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Research Article

PROTECTIVE EFFECT OF ETHANOLIC EXTRACT OF *HORDEUM VULGARE* SEED ON GENTAMICIN INDUCED NEPHROTOXICITY

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ABSTRACT

Objective of the study was to evaluate protective effect the Ethanolic extract of seeds of *Hordeum vulgare* was tested in an animal model of nephrotoxicity. Nephrotoxicity was induced by the addition of Gentamicin (80 mg/kg; i.p) to the normal diet of Wistar albino rats for a period of 10 days. Group I served as a normal control. Group II served as nephrotoxicity control. Group III, IV and V were treated with ethanolic extract of *Hordeum vulgare* (EHV) at 100, 250 and 500 mg/kg respectively. The effects of EHV on various biochemical parameters were studied in Nephrotoxicity rats. Gentamycin induced nephrotoxicity in rats, there were significant elevated kidney weight, body weight loss and blood vessel congestion and scattered mononuclear inflammatory cell within the interstitial in gentamycin induced rats. In vivo antioxidant parameters including protein, malondialdehyde, superoxide dismutase, reduced glutathione and catalase (CAT) were also determined. EHV significantly maintained the renal parameters like serum urea, BUN, creatinine. The extract also induced a significant decrease in MDA which increased in nephrotoxicity control rats. The extract also significantly increased reduced glutathione, superoxide (100,250) and CAT in nephrotoxicity rats which were markedly decreased in gentamicin induced nephrotoxicity in rats. This study demonstrates the protective activity of *Hordeum vulgare* seeds and rationalizes their medicinal use for the treatment of nephrotoxicity.

Key words: Gentamycin; *Hordeum vulgare*; Nephrotoxicity.

INTRODUCTION

Kidney stones are quite common and usually affect people who are between 30 and 60 years of age. They affect men more than women. It is estimated that renal colic (severe pain caused by a kidney stone) affects about 10-20% of men, and 3-5% of women. Currently, no allopathic medications are available for nephroprotective, urolithiasis. Surgery, lithotripsy, and local calculus disruption using a high-power laser are used to treat calculi. However, these procedures are expensive and recurrence is quite common. [1]

As per Ayurveda, the seeds of *Hordeum vulgare* Linn. Are reported to be useful in the treatment of a wide range of aliments including urinary stones.[2] However, no scientific data are available to establish the Nephroprotective property of the seed extract of *H. vulgare* Linn. In the present study, an effort has been made to establish the scientific validity of the Nephroprotective activity of *Hordeum vulgare* seed extract using Gentamicin induced Nephrotoxicity using male Wistar albino rats.

MATERIALS AND METHODS

Animal selection

For acute toxicity studies, albino mice of either sex weighing between 25-30 gm and for the study of Nephroprotective activity adult male albino rats of Wistar strain (150-200 gm) were procured form Zydus Research Centre, Ahmedabad. The animals were acclimatized to standard laboratory conditions (temp: 23 ± 2 °C) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow (VRK Nutritional Solutions, Pune, India) with free access to drinking water *ad libitum* for the period of 28 days. Institutional Animal Ethics Committee (IAEC) approval (Protocol no.: IICP/PH/03-2010/04 dated 29.03.2010) was obtained before the experiment and care of animal was taken as per guidelines of CPCSEA, Ministry of Social Justice and Empowerment, Government of India.

Chemical

Gentamicin was procured from (Cadila Pharmaceutical Ltd) the local market. All other chemicals and reagents used of analytical grade.

Plant material

The dried seeds of *Hordeum vulgare* Linn. were received from commercial supplier, Anand, Gujarat, India. The seeds were identified by Dr. G. C. Jadeja, Department of Agricultural Botany, B. A. College of Agriculture, Anand Agricutlture University, India. A Voucher specimen (voucher no. IICP/11-JGS/03-HV) was deposited in the herbarium of the Department of Pharmacognosy, Indukaka Ipcowala College of Pharmacy, New Vallabh vidyanagar, India.

