changes in silymarin along with paracetamol (C4) treated rats showed mild congestion and tubular degeneration (Figure 11). While histopathological changes in kidney of rats treated with 100 mg/kg PHEM along with paracetamol (T1) showed congestion along with tubular necrosis and loss of parietal epithelium of Bowman's capsule with increased Bowman's space with glumerular atrophy (Figure 12). Histopathological changes in rats treated with 200 mg/kg PHEM along with paracetamol (T2) revealed mild proximal tubular degeneration and necrosis (Figure 13) and rats treated 300 mg/kg PHEM along with paracetamol (T3) show almost normal architecture of tubules with mild congestion (Figure 14).

Abel *et al.* (2015) observed hepatic damage after paracetamol treatment (3 g/kg, PO) to rats characterized by inflammatory lesions in hepatic tissues, including the presence of moderate infiltration of neutrophils. No remarkable pathological lesions were observed in rats treated with silymarin (200 mg/kg) along with paracetamol. Sivakumar *et al.* (2014) reported the hepatoprotective effect of polyherbal formulation containing ethanol extract of *Boerhavia diffusa*. They reported that paracetamol treated rats showed centrilobular necrosis of hepatic cells to central lobular fatty degeneration with inflammation while polyherbal formulation (200 mg/kg, p.o.) attenuated the pathological changes and showed significant protection against paracetamol induced hepatic damage. Abdullah *et al.* (2017) reported effect of long term administration of paracetamol in mice as lymphocyte infiltration, congestion, glomerulus and tubular damage in kidney. Padmalochana and Rajan (2015) reported that treatment with ethanol extract of *A. paniculata* leaves (300 mg/kg, p.o. for 10 days) significantly prevented gentamicin (80 mg/kg, p.o. for 10 days) induced swelling and massive and diffuse cell necrosis in proximal tubules of kidneys. Sawardekar and Patel (2014) also reported reversal of gentamicin induced prominent tubular epithelial necrosis and desquamation of half of cortical tubules in kidney by *Boerhavia diffusa* at 400 mg/kg. These

reports support our observations in the present study. No appreciable histopathological lesions have been observed in the stomach, intestine, spleen, heart and lungs of rats treated with paracetamol and all other groups. Further, PHEM was found to be non-toxic at the dose of 2000 mg/kg as did not cause any mortality or symptoms of toxicity during acute toxicity testing. In acute toxicity testing of PHEM, haematological and biochemical parameters were not altered as compared to those before administration of PHEM (Data not shown).

4. Conclusion

In conclusion, PHEM prepared from leaves of *Andrographis paniculata*, *Gymnosporia montana*, *Boerhavia diffusa*, fruit of *Luffa echinata*, stem of *Capparis deciduas* and peels of *Allium cepa* and when given at dose rate of 200 mg/kg for 21 days has shown amelioration of damage caused to liver and kidney by paracetamol in rats. The amelioration effect of PHEM might be due to antioxidant properties of plant extracts used in the study. However, evaluation of efficacy of PHEM against high dose of paracetamol or following administration for longer duration in rats is required.

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Conflict of interest

We declare that we have no conflict of interest.

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