



Efficacy of parsley (*Petroselinum crispum*) leaves extract and decoction on status of lipid profile and osmotic fragility in gentamicin-induced nephrotoxicity in rats

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ABSTRACT

The clinical usefulness of gentamicin is limited due to the development of nephrotoxicity. The abnormalities in lipid and lipoprotein pattern produce number of pathological diseases including nephrotoxicity. Reactive oxygen species produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation, and DNA damage. Potential therapeutic approaches to protect (or) reverse GM damage would be having very important clinical consequences in increasing the safety of the drug. Several natural agents have been used to ameliorate drugs toxicity. The survey of literature reveals that the parsley (*Petroselinum crispum*) are found to be used in the traditional system of medicine. In addition, the aqueous extract of parsley reduced the number of calcium oxalate deposits and therefore parsley can be used for kidney and bladder stones. However, the nephroprotective and antidyslipidemic effect of *P. crispum* has not been scientifically investigated. So, the present study was design to evaluate the efficacy of parsley (*P. crispum*) extract and decoction on the kidney of gentamicin-induced nephrotoxicity in rats. Freshly prepared ethanolic extract and decoction of *P. crispum* (PCE and PCD) were orally administered to rats. The altered renal markers (Urea, uric acid, creatinine and BUN) after GM administered were normalized in extracts treated animals. The modified levels of lipid profiles (total cholesterol, triglycerides, phospholipids, HDL, LDL, and VLDL) in GM administered rats were normalized in PCE and PCD treated animals. The membrane stabilizing effects were confirmed by erythrocytes osmotic fragility and to RBC morphology. In conclusion, this study revealed that PCE and PCD administered at a dose of 250mg/kg.bwt and 250mg/kg.bwt were effective respectively. Further, the results of the present study indicate that *P. crispum* may emerge as a putative nephroprotective, antihyperlipidemic and membrane-stabilizing agent against nephrotoxicity. Further studies need to be undertaken in order to confirm these findings and its extrapolation in humans.

Keywords: *Petroselinum crispum*, gentamicin, nephrotoxicity, RBC and antihyperlipidemic, osmotic fragility, membrane-stabilizing agent.

INTRODUCTION

Gentamicin (GM; aminoglycoside; Figure 1) is one of the antibiotics used for the treatment of infections caused by Gram-negative aerobes [1]. However, the dose-limiting factors in the use of GM due to serious complications like nephrotoxicity and ototoxicity. It induces nephrotoxicity in 10-25% of therapeutic courses, which is the main limiting factor for its clinical use [2, 3]. GM-induced nephrotoxicity is characterized by morphological alterations including destruction, necrosis and apoptosis of kidney cells, which eventually lead to acute kidney injury (AKI) and

dysfunction [4]. The renal toxicity is due to its selective accumulation in the renal proximal convoluted tubules and its long-term stay subsequently leading to loss of brush border integrity [5]. The abnormalities in lipid and lipoprotein pattern produce number of pathological diseases including nephrotoxicity. To date, there is no specific agent used to protect against GM-induced nephrotoxicity. In this regard, various medications have been used concomitantly with GM to prevent AKI in laboratory animal models [6]. Medicinal plants and herbs play an important role in the prevention and treatment of kidney diseases. Parsley (*Petroselinum crispum*, Family: Umbelliferae/Apiaceae) is used as a culinary, garnishing and medicinal herb in the Mediterranean region of Southern Europe. Parsley extract was reported to produce a diuretic effect and good antioxidant activity [7]. Parsley leaves are rich in apigenin and its glucosidal flavonoids that were found to possess anti-inflammatory especially for renal inflammation; antioxidant and anticancer activities [8]. In addition, the aqueous extract of parsley reduced the number of calcium oxalate deposits and therefore parsley can be used for kidney and bladder stones [9].

