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Uricosuric activity of *Tinospora cordifolia*

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Abstract

Uricosuric activity of different extracts of *Tinospora cordifolia* was studied in hyperuricemia induced in albino Wistar rat using potassium oxonate. The uric acid level in serum and urine were measured. Uricosuric activity was also evaluated using phenol red dye excretion model. Phenol red levels were measured in blood. In potassium oxonate induced hyperuricemia, probenecid, aqueous, hydro-alcoholic, dichloromethane extract and galo satwa (starch of *T. cordifolia*) significantly lowered the serum uric acid levels. All the extracts increased uric acid excretion and decreased the elevated serum uric acid levels induced due to potassium oxonate. Probenecid, aqueous extract and galo satwa significantly increased fractional excretion of uric acid and phenol red levels in blood indicating uricosuria. Polysaccharides in aqueous extract and galo satwa may be responsible for uricosuric action.

Introduction

Tinospora cordifolia (Willd) Miers Ex Hook is a large, glabrous, climbing shrub indigenous to tropical Indian subcontinent and other Asian countries from family *Menispermaceae*. It is widely used especially for immunomodulatory (Desai et al., 2007), cardioprotective (Rao et al., 2005) and hepatoprotective (Adhvaryu et al., 2008) activity. In Ayurveda, it has shown antirheumatic and antigout activity (Gogte, 2000). In humans, it has shown beneficial effect in diabetic foot ulcers (Purandare and Supe, 2007) and allergic rhinitis (Badar et al., 2005). Prolonged and untreated hyperuricemia results into gout, a severe inflammatory condition. Sustained hyperuricemia leads to impaired blood pressure control, renal impairment and nephropathy (Feig et al., 2006). The drugs used in treatment of hyperuricemia and for prophylaxis of gout include xanthine oxidase inhibitors viz. allopurinol and uricosuric agents like probenecid and benzbromarone (Chohan and Becker, 2009). Allopurinol is contraindicated in patients with compromised renal function (Perez-Ruiz et al., 2005) and frequently causes severe hypersensitivity reactions (Halevy et. al., 2008). Febuxostat is contraindicated in liver failure (Rider et. al., 2010). Uricosuric agents furthermore can't be administered in patients with renal stones (Perez-Ruiz et al., 1999). So, it is the need of the today to find out novel drug with minimal adverse effects. Galo satwa is the sedimented starch of *T. cordifolia*, the most widely used preparation in Indian system of medicine.

In this study, we tried to evaluate the uricosuric activity of aqueous, hydro-alcoholic, dichloromethane extracts and galo satwa of *T. cordifolia*.

Materials and Methods

Drugs and chemicals

Dichloromethane (Sisco Research Laboratories Pvt. Ltd., India), phenol red (Merck Ltd., India), potassium oxonate (Acros Organics-Thermo Fisher Scientific, USA), probenecid (Geno Pharmaceuticals Ltd., India), creatinine and uric acid kits (Beacon Diagnostics Pvt. Ltd., India) were used.

Collection of plant

Fresh stems of *T. cordifolia* (thickness of stem at least 1

cm; approximately 5 kg) were collected in summer-rainy season (June to August, that is, flowering season) from the botanical garden of K. B Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India. Plant was authenticated (Taxonomist: Dr. Hitesh Solanki, Department of Botany, University School of Sciences, Ahmedabad, Gujarat, India) and a herbarium specimen (PH/09/0015) was preserved at the Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, India, for future reference.

Preparation of extracts

Stems of *T. cordifolia* were shade dried (weight loss 60%) and reduced into coarse powder with help of ball mill. Aqueous extract, hydro-alcoholic extract and dichloromethane extract from dried powdered stems; galo satwa from fresh stems were prepared in following manner:

Preparation of aqueous extract

Powder (100 g) was boiled in distilled water in 1:3 ratio at 100ºC for 30 min with gradual shaking. After 30 min, the mixture was filtered and the filtrate was evaporated to dryness at 100ºC on water bath in a tared flat-bottomed petridish with occasional shaking. Residue was weighed (yield 16% w/w).

Preparation of hydro-alcoholic extract

Powder (100 g) was defatted with petroleum ether and then extracted with mixture of ethanol (95% v/v) and distilled water (80:20) using a Soxhlet continuous extraction apparatus. The filtrate was evaporated to dryness at 100ºC on water bath in a tared flat-bottomed petridish with gradual shaking, and residue was weighed (yield 13.6% w/w).