Preparation of plant extract

The air-dried powdered seeds (500 g) were extracted with ethanol in soxhlet apparatus for 24 h. The extract was evaporated to dryness under reduced pressure to give solid residues. The residue was stored below 4 ºC for subsequent experiments. The yield of the extract was 4.82 % w/w.

Acute toxicity studies

The acute toxicity was performed as per Organisation of Economic Cooperation and Development (OECD) guideline (no.420) using albino mice of either sex prior to the evaluation of nephroprotective activity. The Ethanolic extract of Hordeum vulgare Seeds (EHV) was tested using graded doses (500, 1000, 2000 and 5000 mg/kg) in mice. Furthermore, the general behavior like changes in awareness, mood, motor activity, posture, motor-coordination, muscle tone and reflexes were mice was recorded continuously for 12 h, and daily for a further 2 weeks for any eventual mortality.[3] The EHV did not show mortality, or any remarkable symptoms of toxicity and or any significant changes in general behavior in mice.

Experimental Design

Male albino rats weighing 175-225 g were used for the experiment. The animals were housed in group (six animals in each cage) and fed with standard diet and water *ad libitum.* The nephrotoxicity induced by the Gentamicin. Thirty rats were randomly assigned to five groups equally as follows.

Group I : Control animals treated with normal saline (5 ml/kg)
Group II : Gentamicin treated (80 mg/kg; i.p)
Group III : Ethanolic extract of *Hordeum vulgare* (100 mg/kg), simultaneously administered Gentamicin (80 mg/kg; i.p)
Group IV : Ethanolic extract of *Hordeum vulgare* (250 mg/kg), simultaneously administered Gentamicin (80 mg/kg; i.p)
Group V : Ethanolic extract of *Hordeum vulgare* (500 mg/kg), simultaneously administered Gentamicin (80 mg/kg; i.p)

The extract was given once orally for 10 days daily.

Collection and analysis of biological samples

At the end of the experiment period, body weight and kidney weight of each animal were measured. Blood sample were collected from animals of each group by retro-orbital plexus under light ether anesthesia. Serum was separated by centrifugation at 10,000 rpm for 10 minutes and serum was determined for biochemical parameters like serum urea, blood urea nitrogen (BUN) and serum creatinine using diagnostic kits in all animals.

The rats were scarified and the abdomen was opened and kidneys were removed. Measure the weight of kidney of each animal. A portion of kidney was taken from all the groups, and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4). The kidney homogenate was estimated for protein, malondialdehyde, superoxide dismutase, reduced glutathione and catalase in each animal of all groups. A portion of kidney was fixed in 10% formalin for histopathological studies.

Statistical analysis

Results were expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Dunnett's multiple comparison tests (Graphpad Prism software for Windows, Version 2.03.1998). $p < 0.05$ was considered to be statistically significant.

RESULTS
General parameters

The data showed that, the changes in body weight was decreased in Gentamicin treated animals compare to normal control animals. The animals treated with EHV 500 produced significant improvement of body weight (Figure 1-A). While, EHV 100 and 250 did not showed significant change in body weight of Gentamicin treated animals. In Gentamicin treated animals, kidney weight was considerably more compared to normal control animals. The treatment with EHV 250 and 500 showed decrease in kidney weight to much extent. (Figure 2-B). Here EHV 100 did not show any significant effect on kidney weight of nephrotoxic animals.

Analysis of serum

Gentamicin treated group of animals the concentration of renal function parameters like serum urea, blood urea nitrogen and creatinine were considerably elevated as compared to normal control animals, this indicates sever nephrotoxicity. Animals treated with EHV 250 and 500 showed statistically significant reduce in concentration of serum urea (Fig.2-A) and blood urea nitrogen (Figure 2-B) compared to Gentamicin treated nephrotoxic animals. Here, EHV 100 was failed to produce significant effect on serum urea and blood urea nitrogen. The serum creatinine level was significantly decreased in animals treated with the EHV 100, 250 and 500 (Figure 2-C).