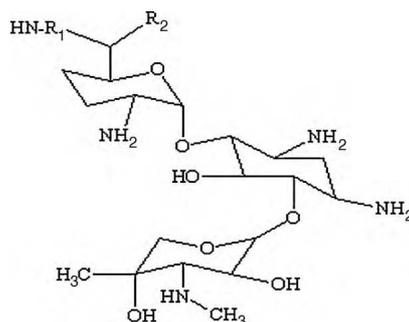


Figure 1. Structure of Gentamicin

P. crispum increases diuresis by inhibiting the Na⁺/K⁺-ATPase pump in the kidney, thereby enhancing sodium and water excretion while increasing potassium resorption [10]. Phytochemically, the leaves and seeds of *P. crispum* has been shown to contain xanthotoxin, ficusin, bergapten, majudin, heraclin and antimicrobial furocoumarins namely 8-methoxypsoralen, 5-methoxypsoralen, oxypuecedanin, isopimpinellin, 6'-acetylopin and a new monoterpene glycoside [11]. Furthermore, the plant is a good source of iron, calcium, phosphorous and antioxidants like luteolin, vitamin C, vitamin A and zinc and these may likely account for its hepatoprotective effect [12]. However, the nephroprotective and antidyslipidemic effect of *P. crispum* has not been scientifically investigated. GM administration in rats could be considered one of the best experimental models to study the AKI, due its availability, low cost and reproducibility of the renal lesions. So, the present study was design to evaluate the efficacy of parsley (*P. crispum*) extract and decoction on the kidney of gentamicin-induced nephrotoxicity in rats.

EXPERIMENTAL SECTION

Preparation of Ethanolic P. crispum extracts [PCE]

The separated leave of *P. crispum* (figure 2) was washed with tap water to remove the dust and other foreign materials. The washed leaves were dried under shade for one week. Approximately about 500 g of air-dried whole leaves were pulverized into powdered form by using heavy duty commercial blender. The powder samples (50 g) were extracted with 95% ethanol (1:3 w/v) by using Soxhlet extractor at 37°C for two days. Thereafter, the extract was filtered and evaporated (Rotavator, Buchi, Switzerland) to dryness under vacuum at 40°C with a rotary evaporator. After the yield was determined, the extract was stored at 4°C until use [13].

Preparation of P. crispum leaves Decoction [PCD]

Plant materials (100 g), cut into small pieces, were boiled in water (1000 mL) by using an apparatus to re-cool for 1 h and were filtered. This mixture is called a decoction. The decoction was then cooled and stored at 4C until use [14].



Figure 2. *Petroselinum crispum*

Chemicals

The antibiotics, GENTAM[®] were purchased from SPIMACO, Al-Qassim, Kingdom of Saudi Arabia and other chemicals and solvents used were of analar grade.

Animals

Healthy, male albino Wister rats (*Rattus norvegicus albinus*) each weighing 150-200 g were used for this study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60 % humidity). Standard pelletized feed (Grain Silos & Flour Mills Organization, Riyadh, KSA) and tap water were provided *ad libitum*. The experimental design was approved by the Institutional animal care and use committee, College of Medicine, University of Hail (EC Ref No.EC#006; date. 22/10/2015).

Induction of kidney damage

Kidney damage was induced in rats by administrating Gentamicin (GM) intraperitoneally at the dose of 100mg/kg body weight for 6 consecutive days [15].

Experimental Design

The animals were grouped as follows and each group contains 6 rats. **Group I:** Normal animals received standard feed and water *ad libitum*. No other treatment. **Group II:** GM-induced group received gentamicin (100mg/kg body weight, ip) for 6 consecutive days along with standard feed and water *ad libitum*. **Group III:** Treatment group received GM as group II for 6 days followed by the treatment with PCE orally (250 mg / kg b.wt per day for 10 days). **Group IV:** Treatment group received GM as group II for 6 days followed by the treatment with PCD orally (250 mg / kg b.wt per day for 10 days). **Group V:** Drug alone treated group received PCE orally (250 mg / kg b.wt per day for 10 days).

The body weight of the animals was recorded throughout the experimental period starting from Day 0. After the experimental regimen, the rats were fasted overnight and were sacrificed by cervical dislocation under light ether anesthesia, and the blood was collected on decapitation.

