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Protective effect of *Pistacia integerrima* galls in nimesulide induced acute hepatorenal toxicity

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Abstract

Herbal medicines have a long history of use in the management of different diseases owing to economy and relatively with few or no adverse effects. Due to the presence of antioxidant potential, *Pistacia integerrima* has been used for various health problems. The objective of the current study was to assess the protective role of the methanolic extract of *Pistacia integerrima* galls at different doses, in the acute toxicity of nimesulide on hepatorenal function. A filtered and dried methanolic (99.8%) extract of galls of *Pistacia Integerrima* was obtained. Rabbits were used as animal model for this study. Vitamin-C and silymarin were used as standard drugs. The result showed that nimesulide at 15mg/kg/day dose elevated the ALT level of liver within seven days of administration as compared to normal group ($p<0.05$). Among all the groups, administered with methanolic extract of *Pistacia integerrima* galls at different doses (100, 300 and 500mg/kg/day respectively), the group at a dose of 300mg/kg/day, have shown some protective effect in the hepatotoxicity as compared to silymarin (control group) $p<0.05$. The renal function is affected insignificantly in the acute toxicity induced by nimesulide at 15mg/kg/day as compared to normal and other groups ($p>0.05$). A moderate increase was observed in the level of TLC in nimesulide-only group as compared to normal. It shows that *Pistacia Integerrima* galls possess potential of reversing the toxic effect, induced by nimesulide with respect to hepatic and renal functions.

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Introduction

Human beings have always been keenly interested in searching the nature since long to discover new drugs especially from plants, to cure diseases (Savithramma *et al.*, 2011). Different parts of plants like bark, leaves, flowers, seeds, etc have various medicinal constituents (Cragg and Newman, 2001). The leaf galls of *Pistacia integerrima* (PI) are used for majority of health conditions like cough, asthma, fever, respiratory problems and liver disorders (Janakat and Al-Merie, 2002). PI galls have antioxidant property due to the presence of phenolic and flavonoids compounds, which have multiple biological effects (Cragg and Newman, 2001, Savithramma *et al.*, 2011).

Liver is an important organ for drug metabolism and its removal from the body (Mahmood *et al.*, 2009). Homeostasis is achieved by the detoxification of drugs and other chemical products in the liver (Wallace and Granger, 1992). Drug induced liver toxicity has caused the withdrawal and rejection of drugs for approval by Food and Drug Administration (FDA) in the US (Cantoni *et al.*, 2003). Numerous chemical compounds have been noted to induce liver injury (Bort *et al.*, 1999). And among them acute liver failure cases are 50% of all for which 5% patients then need hospitalization. More than 75% idiosyncratic reactions are drug induced which caused death and liver transplantation (Masubuchi *et al.*, 2000). About 10% mortality and acute liver diseases have been reported because of drug-induced liver injury (Bort *et al.*, 1999). It has been observed that recipients of renal transplantation with chronic liver disease are more susceptible to drug induced liver intoxication (SINGLA *et al.*, 2000). Drugs like acetaminophen, isoniazid, methyldopa, allopurinol, aspirin, quinidine, sulfonamides, valproate, amiodarone, methotrexate, niacin, rifampicin, diclofenac, halothane-related anesthetics, indomethacin, phenytoin, propylthiouracil, hydrocarbons, methyltestosterone, oral contraceptives etc. shown drug induced hepatotoxicity (Famaey, 1997).

Kidneys remove extra water and waste products from the blood to form urine and thus maintain minerals, salts and water balance (Merlani *et al.*, 2001). Many drug entities caused drug induced systemic intoxication which also includes renal dysfunction. 17- 26% of hospitalization due to acute kidney injury is caused by nephrotoxic agents (Nair *et al.*, 2006, Patel *et al.*, 2017). Some classes like nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, antimicrobial agents, antiviral agents, antifungal agents, Chemotherapeutic agents etc. are having the drugs which causes renal impairment (Van Steenbergen *et al.*, 1998).

