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Nephroprotective effects of *Helianthus annuus* seeds extract in gentamicin induced nephrotoxic male mice

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ABSTRACT

Acute kidney injury (AKI) causes a decrease in renal function which leads to failure in balancing electrolyte, fluid and acid-base homoeostasis. AKI is a damaging and life-threatening disorder, but it can be managed if identified earlier. This study aimed to investigate the possible nephroprotective effect of *Helianthus annuus* seeds extract against gentamicin (GM) induced nephrotoxicity in male mice. The control group (0.5 ml normal saline i.p.,), Gentamycin (GM) group (GM 100 mg/kg i.p), silymarin + GM group (silymarin 50 mg/kg and GM 100 mg/kg i.p.,), H. *annuus* extract (HAE) and GM, group (HAE 250 mg/kg and GM 100 mg/kg i.p), HAE2 + GM group (HAE2; 500 mg/kg and GM 100 mg/kg i.p) and *H. annuus* oil (HAO) + GM (HAO 2.5 ml/kg and GM 100 mg/kg i.p). Serum creatinine, urea and blood urea nitrogen (BUN) were significantly ($P< 0.001$) elevated in the GM group compared to the control group. The elevated level of serum creatinine, urea and BUN were decreased significantly ($P<0.001$) in groups treated with HAE and HAO extracts compared to the GM group. The kidney histopathological study from the GM group showed tubular necrosis, vacuolation and fibrosis. However, the animal that received HAE and HAO showed no tubular necrosis and vacuolation. Only mild inflammation was observed compared to the GM group. In conclusion, the extract caused marked radical scavenger and protected the kidney from oxidative damage of GM. *H. annuus* seeds contain strong antioxidant compounds, including flavonoids, phenolic acids, tocopherols and minerals, which could be responsible for the current show.

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Introduction

Acute kidney injury (AKI) is a familiar and prospectively life taking disease (1). AKI incidence is rising; it has very grave concerns with inadequate therapeutic choices available both in adults and children (2, 3). AKI is generally diagnosed by urea and creatinine levels in serum and urine. Diagnosis of kidney disease is based on a long-term decrease in kidney function and functional kidney injury. The most obvious parameter for measuring kidney function is the glomerular filtration rate (GFR), which is the amount of fluid passing through all of the working nephrons in a given period (4).

There is mounting evidence that many medicinal herbal products have the potential to be valuable therapies in the treatment of a variety of kidney disorders and the prevention of drug-induced nephrotoxicity (5). Many plants therapy are used in traditional systems of medicine around the world, and these are managed through dietary measures. Plants were the primary form of therapy prior to the advent of allopathic medicine. Medicinal plants can be used to avoid the need for dialysis by treating the risk factors and side effects of kidney disorders. It will also lessen the burden of dialysis-related side effects (6).

Helianthus annuus (*H. annuus*) as an oilseed plant that belongs to the family Asteraceae, it has many common names that is Surajmukhi (Urdu, Hindi, Gujerati, and Bengali) and Sunflower (English) (7-9). Talele *et al;* 2012 reported that *H. annuus* seeds, roots and leaves are traditionally used in various regions for treating kidney disorders (10). It has also anti-inflammatory (11), antioxidant (12) and anti-microbial

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(13) properties. This study aimed to determine the acute toxicity of *H. annuus* seed extract plus oil and its nephroprotective effect against gentamicin (GM) induced nephrotoxicity in male mice.

Materials and methods

Chemicals

Gentamicin (Ray Pharma (Pvt) Ltd), Formalin (Haq chemicals Pakistan), Tween-20 (Haq chemicals Pakistan), Chloroform (Haq chemicals Pakistan) and normal saline (Otsuka Pak. Limited).

Plant collection

H. annuus seeds were purchased from the local store in the main Qissa Khawani Bazar of Peshawar in July 2018. Dr. Mohib Shah did the identification and authentication of seeds. The plant specimen was placed in the herbarium botany faculty, Abdul Wali Khan University of Mardan (Khyber Pakhtunkhwa). The voucher specimen number allotted was 895.

