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Research Article

EFFECT OF *CITRUS MEDICA* BRITT. EXTRACT ON SERUM BIOCHEMICAL CHANGES IN ETHYLENE GLYCOL INDUCED RENAL DAMAGE IN WISTAR RATS

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ABSTRACT: Stone in the urinary tract is one of the common conditions associated with urinary system. *Citrus medica* Britt. is known for its protective activity on renal system. A study was performed on 72 Wistar rats to validate the claim. Renal damage was induced in rats using 0.75 % (v/v) ethylene glycol along with drinking water as stone inducing agent. Serum biochemical parameters (Calcium, Urea, BUN, Uric Acid and Creatinine) were measured to know the reno-protective activity of *Citrus medica*.

Key words: *Citrus medica*, Ethylene glycol, Renal damage, Serum biochemistry, Wistar rat.

INTRODUCTION

Renal diseases are common now-a-days. In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys and renal damage (Mohamed *et al.*, 2007). Present study was conducted to find alternative way to protect the renal damage. *Citrus medica* have been used traditionally from ancient time to treat renal diseases (Chavda *et al.*, 2012). Ethylene glycol is a well-known stone inducer chemical in rats and its use is widely accepted as stone inducer chemical. It has been proved that ethylene glycol induces hyperoxalouria in rat (Mike *et al.*, 2009).

MATERIALS AND METHODS

The present work was conducted at the Department of Veterinary Medicine, Ananad Agricultural University, Anand. The study was conducted on adult healthy 72 Wistar rats. All the protocols as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines on the care and use of laboratory animals were followed and approved by the Institutional Animal Ethics Committee (IAEC) of Veterinary College, Anand, Gujarat, India.

Ethylene glycol was used to induce kidney stone (Loba Chemi Pvt. Ltd, Wode house road, Mumbai, India). All other chemicals used in the study were of analytical grade.

Plant

Citrus medica plant was used to study reno-protective activity study. Fresh unripe fruits of *Citrus medica* were procured from National Research Centre for Medicinal and Aromatic Plants

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(NRCMAP), Boriavi, Anand, Gujarat. Aqueous, alcoholic and chloroform extracts were prepared from fresh unripe fruits of *Citrus medica.*

Experimental protocol

Rats were selected randomly and divided in to 9 groups (Group – I, II, III, IV, V, VI, VII, VIII and IX) with eight animals in each group. Group I served as normal control consisted of healthy animals. Urolithiasis was induced in group II, III. IV, V, VI, VII, and VIII animals using 0.75 % (v/v) ethylene glycol along with drinking water as stone inducing agent. Group I and Group IX animals were given normal water. Group II animals were given ethylene glycol but kept untreated with any *Citrus medica* extract. Group IX animals are not given ethylene glycol but treated with *Citrus medica* extract. Group I animals served as normal control, Group II animals served as lithiatic control and Group IX animals served as extract control. Aqueous, alcoholic and chloroform extracts of *Citrus medica* were dispersed in water and administered at to animals of group III, IV, V, VI, VII and IX. Group III animals were administered aqueous extract of *Citrus medica* at dose of 200 mg/kg body weight and group IV animals were administered aqueous extract of *Citrus medica* at dose of 400 mg/kg body weight. Group V animals were administered alcoholic extract of *Citrus medica* at dose of 200 mg/kg body weight and group VI animals were administered alcoholic extract of *Citrus medica* at dose rate of 400 mg/kg body weight. Animals of Group VII and VIII were administered chloroform extract at dose rate of 200 mg/kg and 400 mg/kg as respectively. Group IX animals were administered with aqueous, alcoholic and chloroform extracts of *Citrus medica* as extract control (two animals for each extract). The extracts were administered to rats directly in oesophagus by using rat oral feeding needle with 2 ml BD syringe for 28 days.

Sample collection

After 28 days of dosing period, on the 29th day blood samples were collected from all the animals by retro-orbital plexuses puncture under light ether anaesthesia with the help of capillary tube as described by Sorg and Buckner (1964). Blood samples (2 ml) collected in K_3EDTA test tubes were utilized for haematological evaluation, whereas blood samples (2 ml) collected in centrifuge tubes without anticoagulant were allowed to clot at room temperature (26 ± 2 °C). Serum was harvested by centrifugation at 3000 rpm for 10 minutes at 10^{o}C (Eppendorf 5804 R, Germany) and stored at - 40^{o}C for biochemical analysis and analyzed within 12 hrs.

Biochemical analysis

Serum biochemical parameters (Calcium, Urea, BUN, Uric Acid and Creatinine) were estimated using standard assay kits (Coral Clinical System, Goa, India) with the help of clinical serum biochemistry analyzer (Photometer 5010 $_{V5+}$, Dynalab Enterprize).