It is observed that there is a great controversy about NSAIDs of having both oxidant and antioxidant properties (Mahmood *et al.*, 2009). There is evidence that NSAIDs causes gastro-intestinal damage, nephrotoxicity and hepatotoxicity which involves oxidative stress (Wallace and Granger, 1992, Cantoni *et al.*, 2003). It is investigated that NSAIDs have diphenylamine in its structure which is held responsible for decrease in the content of liver ATP culminating in hepatocyte injury (Bort *et al.*, 1999). Nimesulide is considered a selective cyclooxygenase-2 (COX-2) inhibitor with analgesic, antipyretic and anti-inflammatory properties (Merlani *et al.*, 2001). Some studies showed increase levels of lipid peroxides with nimesulide use (Nair *et al.*, 2006). Hepatobiliary injury, hepatitis, fulminant hepatic failure and end stage renal failure are reported with nimesulide. Various reports of hepatotoxicity (Van Steenbergen *et al.*, 1998) and even fatal hepatic failure is the main cause of the withdrawal of drug in many countries (Weiss *et al.*, 1999, Walker *et al.*, 2008). NSAID produces oxidative stress by the formation of reactive metabolites, which modify proteins covalently and causes the injury of mitochondria (Boelsterli, 2002b). NSAIDs are showing unpredictable and idiosyncratic mechanism of hepatotoxicity. The hepatotoxicity induced by nimesulide may be due to the reactive metabolites formed, as observed in some in-vitro and In-vivo studies (Boelsterli, 2002a).

The toxicity at cellular level induced by nimesulide is not clear yet. Apoptosis and disturbing of mitochondrial physiology have been reported due to intercellular reactions. Nimesulide contains nitro aromatic group, which is reduced through bio-activation at the molecular level and causes oxidative stress with binding of metabolite covalently to cellular proteins (Boelsterli, 2002b).

The galls of the plant have been investigated for the presence of chemical constituents containing tannins, reducing sugars, sterols, alkaloids, and flavonoids. Analgesic, antioxidant and immunomodulatory activities of galls have also been reported (Ahmad *et al.*, 2010).

Keeping in view the importance of PI galls in the traditional medicine in liver disorders the recent study was carried out to investigate the protective effect of the methanolic extract of the galls of the PI in the hepatorenal intoxication at toxic dose (15mg/kg/day) of Nimesulide.

Material and methods

The experimental study was held at animal house Department of Pharmacy, University of Malakand, Khyber Pakhtunkhwa Pakistan, from May to October 2015 and comprised rabbits weighing 1-1.5kg of either sex.

Galls collection and extraction

Methanolic extract of shade dried galls of *P. Integerrima* was extracted by soaking in methanol (99.8%) for 15 days which was subsequently filtered through filter paper, followed by concentration in rotary evaporator.

Standard drugs

For renal protection ascorbic acid (vit-C) was used as a standard, while for hepato-protection silymarin was used.

Animal models

Seven groups of rabbits were used, each group was having six rabbits. All the the groups were fed with water & normal diet for acclimatization.

Table 1 indicates that Group N2 was administered with nimesulide only. G1, G2, and G3 groups were given methanolic extract of PI galls at doses of 100, 300 and 500mg/kg/day respectively, with concomitant administration of nimesulide. Group C2 (Vitamine-C at 40mg/kg/day) (Raafat *et al.*, 2009) and group S2 (silymarin at 50mg/kg/day) (Maryam *et al.*, 2010) were also adminitered along with minseulide. Seventh group (NS) was a normal and received only normal saline as 5ml/kg/day orally.

Table 1. Groups and Drug administraion.

Group	Dose of nimesulide	Dose of Methanolic extract	Dose of Normal saline	Vitamin-C	Silymarin
NS	-	-	5ml/kg/day	-	-
N2	15mg/kg/day	-	-	-	-
G1	15mg/kg/day	100mg/kg/day	-	-	-
G2	15mg/kg/day	300mg/kg/day	-	-	-
G3	15mg/kg/day	500mg/kg/day	-	-	-
C2	15mg/kg/day	-	-	40mg/kg/day	-
S2	15mg/kg/day	-	-	-	50mg/kg/day

Blood Sampling

Blood was collected from ear marginal vein of each group, on day 1st, 4th and 7th. Blood of 4ml was drawn from marginal ear vein of the rabbit with the help of a fine 22G needle and was transferred immediately to EDTA tubes. Xylene was applied on target surface to dilate the vein. The serum was separated from the blood through centrifuge machine and was stored at -20°C till analysed.

Blood parameters studied

Alanine transaminase (ALT), serum bilirubin (S.Br), blood urea nitrogen (BUN), Serum creatinin (S.Cr), hemoglobin (Hb), and total leukocyte count (TLC) were calculated. All parameters were recorded by auto-analyser.

Chemicals & Accesorries

Normal saline 1000ml intravenous (IV) solution, lignocain injection vials, were used. Automated clinical chemical analyser, disposable syringes 5cc

with 23G needle, cotton bags, butterfly needles (22G), scissors, digital balance, EDTA tubes, measuring cylinder 100ml, glass pipette, glass rod were also used.