Maceration and extraction

The seeds were divided into two equal parts, one part of the seeds was macerated in methanol while the other part was macerated in *n*-hexane for two weeks, respectively. After which, the solvent was dried with the help of a rotary evaporator. Both extracts were labeled accordingly.

Acute toxicity

For evaluation of any potential toxic effect acute toxicity test was conceded. Swiss albino mice of either sex (n = 6) were administered with Helianthus methanolic extract (HAE) in different doses (1000mg/kg, 2000mg/kg and 3000mg/kg i.p). Similarly, Helianthus oil extract (HAO) was also administered in different doses (5 ml/kg, 10 ml/kg and 15 ml/kg i.p). The control group was administered with 10 ml normal saline solution. Different time intervals (0 min, 30 min, 60 min, 120 min, and 180 min) were used to observe animal behaviour. However, mortality was noted after 24 h of treatment (14).

Pharmacological activity

Swiss Albino male mice were used in the experiment as research animals. The mice were properly weighed prior to the experiment. The animals' weights ranged from 26 to 35 gra, and their ages ranged from 6 to 8 weeks. These mice were obtained from Khyber Pakhtunkhwa's Peshawar Medical College (PMC). They were kept under a controlled environment (25 ± 2 m°C ambient temperature, 12 h light-dark cycle). Food and water were provided *ad libitum*. The animal handling and experiment were performed according to the standard operating procedures set by the Ethical Committee of Faculty of Pharmacology Department, PMC Khyber Pakhtunkhwa.

Animal dosing

The mice were divided into groups each group containing six animals. For eight days, one group received an isotonic saline injection (0.5 ml), another Gentamicin (GM) 100 mg/kg i.p., and another Silymarin 50 mg/kg. The remaining groups obtained crude extract and hexane fraction of *H. annuus* seed at a dose of 250 mg/kg and 500 mg/kg i.p.

Measurement of biochemical parameters

To prepare the animals for blood collection, chloroform anaesthesia was administered 24 hours after the last injection. After the blood was collected, it was centrifuged to separate the serum, which was then transferred to serum cups. Different commercially available diagnostic kits were used to measure serum creatinine, urea, and BUN. (15).

Histopathology

Following blood collection, each animal's kidneys were removed. Before embedding the kidney samples in paraffin wax, they were fixed in 10% buffered formalin. It was stained with hematoxylin and eosin after being cut into 5 mm slices. The slides were labelled with a unique code and examined by Professor Dr. Fouzia, a histopathology specialist who was unaware of the treatment groups. The nephrotoxic histological features were assigned a score of 0, 1, 2, or 3. A grade of 0 indicates that no tubules are affected, a grade of 2 indicates that two-thirds of the tubules are affected, and a grade of 3 indicates that all tubules are affected. Histopathology of kidney slides revealed the following features: proximal tubular necrosis, haemorrhage, mononuclear infiltrate, vacuolation, tubules hyaline cast, and fibrosis. Each animal in the specified group

was graded 0, 1, 2, or 3 based on the tubules affected (16).

Statistical Analysis

The data was presented in the form of a standard error mean (SEM). The Duncan test and one-way analysis of variance (ANOVA) were used to assess differences in group means. The results were deemed statistically significant at $p<0.05$.

Results and discussion

Effect in acute toxicity

No toxicity or mortality was found after 24 hours of treatment; therefore it was concluded that HAE and HAO are safe up to doses of 3 g/kg (i.p) and 15 ml/kg (i.p) respectively.

Effect on renal biomarkers

The result of the blood sample analysis showed that serum creatinine, urea and blood urea nitrogen (BUN) level was significantly ($P<0.001$) increased with GM against control animals. It was discovered that after the administration of HAE, the levels of these biomarkers were nearly normalized. Figures 1, 2, and 3 show that the elevated levels of these biomarkers were significantly reduced ($P<0.01$) in the HAE+GM and HAE2+GM groups, respectively. Treatment with *H. annuus* extract prevented GM-induced nephrotoxicity; the reduction in serum biomarkers was greater in the HAE2+GM group than in the HAE+GM group, indicating that HAE provides dose-dependent protection of kidney tissue. When compared to the GM group, mice treated with HAO had a significant reduction ($P<0.01$) in serum biomarker levels.