Statistical analysis

One-way-analysis of variance (ANOVA) was used to compare the effects of *Citrus medica* extracts with normal control group, ethylene glycol model group and group given plant extract on different variables like body weight, haematological and biochemical parameters by using software SPSS (Version 20). All the data have been presented as mean ± SE.

RESULTS AND DISCUSSION

Calcium

Mean values of Calcium in groups I, II, III, IV, V, VI, VII, VIII and IX were 7.50±0.32, 12.75±0.36, 10.5±0.42, 8.75±0.49, 10.75±0.36, 8.87±0.39, 10.25±0.36, 8.25±0.36 and 7.87±0.39 mg/dl respectively. Group I (7.50 mg/dl) showed lower mean value of calcium as compare to all other groups. As group I served as normal control. Group II (12.75 mg/dl) showed higher mean value of calcium as compare to all other groups. It is due to induction of calcium containing stones following administration of ethylene glycol. Group III

(10.50mg/dl), V (10.75 mg/dl) and VII (10.25 mg/dl) showed significant (P<0.05) decrease in mean value of calcium as compare to group II (12.75 mg/dl). It is due the treatment given to all these groups with 200 mg/kg of aqueous, alcoholic and chloroform extracts respectively. In these groups mean values of calcium is significant (P<0.05) lower than group II, but it is not in the range of group I (7.50). It indicates that aqueous, alcoholic and chloroform extracts of *Citrus medica* protects against induction of renal damage following ethylene glycol administration. Group IV (8.75 mg/dl) and VI (8.87mg/dl) showed highly significant (P<0.05) decrease in mean values of calcium as compare to group II (12.75 mg/dl) and also compare to group III (10.50 mg/dl) and V (10.75 mg/dl). It suggests that aqueous and alcoholic extracts of *Citrus medica* are more effective at the dose rate of 400 mg/kg. It suggests that increase in dose rate of aqueous and alcoholic extract effectively decrease mean value of calcium. It means aqueous and alcoholic extracts showed dose-dependent relationship in decreasing elevated calcium levels. Group VIII (8.25 mg/dl) showed no significant (P<0.05) decrease in mean value of calcium at dose rate of 400 mg/kg than group VII (10.25 mg/dl). It attributes that increase in dose rate of chloroform extract does not effectively decrease mean value of calcium. Chavada *et al*. (2012) yielded the similar results. These results also agrees with Kishore *et al.* (2013).

Urea

Mean values of Urea in groups I, II, III, IV, V, VI, VII, VIII and IX were 14.75+0.52, 39.75±1.71, 19.37±0.90, 17.25±0.72, 20.37±0.49, 18.37±0.77, 19.87±0.89, 17.75±0.61 and 14.62±0.32 mg/dl respectively. Group II (39.75 mg/dl) showed higher mean value of urea as compare to group I (14.75 mg/dl) and all other groups. Groups III (19.37 mg/dl), IV (17.25 mg/dl), V (20.37 mg/dl), VI (18.37 mg/dl), VII (19.87 mg/dl) and VIII (17.75 mg/dl) showed significant (P<0.05) decrease in mean values of urea as compare to group II (39.75 mg/dl) which suggests that aqueous, alcoholic and chloroform extracts of *Citrus medica* protects against ethylene glycol induced renal damage. Here, there is no significant (P<0.05) decrease in mean value of urea in case of groups IV (17.25 mg/dl), VI (18.37 mg/dl) and VIII (17.75 mg/dl) as compare to groups III (19.37 mg/dl), V (20.37 mg/dl) and VII (19.87 mg/dl) respectively. It indicates that aqueous, alcoholic and chloroform extracts of *Citrus medica* does not have dose-dependent relationship in renoprotective activity against ethylene glycol induced renal damage. This results agrees with Chavada *et al*. (2012). Crystal deposition in kidney decreases glomerular filtration rate (GFR) due to the obstruction to the outflow of urine in urinary system, due to this the waste products particularly nitrogenous substances such as urea get accumulated in the blood.

BUN

Group II (38.75 mg/dl) showed higher mean value of BUN as compare to group I (20.50 mg/dl) and all other groups. Groups III (28.25 mg/dl), IV (26.12 mg/dl), V (29.62 mg/dl), VI (27.75 mg/dl), VII (27.50 mg/dl) and VIII (25.62 mg/dl) showed significant (P<0.05) decrease in mean values of BUN as compare to group II (38.75 mg/dl) which suggests that aqueous, alcoholic and chloroform extracts of *Citrus medica* showed renoprotective effect against ethylene glycol induced renal damage. There is no significant (P<0.05) decrease in mean value of BUN in case of groups IV (26.12 mg/dl), VI (27.75 mg/dl) and VIII (25.62 mg/dl) as compare to groups III (28.25 mg/dl), V (29..62 mg/dl) and VII (27.50 mg/dl) respectively. It indicates that aqueous, alcoholic and chloroform extracts of *Citrus medica* does not have dose-dependent relationship in renoprotective activity against ethylene glycol induced renal damage. This observation agrees with Baheti and Kadam (2013) and Purnima *et al*. (2013).