Analysis of oxidant-antioxidant parameters

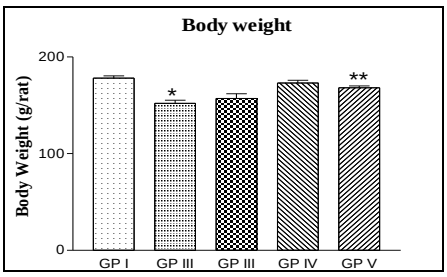
Antioxidant parameters were measured in kidney homogenate of all animals. There was significant decrease in the levels of protein, superoxide dismutase, reduced glutathione and catalase in Gentamicin treated nephrotoxic animals in comparison with normal control animals. The treatment with EHV 100, 250 and 500 showed significantly decrease in the level of protein (Figure 3-A). The EHV 100 and 250 were significantly increased the level of superoxide dismutase in nephrotoxic animals (Figure 3-C). Here, EHV 500 was not increased the superoxide dismutase level significantly on Gentamicin treated nephrotoxic animals as compared to normal control animals. The level of reduced glutathione was also significantly increased by the treatment with EHV 250 and 500 (Fig.4-A). And the concentration of catalase was significantly increased by the EHV 500 (Figure 4-B). Here EHV 100 and 250 did not produced significant effect on concentration of catalase. The concentration of malondialdehyde was significantly increased in Gentamicin treated animals in comparison with normal control animals. The EHV 100, 250 and 500 were significantly decreased the concentration of malondialdehyde in Gentamicin treated animals (Figure 3-B). The presented data showed that the extract having the potent anti-oxidant activity in Gentamicin induced nephrotoxicity.

Histopathology examination

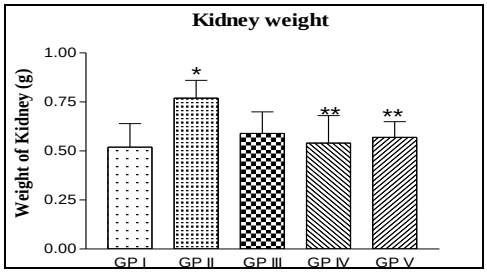
The microscopic examination of kidney from normal control rats showed no structural change in renal tissue. The glomerular and tubular change appear a really determined in normal control rats. The tubular structures were mainly intact without the presence of any mononuclear infiltrates in the interstitium (Figure 5-A). In GM group, there were seen diffuse glomerular congestion, degeneration of tubular epithelial cell and peritubular congestion. The blood vessels congestion and scattered mononuclear inflammatory cell observed within the interstitium. The glomerular changes were quite marked (Figure 5-B). Concurrent treatment with the EHV was found to reduce such changes in kidney histology induced by Gentamicin.In EHV 100 treated animals, the renal tubular cells showed varying

degrees of dilatation with hyaline cast formation in the lumen and the degeneration of tubular epithelial cells, peritubular congestion and cell necrosis (Figure 5-C). Treatment with EHV 250 and 500 showed renal parenchyma with intact architecture. The glomerular and tubular changes appear unremarkable. Some of the blood vessels were dilated and glomerular congestion within the interstitium. Also, seen few scattered mononuclear inflammatory infiltration seen within the interstitium (Figure 5-D & E). According to the pathological result it can be inferred that extracts of *Hordeum vulgare* seeds had protective effect against degenerative injury caused by Gentamicin.

Protective Effect of Ethanolic extract of *Hordeum vulgare* seeds on Body weight (1-A) and Kidney weight (1-B) of Gentamicin induced nephrotoxicity



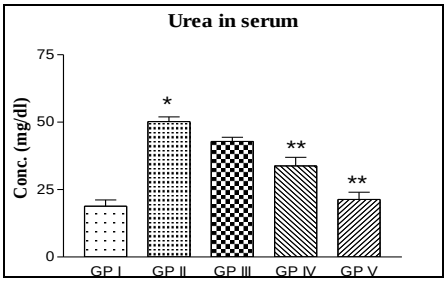
1-A



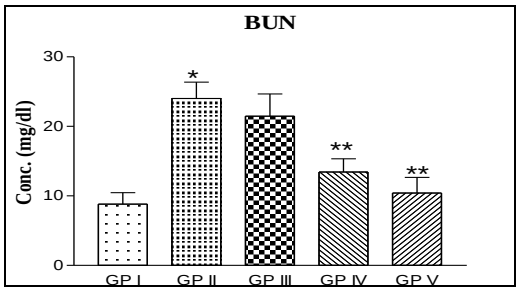
1-B

Values are expressed as Mean ± S.E.M, * significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.