Dissection of rabitts

All the rabbits were sacrified on 7th day, liver and kidney were excised, washed in phosphate buffer and fixed in formalin. The microscopic study of liver and kidney was carried out to observe the histological changes.

Statistical analysis

Data was analyzed using SPSS. The results were presented as mean ± standard deviation (SD). In all statistical analysis, only $p < 0.05$ was considered significant.

Results

Hepatorenal study in nimesulide toxicity, induced at a dose of 15mg/kg/day

Hepatic study

Effect on ALT

The first day results of liver biomarkers revealed that all the groups have almost the same levels and showing insignificant difference ($p>0.05$) as compare to normal. On day 4 and 7, the levels of ALT (Fig. 1) are the highest ($p<0.05$) in group N2 (on day 4^{th} = 144.83 ± 4.643 & day 7^{th} = 152.55 ± 4.732) in comparison to normal NS (on day 4^{th} = 21.50 ± 0.428, day 7^{th} = 20.82 ± 0.320), showing a remarkable acute hepatotoxicity at a dose of 15mg/kg/day of nimesulide. The G2 group received methanolic extract of PI galls at a dose of 300mg/kg/day showed some hepatoprotective effect, as observed from the level of ALT (on day 4^{th} = 99.17 ± 1.195, on day 7^{th} = 101.18 ± 1.020) in comparison to standard group, S2 (on day 4^{th} =114.22 ± 2.198, on day 7^{th} = 114.83 ± 2.182). Other groups G1 (on day 4^{th} =123.83±1.400, day 7^{th} = 128.13 ± 0.775) and G3 (on day 4^{th} = 117.17 ± 1.833, day 7^{th} = 121.83 ± 2.845) have less hepato-protective effect as compared to standard. The groups G1 and G3 exhibited an increase in the level of ALT as compared to standard.

Table 2. Effect of PI on histopathology of liver.

Observation	Normal (NS)	Nimesulide (N2)	Silymarin (S2)	G1	G2	G3
Fibrosis	-	+++	+	+	-	+
Inflammatory foci	-	+++	++	+	+	+
Sinusoidal congestion	-	+++	-	+	-	+
Ballooning degeneration	-	+++	+++	++	-	-
Lymphocyte infiltration	-	+++	++	+	+	+
Central vein degeneration	-	+++	++	+	-	+

(-) absent, (+) mild, (++) moderate, (+++) severe, (++++) extremely severe

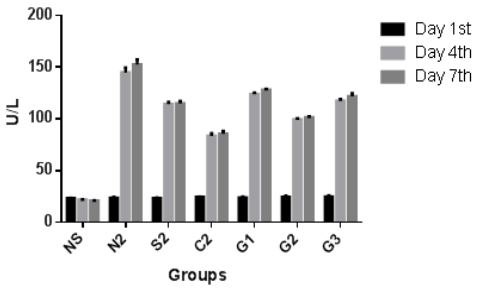

Fig. 1. Effect of nimesulide (N2) at a dose of 15mg/kg/day, silymarin (S2) at a dose of 50mg/kg/day, vitamine- C (C2) at a dose of 40mg/kg/day, normal saline (NS) at a dose of 5ml/kg/day and different doses of Pl galls extracts on ALT.

Effect on bilirubin

The bilirubin levels (Fig. 2) of all the groups on day 4^{th} and 7^{th} are almost the same ($p>0.05$) as compared to normal (on day 4^{th} = 0.55 ± 0.056, day 7^{th} = 0.67 ± 0.071). It can be evaluated that in the acute hepatotoxicity induced by nimesulide there is no significant effect on the level of bilirubin.

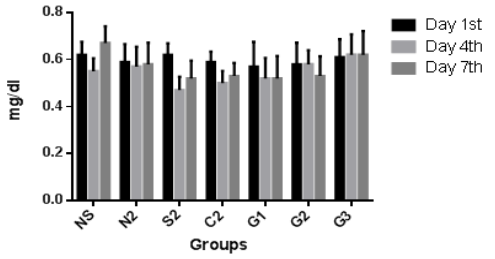

Fig. 2. Effect of nimesulide (N2) at a dose of 15mg/kg/day, silymarin (S2) at a dose of 50mg/kg/day, vitamine- C (C2) at a dose of 40mg/kg/day, normal saline (NS) at a dose of 5ml/kg/day and different doses of Pl galls extracts on S.Br.

