INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

AN EXPERIMENTAL EVALUTION ON NEPHROPROTECTIVE ACTIVITY OF THE FLOWERS OF *SALIX CAPREA* (SALICACEAE)

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Article Received on: 10/12/11 Revised on: 06/02/12 Approved for publication: 29/02/12

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ABSTRACT
Salix caprea.L. (Salicaceae), commonly called Bede-mushk, is a shrub growing in Iran, Kashmir, north-western Himalayas and Punjab. The plant has been used in traditional system of medicine as antioxidant, antifungal, antimicrobial, anti-inflammatory, hemolysis and nephroprotective drug.
KEYWORDS: *Salix caprea*, Blood urea nitrogen (BUN), Creatinine, Nephroprotective activity.

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INTRODUCTION

Salix caprea L. (Salicaceae), commonly known as Bed mushk, Sallow; Willow-bark, is a large shrub or small tree planted for ornamental purpose in the north-western Himalayas, Persia and Afghanistan. It has been reported to have antioxidant, antifungal and antimicrobial and is used to treat hemolysis[1]. The flowers of this plant has been reported for antioxidant activity[2,] hemolysis[3,4], the bark is useful as astringent application for piles. This drug is beneficial as stimulant, aromatic and to cure influenza[5]. The plant is used in Swedish traditional medicine to treat inflammatory disease and wounds. Renal failure is a condition where retention of metabolic by products accumulates in response to deterioration of renal functions. In recent times, the treatment of renal failure has been revolutionized by the development of modern medical and surgical procedure *viz* haemodialysis, renal transplantation and chemotherapy. These procedures are so costly that all patients cannot afford their cost. On the other hand haemodialysis only replaces the toxic metabolites from blood and it does not recover the endocrine function of the kidney. Since no effective therapy is available in the present modern system of medicine for the treatment of renal failure, it is important to search the traditional systems of medicine viz Ayurveda, Unani and Sidha which may provide a solution. The drugs used in these systems of medicine originate from plants, animals and minerals and have minimal side effects. The present study has been carried out to evaluate the nephroprotective activity of *Salix caprea* flowers.

MATERIAL AND METHODS

Plant Material

The dried flowers of *Salix caprea* were purchased from Khari Baoli market of Delhi. The authenticity of the material was established by Prof.M.P.Sharma, the Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen No-PRL/ JH/08/37 in deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Animals

Albino rats of wistar strain, used in the experiments, were obtained from Central Animal House Jamia Hamdard. The animals were kept under standard laboratory condition 28 ±1 °C. The animals were fed with standard diet. Healthy albino rats of either sex (180-200 g) were divided into three groups of six animals each group. The same quantity of food in the form of pallets was given to all animals. Plant extract was administrated in the form of suspension. The extract fraction were tested for their nephroprotective activity in two experimental activities, Blood urea nitrogen (BUN) and Creatinine models. The renal effects of these extracts were studied by monitoring blood urea nitrogen and serum creatinine level.

Preparation of extract

The dried plant material (1.32 kg) was crushed thoroughly and extracted exhaustively with alcohol in a Soxhlet apparatus. The solvent was removed under reduced pressure to get a dark viscous mass (18.86 %) The alcoholic extract was fractionated by treating successively with petroleum ether and water soluble and water insoluble fraction and the solvents were removed as usual process to give PET (50 g) WS (38 g) and WIS (20 g).

Animal group I in the water containing 1% CMC served as control while the animal of group II and III received toxicant (3mg/kg) and *Salix caprea* (100g/kg), respectively. In another set of experiment the effects of petroleum ether soluble (PET) 50 g, water soluble (WS) 38 g and water insoluble (WIS) 20 g were evaluated. These treatments orally were given to animal for 10 days. A single dose of mercuric chloride 3 mg/kg body wt was administrated s.c (subcutaneously) in the neck region in volume of 1ml/kg to all groups of animals except of group I (control) on the 10th day of study. After 48 hrs of $HgCl_2$ administration, blood samples were collected from retro orbital pluxes under light anesthesia with the help of glass capillary. The blood samples were centrifuged. The serum was separated and estimated for BUN and creatinine. On the next day the blood samples were collected and kept at room temp for two hrs and then samples were centrifuged. The serum was then separated and estimated for BUN and creatinine.