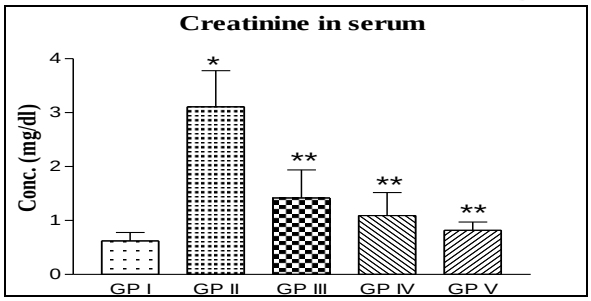
Protective Effect of Ethanolic extract of *Hordeum vulgare* seeds on Serum urea (2-A), blood urea nitrogen (2-B) and creatinine (2-C) of Gentamicin induced nephrotoxicity



2-A



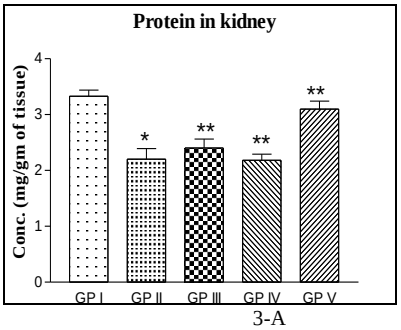
2-B



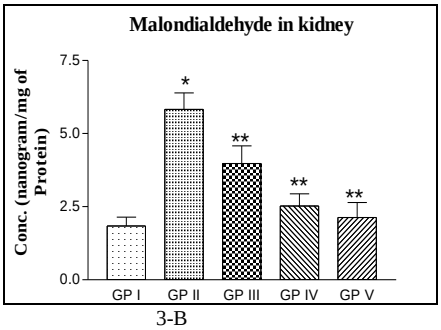
2-C

Values are expressed as Mean ± S.E.M, * significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.

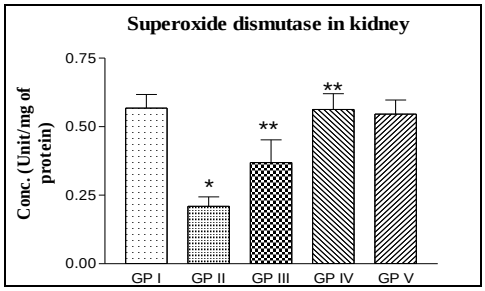
Protective Effect of Ethanolic extract of *Hordeum vulgare* seeds on Tissue protein (3-A), malondialdehyde (3-B) and superoxide Dismutase (3-C) of Gentamicin induced nephrotoxicity



3-A



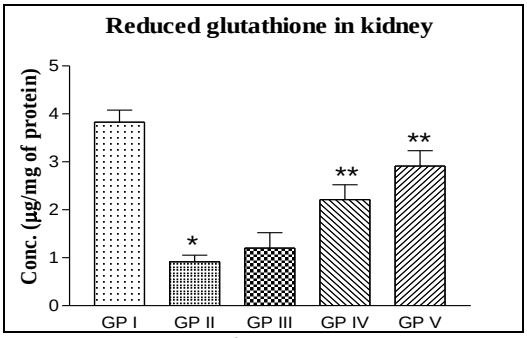
3-B



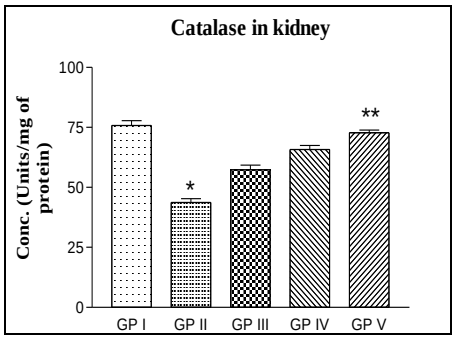
3-C

Values are expressed as Mean ± S.E.M, * significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.