Renal Study

Effect on BUN

The level of BUN (Fig. 3) of all the groups shows that there is no observed acute renal toxicity with the toxic dose of nimesulide at 15mg/kg/day within seven days of administration. All the groups have the same range ($p>0.05$) of BUN as those of normal (on day 1^{st} = 12.03±0.470, day 4^{th} = 11.20±0.449, day 7^{th} = 11.37±0.467).

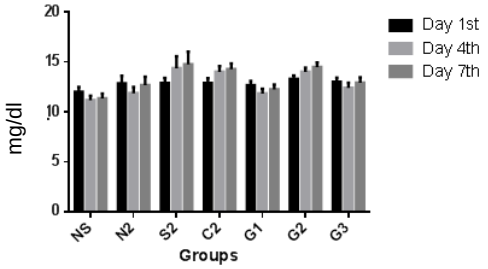

Fig. 3. Effect of nimesulide (N2) at a dose of 15mg/kg/day, silymarin (S2) at a dose of 50mg/kg/day, vitamine- C (C2) at a dose of 40mg/kg/day, normal saline (NS) at a dose of 5ml/kg/day and different doses of Pl galls extracts on BUN.

Effect on serum creatinine (S.Cr)

As shown in Fig. 4, S.Cr in all the groups are in the same range ($p>0.05$) as those of normal (on day 1^{st} = 0.91±0.076, day 4^{th} = 1.08±0.087, day 7^{th} = 1.08±0.060) throughout the study except Vit-C (on day 1^{st} = 0.78±0.048, day 4^{th} = 0.63±0.049, day 7^{th} = 0.78±0.66) which showed low level ($p<0.05$) of S.Cr than normal. It indicates that vitamin C may have some prominent reno-protective effect, regarding S.Cr, than all the other groups (G1, G2, G3).

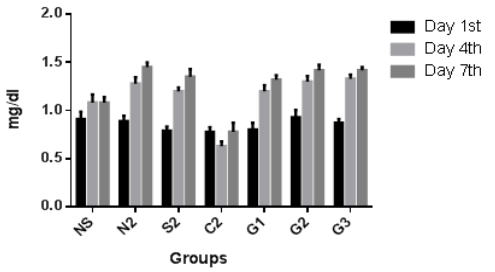

Fig. 4. Effect of nimesulide (N2) at a dose of 15mg/kg/day, silymarin (S2) at a dose of 50mg/kg/day, vitamine- C (C2) at a dose of 40mg/kg/day, normal saline (NS) at a dose of 5ml/kg/day and different doses of Pl galls extracts on S.Cr.

Hematological Study

Effect on Hb

All the groups showed no prominent effect on the level ($p>0.05$) of Hb (Fig. 5) throughout the study and are comparable to those observed in normal (on day 1^{st} = 12.97±0.240, day 4^{th} = 13.33±0.369, day7^{th} = 13.55±0.348).

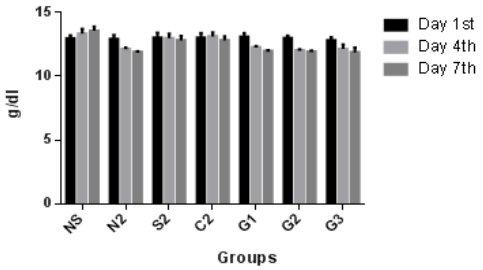

Fig. 5. Effect of nimesulide (N2) at a dose of 15mg/kg/day, silymarin (S2) at a dose of 50mg/kg/day, vitamine- C (C2) at a dose of 40mg/kg/day, normal saline (NS) at a dose of 5ml/kg/day and different doses of Pl galls extracts on Hb.

Effect on TLC

In the case of TLC (Fig. 6), group N2 showed a moderate increase of the TLC levels (on day 4^{th} = 9.27 ± 0.225, day 7^{th} = 9.13 ± 0.216). In comparison with normal (on day 4^{th} = 7.77±0.169, day 7^{th} = 7.72 ± 0.160), N2 group showed some effect on the total leukocyte count ($p<0.05$) in nimesulide toxicity at a dose of 15mg/kg/day. G1, G2 and G3 showed a very minute increase in the level of TLC than standard and normal which is almost insignificant increase ($p>0.05$) in the level of TLC.