Blood samples

Preparation of serum and plasma

Serum was separated by centrifugation (20 min at 2000 rpm) and stored at -20°C for biochemical assays. Plasma was separated from heparinized blood by centrifugation at 1000g for 15 min.

Preparation of Hemolysate

The erythrocytes remaining after the removal of plasma were washed three times with 310mM isotonic Tris-HCl buffer (pH 7.4). Hemolysis was carried out by pipetting out the washed erythrocyte suspension into polypropylene

centrifuge tubes which contained 20mM hypotonic Tris-HCl buffer (pH 7.2). The haemolysate was separated by centrifugation at 3500g for 15min at 20°C.

Isolation of Erythrocyte Membrane

The erythrocyte membrane was prepared by the method of Dodge *et al* [16] modified by Quist [17].

Preparation of kidney tissue homogenate

Tissue samples from animals were washed with ice cold saline and dried between folds of filter paper, weighed and homogenized using appropriate buffer in an all glass homogenizer with teflon pestle. The homogenate was centrifuged at 1000g for 5 minutes and the supernatant was then used for the biochemical estimations.

Biochemical estimations

Biochemical estimations were carried out in blood and kidney tissue samples of control and experimental animals in each group.

Estimation of kidney markers

Urea concentration in blood was estimated by NED Dye method (colorimetric Fix Time test) [18]. Concentration of serum creatinine was measured by alkaline picrate method [19]. Blood urea nitrogen (BUN) was measured with the commercial kit developed by the Parsazmoon Company (Tehran, Iran) based on the method described by Talke and Schubert [20]. Serum uric acid was measured by using commercially available reagents [21].

Estimation lipid and lipoproteins

Lipid extraction was done from plasma, erythrocyte membrane and tissue by the method of Folch *et al*[22]. The lipid extractions were used to estimate lipid profile of plasma. Total cholesterol was estimated by the method of Parekh and Jung [23]. Phospholipids were estimated by the method of Zilversmit and Davis [24]. Triglycerides were estimated by the method of Foster and Dunn [25]. The HDL cholesterol was estimated by the heparin-manganese chloride precipitation method [26] whereas, LDL-Cholesterol and VLDL-Cholesterol was calculated according to Friedewald *et al* [27].

Statistical analysis

The values are expressed as mean±SD. The statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT), using SPSS version 16.0 for windows (SPSS Inc.Chicago; <http://www.spss.com>). The values are considered statistically significant if the p value was less than 0.05.

RESULTS

Effects of *P.crispum* on body weight and kidney weight in gentamin-induced nephrotoxicity

Figure 3 shows the effect of *P.crispum* on the physical parameters. In the present study, the body weight of rats administered with GM was reduced significantly ($p < 0.05$) in comparison to the normal control group. Although there was increased in body weight of group III and group IV, the increased in body weight was not significant ($p < 0.05$) compared to normal group. Rats treated with PCE (Group V) alone showed no significant difference in body weight status compared to control animals. The levels of kidney weights of rats in control and all treated groups are showed in figure 3. GM treatment induced a significant increase in the relative weight of kidneys with respect to normal controls ($p < 0.05$). However, oral administration of PCE (250 mg/kg b.wt) (Group III) and PCD (250 mg/kg b.wt) (Group IV), reverted the weight of kidney to near normal range. Animals treated with PCE (Group V) alone showed no significant difference in kidney weight as compared to control animals.