Protective Effect of Ethanolic extract of *Hordeum vulgare* seeds on Kidney reduced glutathione (4-A) and catalase (4-B) of Gentamicin induced nephrotoxicity



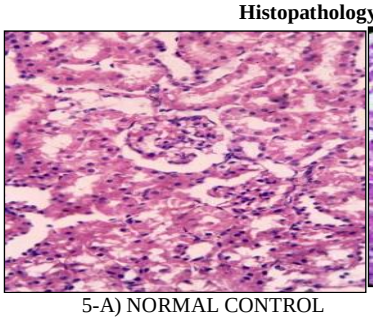
4-A



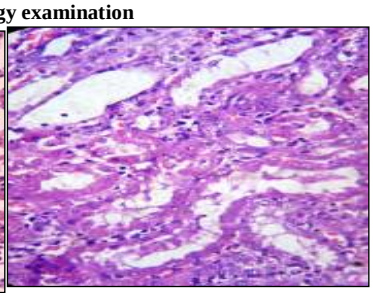
4-B

Values are expressed as Mean ± S.E.M, * significantly different from normal control group, p<0.05 ** significantly different from calculi control, p<0.05.

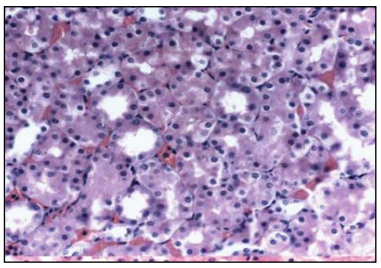
Histopathology examination

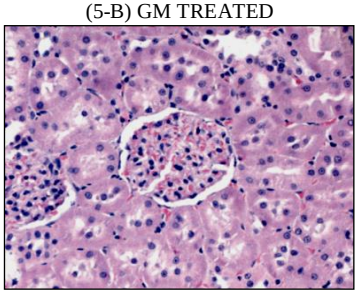


5-A) NORMAL CONTROL

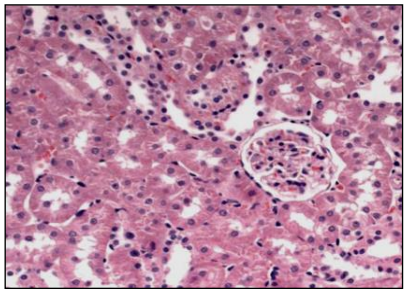


(5-B) GM TREATED





(5-C) GM+EHV 100 TREATED (5-D) GM+EHV 250 TREATED



(5-E) GM+EHV 500 TREATED

DISCUSSION

In the present study, nephrotoxicity induced by Gentamicin was evidenced by depletion of urine volume and increases in kidney weight, serum creatinine, serum urea and blood urea nitrogen concentrations. Nephrotoxicity occurs as a disturbance in renal function due to various adverse drug interactions, inadequate elimination of radioactive contrast materials and chemicals. It is of great concern in patients with renal failure. Nephrotoxicity may limit the clinical usefulness of many diagnostic and therapeutic agents; recognition of factors associated with higher risk for renal injury is of great importance. However, the end point of nephrotoxicity is always cell death; therefore, it is important to identify the mechanism in addition to the site of action, in order to formulate a strategy for damage prevention. In kidney proximal tubular cells are the large part change for the worse in patient medical care with the Gentamicin [4]. Gentamicin binds with cell wall phospholipids, obstructing the chain reactions of phosphotidyl inositol which impairs cell admired. It results by production of reactive oxygen species (ROS) [5]. The strategies aimed at ameliorating the nephrotoxicity are of clinical interest [6]. The use of Gentamicin, an amino glycosides antibiotic with a wide spectrum of activity against gram-positive and gram-negative bacterial invasion but with major advantage for latter is equally associated with nephrotoxicity as its side effect [7]. The Gentamicin made nephrotoxicity is well established experimental model for drug in made renal injury [8]. Many animal experiments have verified irresistibly, the positive correlation between oxidative stress and nephrotoxicity [9]. Gentamicin induced nephrotoxicity by stimulating renal phospholipidosis through suppression of lysosomal hydrolases such as sphingomylinase and phospholipases in gain to causing oxidative stress [10].