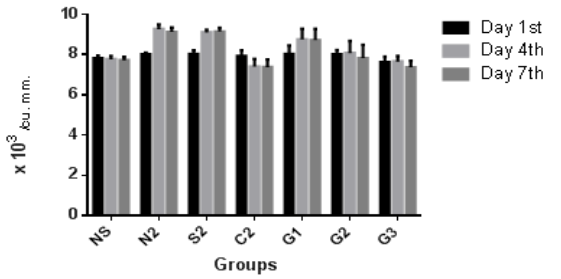

Fig. 6. Effect of nimesulide (N2) at a dose of 15mg/kg/day, silymarin (S2) at a dose of 50mg/kg/day, vitamine- C (C2) at a dose of 40mg/kg/day, normal saline (NS) at a dose of 5ml/kg/day and different doses of Pl galls extracts on TLC.

Histopathology of liver

The histopathological study of liver (Table 1) showed moderate fibrosis, inflammatory foci, sinusoidal degeneration, ballooning degeneration, lymphocyte infiltration and central vein degeneration in N2 group as compare to normal. A better hepatoprotective histology has been shown by G2 group with mild inflammatory foci and lymphocyte infiltration, while no fibrosis, sinusoidal congestion, ballooning degeneration and central vein degeneration as compared to standard (S2). G1 and G3 groups showed not much better result than G2 and have mild fibrosis, inflammation, sinusoidal congestion, ballooning degeneration, lymphocyte infiltration and central vein degeneration. S2 group showed worsen histopathology than normal and other groups (G1, G2 and G3) with severe ballooning degeneration, mild fibrosis and moderate inflammation and central vein degeneration. It is proved through histopathology of liver that G2 group (received methanolic extract of PI galls at a dose of 300mg/kg/day) has some better protective role in nimesulide (15mg/kg/day) toxicity as compared to other groups (S2, G1 and G3). The result of histopathology has clearly supported the biochemical investigations regarding the better hepatoprotective role of methanolic extract of PI galls at 300mg/kg/day).

Histopathology of kidney

Histological observations of kidneys revealed no marked damage induced by nimesulide. All other groups showed no profound effect on the histology of kidney while Vit-C and G2 group (300mg/kg/day of methanolic extract of PI galls) showed much better and comparable histology to normal group.

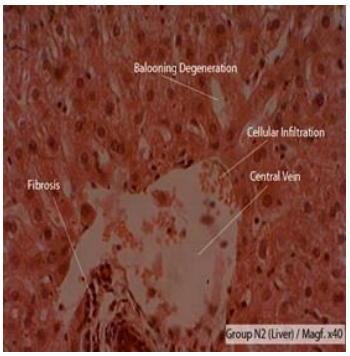

Fig. 7. N2 group (administered with nimesulide only at 15mg/kg/day).

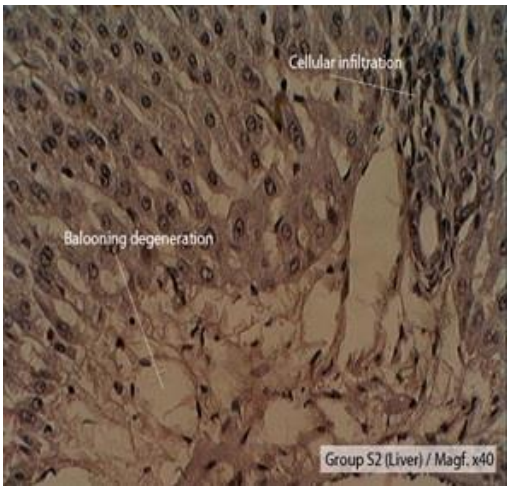

Fig. 8. S2 group (administered with silymarin at 50mg/kg/day and nimesulide at 15mg/kg/day).

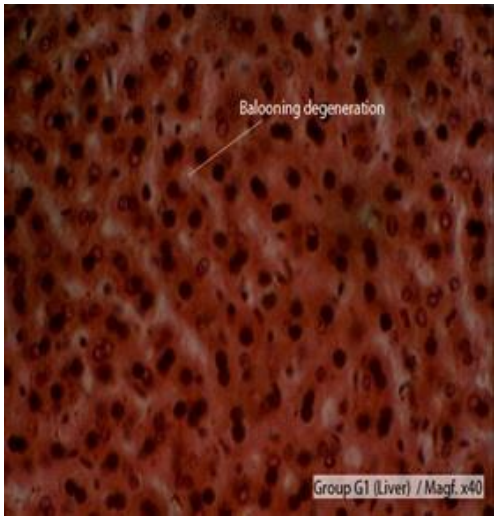

Fig. 9. G1 group (administered with Pl galls extract at 100mg/kg/day and nimesulide at 15mg/kg/day).