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the nephroprotective activity of flowers of *Salix caprea* alcoholic extract against Blood urea nitrogen and Creatinine. The alcoholic extract of the flowers of *Salix caprea* inhibited

28.52 % [Table-1 BUN in rats. Water soluble fraction exhibited significant reduction of the BUN in rat and it was up to 68.64 % [Table-3]. However, water soluble portion of the flowers showed slight inhibition up to 31.36 % [Table-3] of the nephroprotective. The alcoholic extract presented 24.75 % [Table-2] inhibition of the Creatinine in rats. The water soluble and water insoluble fractions of the extracts showed significant reduction of the Creatinine in rats which were 50.97 % [Table-4] and 39.67 % [Table-4], respectively. The renal effects and its fractions were studied by monitoring blood levels of urea and creatine. Acute exposure to Hg^{+} Dosage of 3mg/kg is known to produce a highly selective necrosis of S_3 segment of the proximal tubule. Results of this study confirmed that mercuric chloride at 3 mg/kg produced significant nephrotoxicity as evident by increase in the levels of blood urea nitrogen BUN and serum creatinine. Rats of this model, which received alcoholic extract showed a significant prevention in the rise of the serum markers like BUN and creatinine. In order to identify the fractions containing active constituents, alcoholic extracts were fractionated into petrol soluble (PS) and petrol insoluble (PIS). Petrol soluble fractions were resolved into water soluble (WS) and water insoluble (WIS). The present work indicates that all fractions (PS, WS, WIS) significantly inhibit the rise of BUN and serum creatinine but the effect of WS is pronounced.

The effects of toxic metals on kidney due to occupational or therapeutic exposure to these agents have been known for many years. Renal effects of heavy metals are important as they tend to accumulate in the kidneys and may produce a broad spectrum of morphologic and functional effects on the kidney. The kidney in two ways may excrete metals, they may either be filtered from blood by renal glomerulus or secreted directly into urine by transport across renal tubular lining cells from peritubular capillaries. Metals which are filtered by the glomerulus may be reabsorbed again by the tubular cells and concentrate within the cells. Therefore, there are two initial events that enhance the vulnerability.One of the first clinical manifestations of aminoglycosides induced renal toxicity is the presence of non -oliguric renal failure with the elevation of serum creatinine concentration. Enzymuria with N- acetylglucosaminidase, proteinuria, β_2 – microglubin and lysozymuria may also be observed. As no effective therapy is available in the present modern system of medicine for the treatment of renal failure, it is important to search the traditional systems of medicine viz Ayurveda, Unani and Sidha which may provide a solution. The drugs used in these systems of medicine originate from plants, animals and minerals and have minimal side effects.

ACKNOWLEDGMENT

We thank to Professor M.P. Sharma for technical assistance. Jamia Hamdard Delhi for providing authentic plant material.

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Table1: Effect of alcoholic extract of *Salix caprea* on BUN

Groups	Treatment	BUN(mg/dl) Mean ± S.E.	% changes	% inhibition
I	Control (1%CMC)	0.155 ± 0.011	-	-
II	Toxicant($HgCl_2$)3 mg / kg (1ml/kg)	0.298 ± 0.0054	100%	-
III	Alcoholic extract (100 mg) + $HgCl_2$ (3 mg/kg).	0.213 ± 0.0054	71.48%	28.52%

Table2: Effect of alcoholic extract of *Salix caprea* on creatinine

Groups	Treatment	Creatinine(mg/dl) Mean ± S.E.	% changes	% inhibition
I	Control(%CMC)	6.08 ± 0.219	-	-
II	Toxicant ($HgCl_2$)(3 mg / kg) (1ml/kg)	9.21 ± 0.187	100%	-
III	Alcoholic extract (100mg) + $HgCl_2$ (3mg/kg).	6.93 ± 0.092	75.25%	24.75%

Table3:Effect of fractions of alcoholic extract of *Salix caprea* on BUN

Groups	Treatment	BUN (mg/dl) Mean ± S.E.	% changes	% inhibition
I	Control (%CMC)	0.1317 ± .0060	-	-
II	Toxicant ($HgCl_2$) (3 mg / kg) (1ml/kg)	0.2283 ± .010	100%	-
III	PET+$HgCl_2$, (100 mg + 3mg/kg)	0.1800 ± .0058	78.85%	21.15%
IV	WS+$HgCl_2$, (100 + 3 mg/kg)	0.1567 ± .0061	68.64%	31.36%
V	WIS+$HgCl_2$, (100 + 3mg/kg)	0.1783 ± .0048	78.1%	21.90%