Drug induced nephrotoxicity are frequently related with marked increase in blood urea, serum creatinine and acute tubular necrosis [11]. In the present study, drug induced nephrotoxicity were established by single daily intraperitoneal injection of Gentamicin. This toxicity characterized by pronounced raise in circulating levels of urea, serum creatinine and histological features of tubule nephritis in model control rats when compared with untreated normal rats. However, these changes were reduced by pretreatment with single daily stratified dose of *Hordeum vulgare* seeds for 10 days. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rat of clearance. Elevation of urea, blood urea nitrogen (BUN) and creatinine levels in serum was taken as the index of nephrotoxicity. The serum urea concentration is often considered a more reliable function prediction than serum creatinine. The cationic form of aminoglycosides attaches to the acidic phospholipids in brush border enzymes this results in the leakage of intracellular ions (K^+, Mg^+ and Ca^{++}), proteins and enzymes this results in the decreased glomerular filtration rate [12]. As per Ali Gentamicin is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to reduction of GFR[13]. The decreased glomerular filtration rate which is shown by an increase serum urea, blood urea nitrogen and creatinine [14]. The results of this study show that GM administration to rats produced a typical pattern of nephrotoxicity which was manifested by significant increase in serum urea, blood urea nitrogen and creatinine as compared to normal control. Administration of EHV 100 mg/kg, 250 mg/kg and 500 mg/kg has showed significant decrease in serum creatinine whereas EHV 250 mg/kg and 500 mg/kg was showed significant decrease serum urea and serum BUN as compared to GM group.

In the pathogenesis of GM nephrotoxicity oxidative stress is probably the most common pathogenic [15]. Recently, ROS were considered to be important mediators of Gentamicin-induced nephrotoxicity [16]. It has been found that the Gentamicin-treatment increases H_2O_2 production and it is known that H_2O_2 and O_2^- induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate (GFR). O_2^-, this radical can react with nitric oxide (NO) to form peroxynitrite, cytotoxic oxidant radical species. The inactivation of NO by O_2 - could also lead to a decrease in the GFR. It has been suggested that the oxidative stress induces tubular damage. It is known that the increase in ROS levels induces cytotoxicity due to a concerted action of oxygen and nitrogen-derived free radicals. The reduction in renal antioxidant enzymatic defense could aggravate the oxidative damage in rats. In the present study Gentamicin has significant increased the malondialdehyde levels while decrease Superoxide dismutase, reduced glutathione and catalase activities as compared to normal control. The administration of *Hordeum vulgare* seeds showed significant decrease in malondialdehyde level whereas increase the superoxide dismutase, reduced glutathione content and catalase activities as compared to Gentamicin control animals. *Hordeum vulgare* seeds attenuate the Gentamicin induced nephrotoxicity probably mediated by its anti-oxidant properties.In histopathological study of normal group some blood vessels were dialated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seed within the interstitium. GM treated group showed sever proximal tubular necrosis, loss of lining epithelium along with mononuclear cell infiltrations, diffuse glomerular and blood vessels congestion. In Ethanolic extract of HV 500 mg/kg treated group, varying degrees of regeneration and only small foci of mononuclear infiltration could be seen within the interstitium. From the histopathological studies we can conclude confirm the Ethanolic extract of *H. vulgare* seeds showed predominantly regenerative and protective effect on gentamicin induced nephrotoxicity.

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