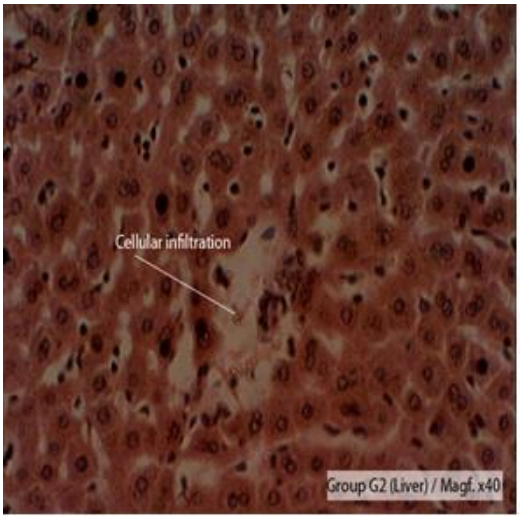

Fig. 10. G2 group (administered with Pl galls extract at 300mg/kg/day and nimesulide at 15mg/kg/day).

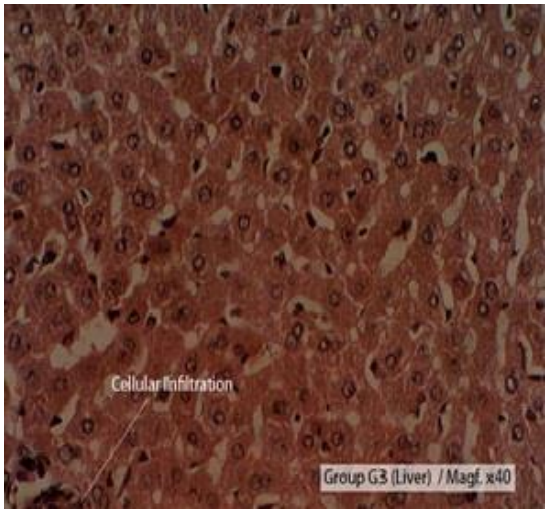

Fig. 11. G3 group (administered with Pl galls extract at 500mg/kg/day and nimesulide at 15mg/kg/day).

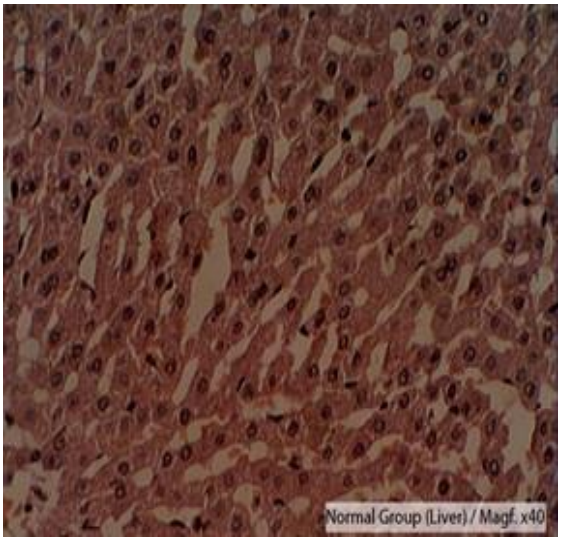

Fig. 12. NS group (administered with normal saline at 5ml/kg/day).

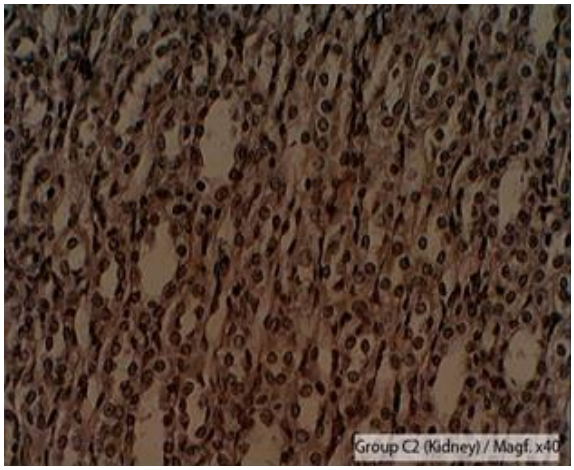

Fig. 13. C2 group (administered with vitamin-C at 40mg/kg/day and nimesulide at 15mg/kg/day).