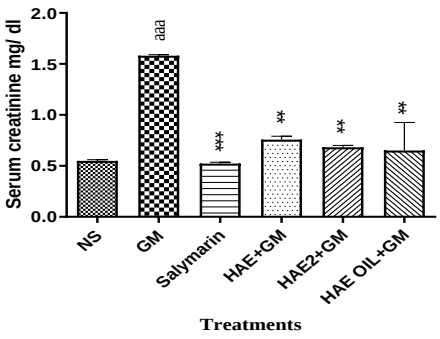

Figure 1. Effect of methanolic and oil extract of *H. annuus* on serum creatinine level of mice. Data were conveyed as mean ± SEM (n=6); $^{aaa}P<0.001$ (against control group); $^{**}P<0.01$, $^{***}P<0.001$(against gentamicin group).

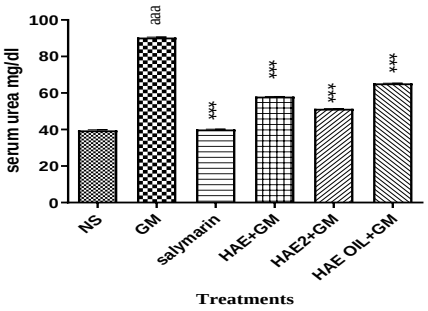

Figure 2. Effect of methanolic and oil extract of *H. annuus* on serum urea level of mice. Statistics were articulated as mean ± SEM (n=6); $^{aaa}P<0.001$ (against control group); $^{***}P<0.001$(against Gentamicin group).

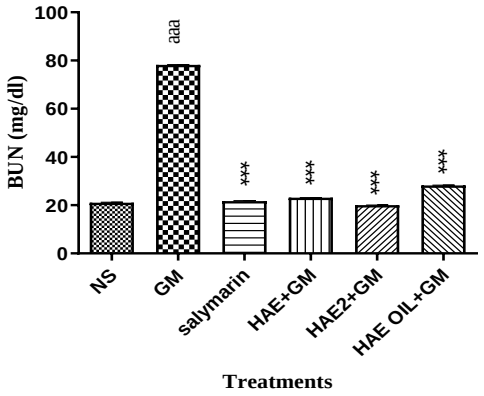

Figure 3. Effect of methanolic and oil extract of *H. annuus* on serum blood urea nitrogen (BUN) level of mice. Data were expressed as mean ± SEM (n=6); $^{aaa}P<0.001$ (compared to control group); $^{***}P<0.001$(compared to gentamicin group).

Histopathological Findings

The histological variations in the kidneys of all groups were classified, and the results were recorded as described in the materials and methods section and summarised in table 1. No changes were observed in the N/S group, with proximal tubular necrosis, inflammation, vacuolation, hyaline cast, and fibrosis all scoring 0. Figure 4 depicts the normal cortex of the kidney area (A) and the normal medulla of the kidney (B).

In the kidney cortex of GM-treated mice, there was obvious tubular necrosis and desquamation of tubular epithelial cells. Tubule necrosis is a common cause of kidney damage; its mechanism includes intra-renal vasoconstriction caused by drug toxicity (17). Figure 4 (D) depicts tubular necrosis with severe glomerular degeneration. In addition, GM-treated mice showed proximal tubule necrosis, the formation of hyaline

casts, and the formation of vacuoles. Figure 4 (E) depicted vacuolation of grade 3 on a scale, which is a common cause of degeneration and a reflection of renal cytoplasmic organelle alteration.