Table4: Effect of fraction of alcoholic extract of *Salix caprea* on creatinine

Groups	Treatment	Creatinine(mg/dl) Mean ± S.E.	% changes	% inhibition
I	Control (%CMC)	0.1983 ±.006	-	-
II	Toxicant ($HgCl_2$,)(3 mg / kg) (1ml/kg)	0.5167 ±.0067	100%	-
III	PET+ $HgCl_2$, (100 mg + 3mg/kg)	0.3250 ± .0076	62.9%	37.10%
IV	WS+$HgCl_2$, (100 + 3 mg/kg)	0.2533 ±.0123	49.03%	50.97%
V	WIS+$HgCl_2$, (100 + 3mg/kg)	0.3117 ± .0119	60.33%	39.67%

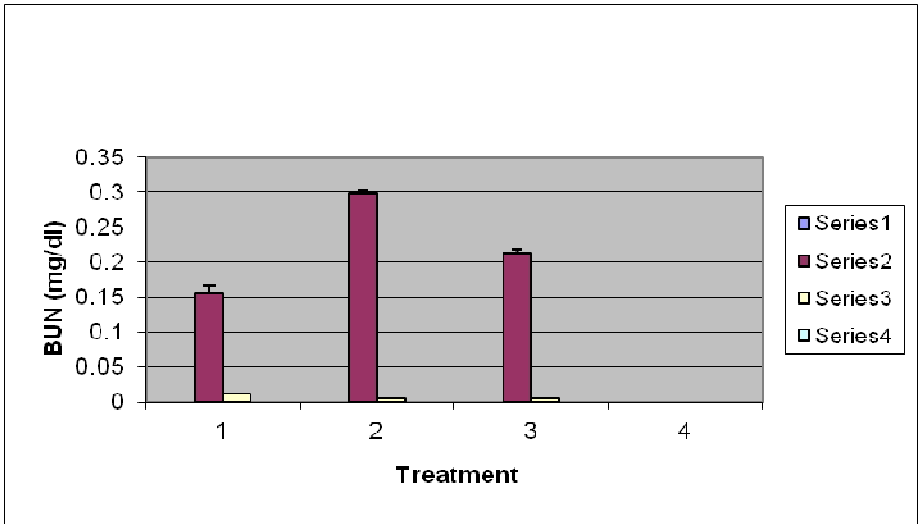

Fig 1: Effect of alcoholic extract of *Salix caprea* on BUN in rat model

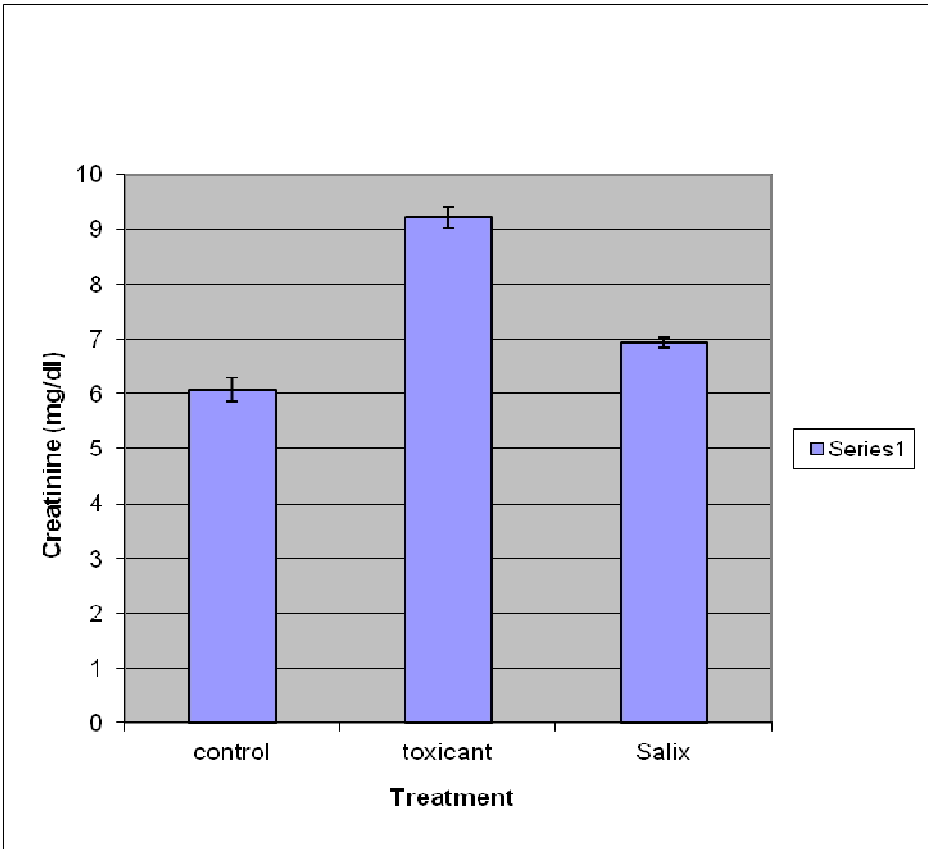