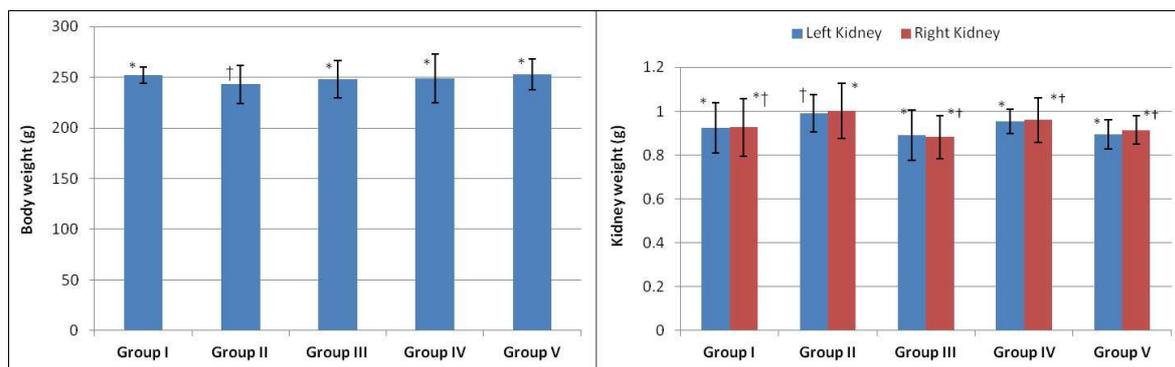


Figure 3. Shows the effects of *P.crispum* on physical parameters of normal and experimental animals (Each bars are expressed as mean \pm SD for 6 animals in each group; Values not sharing a common symbol differ significantly at $p < 0.05$ (DMRT))

Efficacy of *P.crispum* on renal function of gentamicin-induced nephrotoxicity

Figure 4 shows the status of kidney markers in serum of the control and experimental groups. The concentration of serum creatinine, urea, uric acid and blood urea nitrogen were increased significantly in Group II (GM alone) as compared to control animals. Oral administration of PCE (250 mg/kg b.wt) and PCD (250 mg/kg b.wt) significantly decreased the levels of kidney markers. PCE (Groups V) alone treated animals showed no significant difference in kidney markers as compared to control animals.

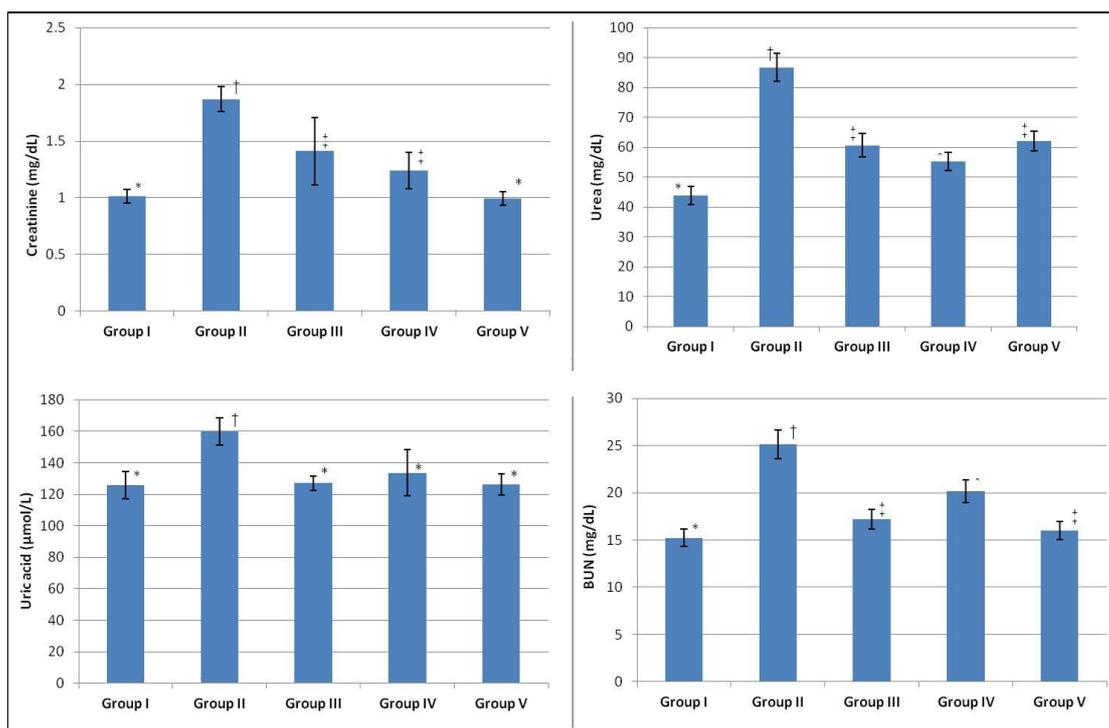


Figure 4. Shows the effects of *P.crispum* on kidney markers of normal and GM-induced nephrotoxicity animals (Each bar are expressed as mean \pm SD for 6 animals in each group; Values not sharing a common symbol differ significantly at $p < 0.05$ (DMRT))

Effect of *P.crispum* leaves on status of lipid, lipoprotein profile and osmotic fragility in GM-induced nephrotoxicity in rats.