Preparation of dichloromethane extract

Powder (100 g) was defatted with petroleum ether followed by chloroform and finally extracted with dichloromethane using a Soxhlet continuous extraction apparatus for 1 week. The filtrate was evaporated to dryness at 100ºC on water bath in a tared flat-bottomed petridish, and residue was weighed (yield 2.4 % w/w).

Preparation of galo satwa

The fresh stems (250 g) were cut, crushed to coarse and crush was socked in distilled water in sufficient amount for 8 hours. It was properly macerated and filtered with nylon cloth. The filtrate was kept to allow the starch to settle down the supernatant was decanted and the sediment was further washed with fresh distilled water till it becomes whitish. Finally it was dried and residue was weighed (yield 10.2% w/w).

Phytochemical analysis of T. cordifolia extracts

Phytochemical tests, for the presence of alkaloids, reducing and non-reducing sugars (polysaccharides), tannins, flavonoids, sterols and terpenoids were performed for all four extracts (Harborne, 1998). Dragendorff's test was carried out for presence of alkaloids. Orange colored precipitates were observed. For the presence of polysaccharides, Molisch's test (purple color ring) and Fehling test (yellow and then brick red precipitates) were performed. For tannins, lead acetate was added and white precipitates were observed. In Shinoda test for flavonoid, to the extract solutions, small piece of magnesium ribbon and 3 to 4 drops of concentrated sulfuric acid were added and red color was observed. For phenolic compounds, drop of freshly prepared $FeCl_3$ solution was added to extracts and brownish green color precipitates were observed. For sterols and triterpenoids, Liberman Buchardt test was performed. To the extracts, acetic anhydride and 2 drops of sulfuric acid were added. Purple to violet color was observed.

Animals

Female albino Wistar rats (body weight 200 to 250 g) were used. The animals were acclimatized to standard condition of temperature (20-22ºC) with relative humidity (30 to 70%) and 12 hours alternate light and dark cycle in poly propylene cages. Animals were kept on free access to food and water *ad libitum* during the course of experiment.

Uricosuric activity in oxonate induced hyperuricemia

Hyperuricemia was induced using potassium oxonate, a uricase inhibitor to study *in vivo* uricosuric effect of extracts. Food and water were withheld overnight prior to study. Hyperuricemia was induced as described by Murugaiyah and Chan, 2009. Based on pilot study previously performed, the dose of each extract was decided. The doses for extracts were aqueous (500 mg/kg), hydro-alcoholic (100 mg/kg), dichloromethane (100 mg/kg) and galo satwa (1,000 mg/kg). Animals were divided into 7 groups of 6 animals in each. All the animals received either extract or standard via oral route. Extracts and potassium oxonate were administered via two different routes to avoid any possible interaction between them. Animals in all the groups except control Group I were administered potassium oxonate (250 mg/kg, i.p, oid) and uric acid (UA) (1 g/kg, p. o, oid), 1 hour prior to administration of test or standard, on day 1, 3 and 7. Group II served as disease group (received potassium oxonate and UA). Standard group (III) received probenecid (50 mg/kg, oid). Group IV, V, VI and VII received, aqueous extract (500 mg/kg, oid), hydro-alcoholic extract (100 mg/kg, oid), dichloromethane extract (100 mg/kg, oid) and galo satwa (1,000 mg/kg, oid) respectively. Extracts and probenecid were administered from day 1 to 7. Probenecid, aqueous extract and galo satwa were suspended in distilled water using carboxymethyl cellulose (1% w/v). Hydro-alcoholic extract was dissolved in distilled water. Dichloromethane extract

was suspended using 20% tween 20 aqueous solution. After 2 hours of administration of either extract or probenecid, blood was collected using retro orbital puncture. Urine was collected at 5 hours and volume was measured. Blood was centrifuged at 2817 xg using cooling centrifuge and serum was separated. Serum uric acid and urinary uric acid levels were estimated (on day 1, 3 and 7) using kits based on uricase method (Fossati et al., 1980). Serum creatinine (mg/dL) and urine creatinine (mg/dL) were measured (on day 1, 3 and 7) using kits based on Jaffe's method (Murray, 1989) respectively. Fractional excretion of uric acid (FEUA) was calculated using given equation to assess the uricosuric effect of the extracts (Dan et al., 1994). FEUA = ([urine urate]/[plasma urate])/([urine creatinine]/[plasma creatinine]).