Table 1. Serum biochemical changes in different groups of Wistar rats.

	Mean ± SE (mg/dl) (n=72)				
Group	**Calcium**	**Urea**	**BUN**	**Uric Acid**	**Creatinine**
I	7.50±0.32	14.75 ± 0.52	20.50 ±0.77	4.25 ±0.72	0.53 ±0.06
II	12.75 ±0.36	39.75 ± 1.71	38.75 ±0.79	7.12 ±0.44	2.70 ±0.10
III	10.50 ±0.42	19.37 ± 0.90	28.25 ±0.90	5.87±0.22	1.40 ±0.07
IV	8.75 ±0.49	17.25 ± 0.72	26.12 ±0.63	5.37 ±0.32	1.25 ±0.05
V	10.75 ±0.36	20.37 ± 0.49	29.62±0.65	5.62 ±026	1.60 ±0.08
VI	8.87 ±0.39	18.37 ± 0.77	27.75 ±0.45	5.25±0.25	1.30 ±0.06
VII	10.25 ±0.36	19.87 ± 0.89	27.50 ±1.16	5.75±0.25	1.65±0.04
VIII	8.25 ±0.36	17.75± 0.61	25.62±1.10	5.12±0.22	1.27±0.05
IX	7.87±0.39	14.62± 0.32	20.25±0.64	4.62±0.26	0.56 ±0.04

Uric acid

Group II (7.12 mg/dl) showed higher mean value of uric acid as compare to group I (4.25 mg/dl) and all other groups. Groups III (5.87 mg/dl), IV (5.37 mg/dl), V (5.62 mg/dl), VI (5.25 mg/dl), VII (5.75 mg/dl) and VIII (5.12 mg/dl) showed significant ($P<0.05$) decrease in mean values of uric acid as compare to group II (7.12 mg/dl), so we can say that aqueous, alcoholic and chloroform extracts of *Citrus medica* have renoprotective effect against induction of renal damage following ethylene glycol administration. There is no significant decrease in mean value of uric acid in case of groups IV (5.37 mg/dl), VI (5.25 mg/dl) and VIII (5.12 mg/dl) as compare to groups III (5.87 mg/dl), V (5.62 mg/dl) and VII (5.75 mg/dl) respectively. It indicates that aqueous, alcoholic and chloroform extracts of *Citrus medica* does not enhance the nephron-protective activity with increase in dose rates. Vidhya *et al.* (2013) and Ingale *et al.* (2010) observed the similar results. Increased uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation.

Creatinine

Group II (2.70 mg/dl) showed higher mean value of creatinine as compared to group I (0.53 mg/dl) and all other groups. In case of groups III (1.40 mg/dl), IV (1.25 mg/dl), V (1.60 mg/dl), VI (1.30 mg/dl), VII (1.65 mg/dl) and VIII (1.27 mg/dl), there is significant ($P<0.05$) decrease in mean values of creatinine as compare to group II (2.70 mg/dl) which attributes that aqueous, alcoholic and chloroform extracts of *Citrus medica* exhibit renoprotective activity against ethylene glycol induced renal damage. There is no significant ($P<0.05$) decrease in mean value of creatinine in case of groups IV (1.25 mg/dl), VI (1.30 mg/dl) and VIII (1.27 mg/dl) as compare to groups III (1.40 mg/dl), V (1.60 mg/dl0 and VII (1.65 mg/dl) respectively (Table 1). It indicates that

aqueous, alcoholic and chloroform extracts of *Citrus medica* does not show dose-dependent relationship in renoprotective activity against ethylene glycol induced renal damage. Purnima *et al.* (2013) found the similar results. In renal damage the glomerular filtration rate (GFR) due to obstruction to the outflow of urine by stones in urinary system. Due to this the waste product, particularly nitrogenous substances such as creatinine get accumulated in blood.

CONCLUSION

Citrus medica has significant effect on serum biochemical parameters in Ethylene glycol induced renal damage. *Citrus medica* extract can be used with no side effect to prevent the incidence of renal damage. Study data revealed that *Citrus medica* possesses renoprotective activity. Medicinal plants can be used to prevent incurable diseases.

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