Table 1, 2 and 3 shows the lipid and lipoprotein levels in plasma, erythrocyte membrane and kidney tissues respectively of control and experimental animals in each group. The levels of total cholesterol, phospholipids, triglycerides and LDL-cholesterol were increased whereas the HDL-cholesterol levels were decreased in plasma of GM induced animals as compared to control animals. The total cholesterol was significantly increased whereas

phospholipids were slightly decreased in RBC membrane of GM induced animals as compared to control animals. The levels of total cholesterol was significantly increased whereas the phospholipids were decreased in kidney tissues of GM administered (group II) animals as compared to control (group I) animals. Increased in c/p ratio was observed in plasma, RBC membrane and the kidney tissues in group II animals compared with group I animals. Oral administration of PCE and PCD normalized the altered levels of lipids and lipoproteins significantly prevented hyperlipidemia in nephrotoxicity animals. Animals were treated with PCE alone (groups V) showed no significant difference in the levels of lipid and lipoproteins as compared to control animals. Osmotic fragility curves for control and experimental animals in each group are shown in figure 5. The fragility curve of GM administered animals was shifted to the right for the control animals. Treatment of GM induced animals with PCE and PCD shifted the curve to the left, those of nephrotoxicity animals.

Table 1. Effects of *P.crispum* on serum lipid and lipoprotein profile in gentamicin induced nephrotoxicity rats

Parameters	Group I Normal	Group II GM	Group III GM + PCE	Group IV GM + PCD	Group V PCE
Cholesterol(mg/dl)	144.35±10 ^a	253.10±13.6 ^b	171.61±10.90 ^c	182.20±10 ^c	155.20±8.16 ^a
Phospholipid (mg/dL)	23.15±1.38 ^a	32.02±1.92 ^b	28.18±1.69 ^c	25.38±1.519 ^d	24.90±1.49 ^{ad}
Triglyceride(mg/dl)	149.30±8.13 ^a	199.44±10.90 ^b	172.34±9.39 ^c	175.13±9.54 ^c	165.03±8.99 ^c
LDL(mg/dL)	62.33±6.53 ^a	183.30±11.19 ^b	103.97±8.58 ^c	106.95±5.61 ^c	71.15±3.31 ^a
HDL (mg/dL)	52.16±3.12 ^a	29.88±1.79 ^b	33.18±1.99 ^c	40.18±2.41 ^d	51.02±3.05 ^a
VLDL mg/dL	29.86±1.63 ^a	39.89±2.17 ^b	34.47±1.88 ^c	35.03±1.91 ^c	33.01±1.80 ^c
c/p	6.27±0.23 ^a	7.91±0.36 ^b	6.09±0.27 ^a	7.18±0.04 ^c	6.23±0.05 ^a

Values are expressed as mean±SD for 6 animals in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

PCE- Ethanolic *P.crispum* extracts;PCD- *P.crispum* leaves Decoction

Table 2. Effects of *P.crispum* on RBC membrane lipid profile in gentamicin induced nephrotoxicity rats

Parameters	Group I Normal	Group II GM	Group III GM + PCE	Group IV GM + PCD	Group V PCE
Total Cholesterol (mg/10 ⁸ cells)	7.06±0.35 ^a	17.71±1.21 ^b	8.53±0.57 ^c	11.10±0.51 ^d	7.90±0.51 ^c
Phospholipid (mg/10 ⁸ cells)	1.47±0.09 ^{ad}	1.23±0.08 ^b	1.43±0.06 ^{bc}	1.51±0.07 ^{acd}	1.51±0.08 ^a
c/p ratio	4.65±0.02 ^a	15.01±0.09 ^b	7.02±0.05 ^c	7.24±0.06 ^d	5.36±0.03 ^c