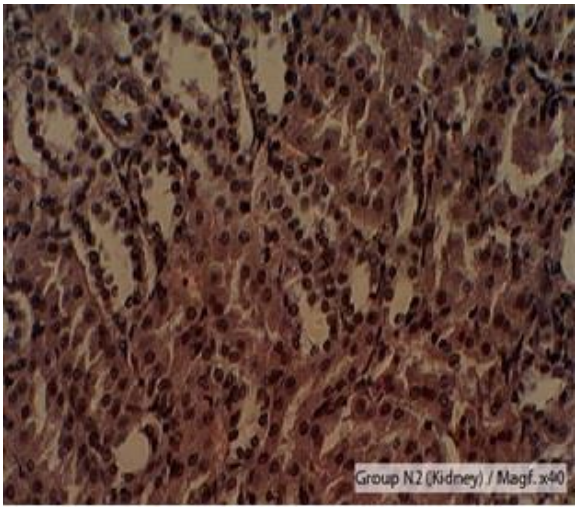

Fig. 14. N2 group (administered with nimesulide on at 15mg/kg/day).

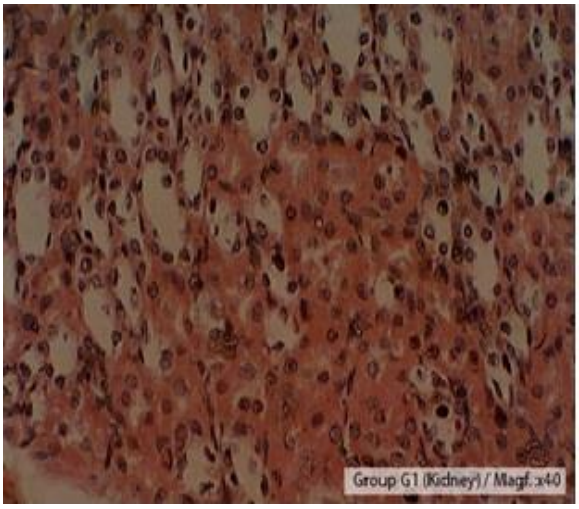

Fig. 15. G1 group (administered with Pl galls extract at 100mg/kg/day and nimesulide at 15mg/kg/day).

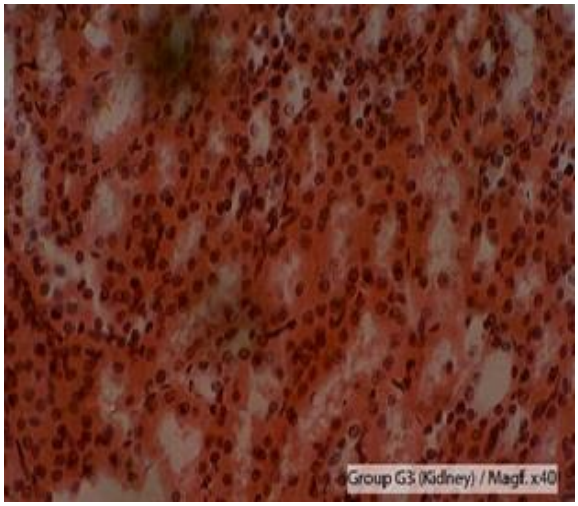

Fig. 16. G3 group (administered with Pl galls extract at 500mg/kg/day and nimesulide at 15mg/kg/day).

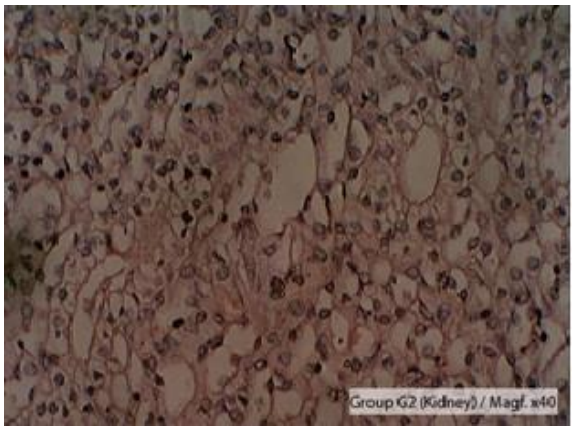

Fig. 17. G2 group (administered with Pl galls extract at 300mg/kg/day and nimesulide at 15mg/kg/day).

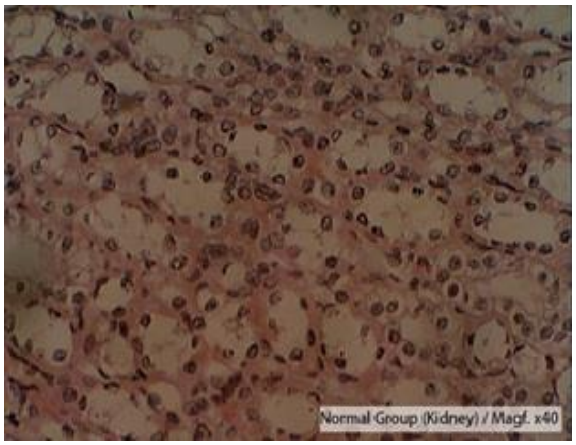

Fig. 18. NS group (administered with normal saline at 5ml/kg/day).