Fig 2: Effect of alcoholic extract of *Salix caprea* on creatinine in rat model

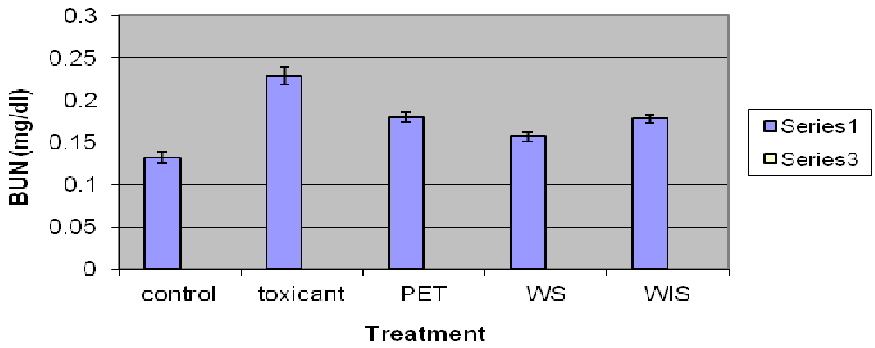

Fig 3: Effect of petroleum ether soluble (PS), water soluble (WS), water insoluble (WIS) Portion of alc extract of *Salix caprea* on BUN in mercuric chloride induced nephrotoxicity in rat model

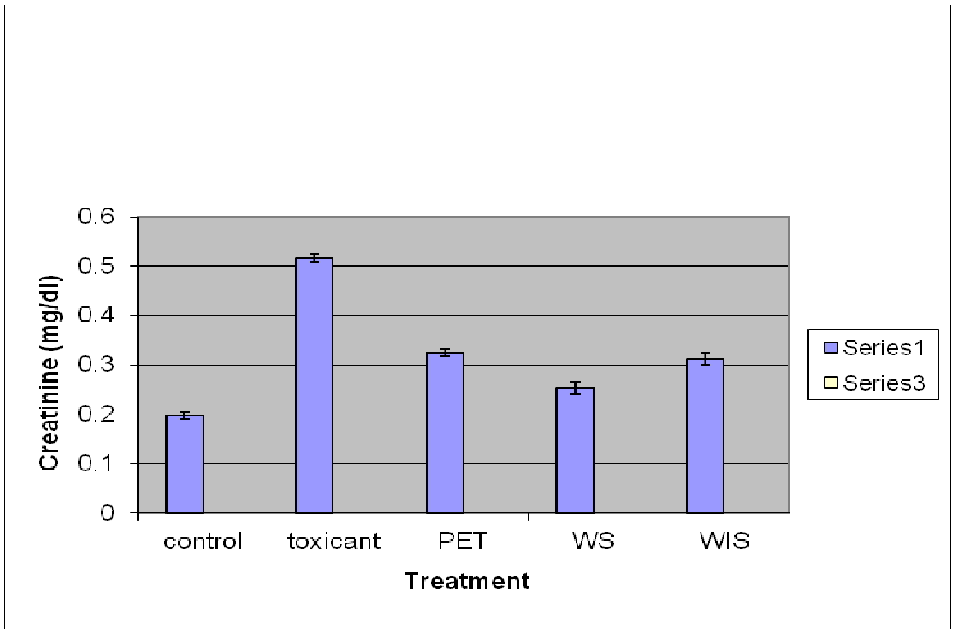

Fig 4: Effect of petroleum ether soluble (PS), water soluble (WS), water insoluble (WIS)Portion of alc extract of *Salix caprea* on creatinine in mercuric chloride induced nephrotoxicity in rat model

Source of support: Nil, Conflict of interest: None Declared