Values are expressed as mean ± SD for 6 animals in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

PCE- Ethanolic *P.crispum* extracts;PCD- *P.crispum* leaves Decoction

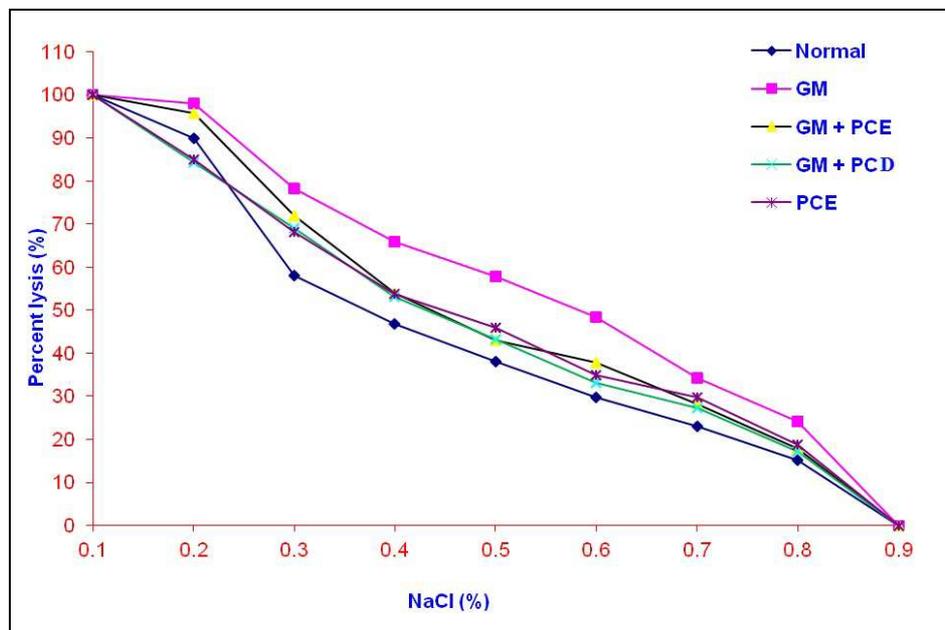


Figure 5. Erythrocyte osmotic fragility curves for normal and experimental animals

Table 3. Effects of *P.crispum* on kidney tissue lipid profile in gentamicin induced nephrotoxicity rats.

Parameters	Group I Normal	Group II GM	Group III GM + PCE	Group IV GM + PCD	Group V PCE
Total cholesterol (mg/g tissue)	4.06±0.28 ^a	9.18±0.49 ^b	5.02±0.32 ^c	5.01±0.27 ^c	3.97±0.21 ^a
Phospholipid (mg/g tissue)	13.30±0.99 ^a	9.05±0.48 ^b	11.06±0.70 ^c	12.97±0.71 ^{ad}	12.38±0.65 ^d
c/p	0.30±0.00 ^a	1.01±0.00 ^b	0.45±0.00 ^c	0.39±0.00 ^d	0.32±0.00 ^c

Values are expressed as mean ± SD for 6 animals in each group.

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

PCE- Ethanolic *P.crispum* extracts; PCD- *P.crispum* leaves Decoction

Photographs of RBC

Figure 6 shows the photomicrography of Red Blood Cells smears from blood samples of normal and experimental animals. In group I animal's erythrocytes is shows within normal limits. There were no morphological changes. But in group II gentamicin administered animals' erythrocyte showed an altered cell structure and formation of acanthocytes. The structure of erythrocytes in PCE and PCD treated group of rats was found to be significantly normal. There are no morphological structural changes found in *P.crispum* leaf extract alone treated group of animals.