Discussion

The liver plays an important role in regulatig the homeostasis of the body. Many drugs like anti-tubercular, and some NSAIDs have been reported to cause toxic effects on liver. Consequnetly, those subsatnces which possessing sever toxicities were withdrawn or disapproved. These hepatotoxic drugs have been investigated and found as strong agents of oxidative stress and mitochondrial damage (Xu *et al.*, 2008). Nimesulide, like other NSAIDs, unpredictably causes severe toxic effects. Nimesulide exert toxicity in the form of increased bilirubin level, when used at 100mg twice daily dose, followed by liver damage and sever haemolytic anemia with in three weeks of use (Rodrigo *et al.*, 2002). Mitochondria are the power houses of the cell providing energy. Inside mitochondria, numerous biochemical rections occur. The nitro group present in nimesulide and the resultant metabolite produced after the reduction, possess deleterious effects on mitochondria. Moreover, due the presence of nitro group, nimesulide act as oxidant on liver cell mitochondria (Tan *et al.*, 2007). Fuliminant hepatic failure has been documented after nimesulide use. About 10% of all drug induced hepatotoxicities are associated with the use of NSAIDs. However, other common adverse effects of nimesulide are hypoalbuminaemia, coagulopathy, inflammatory infiltrate, hepatocytes necrosis and renal failure (Agúndez *et al.*, 2011).

Keeping in view the toxic effects of nimesulide, various natural sources have been utilized to manage the adverse effects. Nimesulide induced mitochondrial dependent apoptosis are modulated by an extratct of *Fumaria parviflora* (Tripathi *et al.*, 2010). An antioxidant activity has been shown by Phyllanthus niruri, which has hepatoprotective role against liver toxicity induced by nimesulide (Chatterjee and Sil, 2007). Hepatotoxicity induced by nimesulide can also be prevented by natural terpenes like camphene and gerniol (Singh *et al.*, 2012). The glycosidal extract of *Picrorhiza kurroa* has nephroprotective activity against the nimesulide induced renal toxicity (Siddiqi *et al.*, 2015). Polysaccharide was observed and has shown liver protection by the reduction of oxidative stress induced by nimesulide in hepatotoxicity (Nguyen *et al.*, 2015). The leaf extract obtained from the

Cichorium intybus also has shown hepatoprotective activity against nimesulide induced liver toxicity.

In this study the liver biomarkers showed hepatotoxicity observed from the level of ALT. The group received nimesulide only has increased level of ALT more than all the groups, which showed severe hepatotoxicity than normal and standard. The protective effect has been shown by group G2 (methanolic extract at 300mg/kg/day) as compared to standard. The other groups G1 (methanolic extract at 100mg/kg/day) and G3 (methanolic extract at 500mg/kg/day) showed no protective effect as compared to standard. Group G2 produces an optimal control on the elvation of ALT. Other groups G1 & G3 has no control on the elevated ALT as compared to G2. It may be stated that methanolic extract at 100mg/kg/day is lower dose for the protrctive effect of liver while G3 may have shown some extra toxic effect on liver, along with nimesulide. The hepatoxicity can also be confirmed from the histopathology of the liver of the respective groups. Better histopathology has been shown by group G2 as compared to N2, G1, G3 and standard (S2). There is no remakable effect on the bilirubin level of all the groups.

There is no prominent effect on the renal biomarkers. All the groups have almost the same level of BUN and S.Cr except as that of normal, while group C2 (Vitamin-C at 40mg/kg/day) has decreased the level of S.Cr. The effect on TLC observed in all the groups remain the same as that of normal. Only N2 group has shown increased level of TLC as compared to all other groups. From the level of renal biomarker it is clear that the toxic dose of numesulide has no signaficant effect on renal function with in seven days. P. Integerrima have antioxidant activity for free radicals as it contains phenolics and flavonides. The demaged liver by drugs can also be treated with P. Integerrima (Joshi, 2010).

Conclusion

PI galls have shown protective effect on liver. However, it showed insignificant effect on the kidney's function, because the nimesulide has shown no prominent renal toxicity at 15mg/kg/day.

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