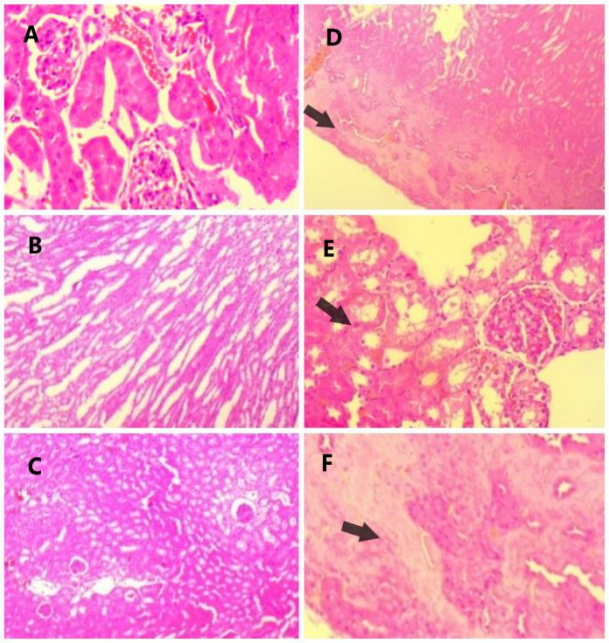

Figure 4. (A) is renal cortex while (B) renal medulla (C) shows histopathology of Silymarin group (50 mg/kg) normal histopathology. “D”, “E” and “F” Showing the histopathology of the GM group receiving 100 mg/kg gentamicin, (D) shows the necrosis of cells it’s graded 3, (E) shows vacuolation graded 3 on the scale while (F) shows fibrosis graded 3.

Figure 4 (F) depicted grade 3 fibrosis, which is a common response to renal injury associated with inflammation. GM completely destroys the morphology of the cell, leaving no visible cytoplasm. The documentation of individual inflammatory cells is difficult due to the cells' fulminant necrosis. There were no discernible differences in the silymarin group (Figure 4C), indicating that silymarin prevented GM nephrotoxicity. While the tubular injury was significantly reduced in the HAE+GM and HAE2+GM groups, the grade of the vacuole, haemorrhage, and inflammation was all 1 as shown in Figure 5 A and B, respectively. Similarly, the tubular injury was significantly reduced in the HAO+GM group, as evidenced by a grade of haemorrhage and a mononuclear infiltrate of 2, as shown in figure 5. (C).

Gentamycin, an aminoglycoside, is well-known for causing nephrotoxicity. Gentamicin affects renal tubules by causing necrosis of epithelial cells in the proximal portion of the tubules and functional changes in cell membrane transporters, affecting solute and water transport.

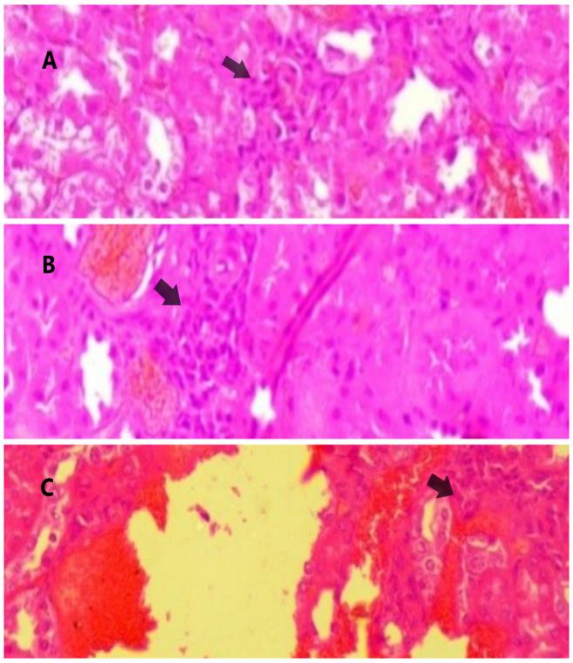

Figure 5. (A) Showing the histopathology of treatment group (HAE 250 mg/kg) mild inflammation (B) showing the histopathology of treatment group (HAE2 500 mg/kg) mild inflammation. (C) Showing histopathology of treatment group (HAE oil 5 ml/kg) moderate inflammation.

Gentamicin treatment causes apoptosis and necrosis in the epithelial cells of the renal tubules in animals (18). Gentamycin, an aminoglycoside, is well-known for causing nephrotoxicity. Gentamicin affects renal tubules by causing necrosis of epithelial cells in the proximal portion of the tubules and functional changes in cell membrane transporters, affecting solute and water transport. Gentamicin treatment causes apoptosis and necrosis in the epithelial cells of the renal tubules in animals (19). Gentamycin, an aminoglycoside, is well-known for causing nephrotoxicity. Gentamicin affects renal tubules by causing necrosis of epithelial cells in the proximal portion of the tubules and functional changes in cell membrane transporters, affecting solute and water transport. Gentamicin treatment causes apoptosis and necrosis in the epithelial cells of the renal tubules in animals (20). According to Juan et al., 2007, GM causes renal damage *in vivo* and *in vitro* via oxidative stress. GM directly increases the level of reactive oxygen species in mitochondria, causing a respiratory chain reaction and the release of cytochrome C.