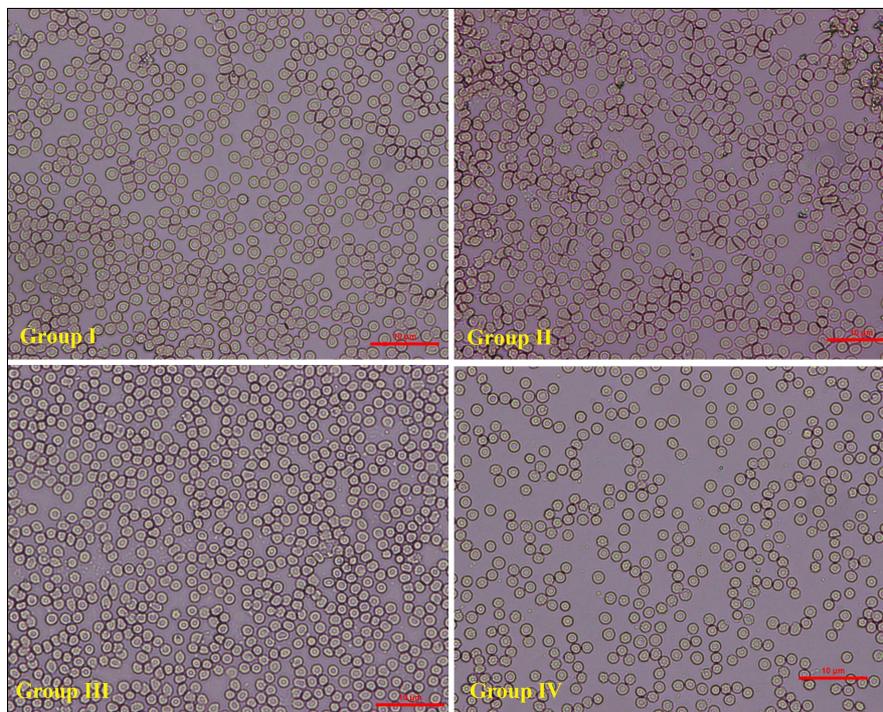


Figure 6. Photomicrography of Red blood cells smears from blood samples of normal and experimental animals. Blood smears were prepared, dried. The morphology of red blood cells was evaluated under optical microscopy (40X) after image capture

DISCUSSION

Nephrotoxicity is a poisonous effect due to drugs and it's over dose on the kidneys. Drug-induced nephrotoxicity is one of the most common side effects of some diagnostic and therapeutic agents, primarily aminoglycoside antibiotics. About 20 % of hospital admissions due to AKI are related to drug-induced nephrotoxicity. Nephrotoxic drugs (gentamicin) can lead to alteration in intra glomerular hemodynamics, tubular epithelial cell damage, tubulointerstitial disease, glomerular disease, renal vasculitis and thrombosis, and obstructive nephropathy [28]. The current study was aimed to explore the nephroprotective and antidyslipidemic effects of *P.crispum* against gentamicin-induced renal damage.

As a measure of renal function status, blood urea and creatinine are often regarded as reliable markers [29]. The gentamicin induced nephrotoxicity were confirmed by an increase in serum creatinine, uric acid, urea and blood urea

nitrogen levels and severe proximal renal tubular necrosis, followed by deterioration and renal failure [30] in group II animals (figure 4), are in agreement with a previous study done by Begum *et al* [31]. High values of blood urea and serum creatinine indicate renal damage [32] and this may be correlated with the significant and progressive body weight loss and kidney weight gain in the GM administered group II (figure 3). These parameters were almost significantly normalized by oral administered PCE and PCD (groups III and IV) animals. This result is consistent with many previous studies done using other traditional plants [33].

Lipids are major cell membrane components, essential for various biological functions including cell growth and division of normal tissues. The abnormalities in lipid and lipoprotein pattern produce number of pathological diseases including nephrotoxicity. Kidneys are highly vulnerable to damage caused by reactive oxygen species (ROs), likely due to oxidative stress by polyunsaturated fatty acids in the composition of renal lipids [34]. GM is the most potent nephrotoxic substance because of its rapid elimination and cumulative injury when it is given intermittently, presumably by free radical-mediated lipid [31, 35]. Lipoproteins are responsible for the transport of lipids through the vascular and extracellular tissue from their site of synthesis or absorption to peripheral tissues. An altered level of HDL, VLDL, and LDL has been implicated in the pathogenesis of several diseases including nephrotoxicity [36]. Membrane lipids constitute about 50 % of the mass of most animal cell plasma membranes. They play an important role in determining the various function and properties of red cells such as maintaining the integrity, permeability, fluidity, and function. Membrane fluidity is known to be dependent on the molar ratio of cholesterol to phospholipid [37]. Measurement of mean corpuscular fragility of erythrocyte membranes is useful to assess the alterations in the integrity of cell structure and function [37]. Alteration in membrane fragility (figure 5) has been documented in nephrotoxicity [37] in gentamicin administered group II animals.

The levels of total cholesterol, triglycerides, and LDL-cholesterol and VLDL-cholesterol were significantly increased whereas HDL-cholesterol was decreased in plasma of GM administered animals. The level of total cholesterol was increased whereas phospholipids were moderately decreased in erythrocyte membrane of GM administered animals. The level of cholesterol was increased whereas phospholipids were decreased in kidney tissues of group II animals as compared to control animals. Oral administration of *P.crispum* leaf extract brought back the values to near normal range in GM administered rats. It has been reported that nephrotoxicity subjects showed body weight loss accompanied with hyperlipidemia. Cholesterol is essential for maintenance of the structural and functional integrity of the biological membranes. It is also involved in the activity of membrane bound enzymes [38]. Alterations in membrane fluidity are determined by the amount of cholesterol and cholesterol/phospholipid molar ratio [38]. The observed increase in cholesterol and c/p ratio indicates the loss of membrane fluidity [38] in AKI animals. Alterations in the erythrocyte lipid composition may be a reflection of altered plasma lipid, due to an ineffective exchange mechanism with plasma.

Erythrocytes and erythrocyte membrane are more vulnerable to lipid peroxidation due to constant exposure to high oxygen tension and richness in polyunsaturated fatty acids respectively [39]. Increased osmotic fragility in AKI animals (group II) can be due to the increased oxidative stress in erythrocytes. Over production of reactive oxygen species has been implicated in the alterations of membrane structure and function. Increased lipid peroxidation observed in present study (results not showed) is therefore responsible for the increase in osmotic fragility [40] in AKI animals (figure 5). Increased erythrocyte fragility and permeability in group II animals are probably due to their altered lipids, lipid peroxidation and antioxidant status. Oral administration of *P.crispum* to these AKI animals prevented the alterations in red cell fragility and lipid profile, which indicates the role of *P.crispum* in maintaining the structural integrity of erythrocytes during AKI. The membrane stabilizing effect of ethanolic extract of *P.crispum* is more potent than that of the decoction. During in the cholesterol accumulation process the structure of the membrane is slowly changed. At low concentrations spicules were formed on the membrane. With increase in cholesterol acquire an echinocytic appearance [41] in gentamicin-administered animals (figure 6). The observed biochemical changes also resulted in significant morphological changes in the erythrocytes of gentamicin administered rats. Changes in membrane lipid composition lead to morphological changes, the prominent changes were the distortions in normal discocyte shape, appearance of central and peripheral protuberances and formation of acanthocytes [42] in group II animals. The drug treatment (*P.crispum* leaf extracts) had potent therapeutic efficacy in modulating erythrocyte function and structural abnormalities by this remarkable hypocholesterolaemic property.

CONCLUSION

This study revealed that the ethanolic and decoction of *P. crispum* administered at a dose of 250 mg/kg.bwt and 250mg/kg.bwt were effective respectively. Alterations in mean body weight, blood urea nitrogen, creatinine and uric acid associated with gentamicin were reduced by treating animals with extract of *P. crispum*. In conclusion, the results of the present study indicate that *P. crispum* may emerge as a putative nephroprotective, antihyperlipidemic and membrane stabilizing effects agent against nephrotoxicity. Further studies need to be undertaken in order to confirm these findings and its extrapolation in humans.

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