Together, these cause protein, nucleic acid, and fatty acid damage, interfering with cell function. Furthermore, it causes mesangial contractions, which cause oxidative stress. As a result, sodium transmembrane transport is inhibited, resulting in cell necrosis and swelling (21).

Antioxidant-rich medicinal plants are the best sources for preventing oxidative kidney damage. Medicinal plants protect against oxidative damage by strengthening the body's antioxidant defenses and lowering lipid peroxidation (22). The extract of *H. annuus* seeds, which contain many natural antioxidants such as flavonoids, phenolic acids, tocopherols, vitamins, and minerals, is being tested experimentally against GM-induced nephrotoxicity in this study. The administration of *H. annuus* extract reversed the increase in serum creatinine, urea, and BUN caused by GM. These findings were consistent with histopathological analysis, which revealed that the GM group had significant changes, as summarized in table 1. These changes were not observed in the *H. annuus* extract groups, indicating that the GM nephrotoxic effect had been reduced.

Table 1. Showing kidney histopathology comparison in animal groups; Animals group (A), Proximal tubular necrosis (B), Mononuclear infiltrate (C), Vacuolation (D), Tubules Hayline cast (E), Fibrosis (F)

A	B	C	D	E	F
N/S group	0	0	0	0	0
GM	3	1	3	0	3
Salymarin group	0	0	0	0	0
HAE+GM	0	1	1	1	0
HAE2+GM	0	0	0	1	0
HAE oil+GM	0	2	2	1	0

H. annuus contains various constituents with antioxidant and radical scavenging properties, such as Vitamin E, which Abdel-Naim and coworkers (1999) found to significantly reduce higher serum creatinine levels. Vitamin-E also improved the rise in renal MDA content ((23)). According to one study, the total tocopherol content of *H. annuus* seed is 669 mg/kg seed, with 92 percent α-tocopherol, 6 percent β-tocopherol. *H. annuus* seeds contain 90 percent γ -tocopherol, followed by β and ϱ tocopherol in amounts less than 5 percent (24). The α-tocopherol was found to cause a significant decrease of renal function and renal malondialdehyde (MDA) together with a significant increase of renal superoxide dismutase (SOD) (25). The main phenolic acid present in *H. annuus* seeds is 5-*o*-caffeoylquinic acid (5CQA), the second most is di-caffeoylquinic acids besides gallic and ferulic acids are also present (26). Amakura *et al;* 2013 have indicated that the antioxidant quality of *H. annuus* seeds is mainly due to polyphenols which comprise ferulic, gallic, sinapic acids and others, and this antioxidant property remains intact when the seed is processed into oil (27). *H. annuus* seeds also contain flavonoids as mentioned by Cho *et al;* 2017 it contains 534 ng/g of Isoflavone, this weight increases to 613 ng/g when it is macerated in water and to 686 ng/g after sprouting. The study also mentioned that anthocyanins one of the flavonoids present in *H. annuus* seeds have a nephroprotective role and are effective against cisplatin-induced nephrotoxicity (28).

Considering the phytochemistry of *H. annuus* seeds it contains many components which have a proven role as strong antioxidants with additional pharmacological properties as well. The nephroprotective role provided by *H. annuus* seeds extract may be due to the one its ingredient or a combination of it.

In conclusion, this study demonstrated that the methanolic and *n*-hexane extract of *H. annuus* seeds possesses nephroprotective activity against gentamicin-induced nephrotoxicity due to its antioxidant and anti-inflammatory action. For the exact mechanism of our finding in this study, further detailed studies are necessary including dose standardization for effective clinical uses.

Acknowledgments

None

Conflict interest

The authors declare no conflict of interest.

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