Uricosuric activity in phenol red model

Extracts were studied further for evaluation of uricosuric activity in phenol red model as described by Turner, 1965. Animals were divided into 6 groups of 6 animals in each. All the animals received either extracts or probenecid orally (one time only) 30 min prior to intravenous injection of phenol red. Aqueous solution of phenol red (3%, 2.5 mL/kg) was injected via tail vein followed by 0.5 mL normal saline. Group I (control) received saline and Group II (standard) received probenecid (50 mg/kg, oid). Test Group III, IV, V and VI received aqueous extract (500 mg/kg, oid), hydro-alcoholic extract (100 mg/kg, oid), dichloromethane extract (100 mg/kg, oid) and galo satwa (1000 mg/kg, oid) respectively. Extracts and probenecid were dispensed as mentioned previously. By retro-orbital puncture, blood was withdrawn at interval of 1 hour and 3 hours after administration of phenol red. Blood (0.2 mL) was diluted with 2 mL of 0.9 % NaCl solution, centrifuged and supernatant was collected. To 1 mL of the supernatant, 1 mL of 1% sodium carbonate solution and 8 mL of saline were added. Absorbance was measured at 546 nm using spectrophotometer. Concentration (µg/mL) of phenol red in blood sample was determined from standard graph. At each time interval the values in treated rats were compared statistically with those of controls.

Statistical analysis

All the data were expressed as mean ± SEM (n=6). $p<0.05$ was considered as statistically significant. Data were analyzed using one-way ANOVA followed by post hoc Tukey test using SPSS computer software version 7.5.

Results

Phytochemical study

Aqueous extract and galo satwa showed the presence of polysaccharides. Hydro-alcoholic extract showed the presence of polysaccharides and terpenoids. In dichloromethane extract, alkaloids and terpenoids were found.

Effect on serum uric acid levels

Administration of potassium oxonate and uric acid resulted into significant (4.5 ± 0.0, 5.0 ± 0.0, 4.0 ± 0.0) ($p<0.05$) increase in serum uric acid as compared to normal control on day 1, 3 and 7 (1.5 ± 0.0, 1.5 ± 0.0, 1.3 ± 0.0). Treatment with probenecid (3.2 ± 0.1, 3.7 ± 0.1, 3.4 ± 0.1), aqueous extract (3.3 ± 0.0, 3.9 ± 0.0, 3.6 ± 0.0), hydro-alcoholic extract (3.6 ± 0.1, 4.2 ± 0.0, 2.7 ± 0.1) and galo satwa (3.3 ± 0.0, 3.7 ± 0.1, 3.5 ± 0.1) significantly decreased the serum uric acid level ($p<0.05$). Dichloromethane extract significantly decreased the serum uric acid only on day 1 and 3 (3.7 ± 0.1, 4.4 ± 0.0). Uric acid levels were significantly less in galo satwa and aqueous extract treated animals as compared to dichloromethane extract (day 1, 3 and 7) and hydro-alcoholic extract (day 1 and 3) treated animals ($p<0.05$) (Figure 1).

Effect on urinary uric acid levels

There was significant increase in urinary uric acid level in hyperuricemic animals (153.3 ± 3.4, 176.8 ± 4.6, 182.8 ± 3.8) as compared to normal controls (106.1 ± 2.4, 122.2 ± 2.8, 131 ± 3.6) on day 1, 3 and 7. Probenecid (283.5 ± 4.0, 294.2 ± 5.6, 292.3 ± 5.4) and all the extracts i. e aqueous extract (230.2 ± 3.3, 244.7 ± 5.5, 252.8 ± 5.1), hydro-alcoholic (199.2 ± 4.6, 216.3 ± 2.578, 228.2 ± 4.4), dichloromethane extract (222.2 ± 6.0, 219 ± 5.5, 217.5 ± 4.5) and galo satwa (264.8 ± 9.1, 255.3 ± 8.4, 258 ± 4.4) significantly increased the urinary uric acid level as compared to hyperuricemic animals ($p<0.05$). Treatment with aqueous extract and galo satwa resulted into significant increase in urinary uric acid level as compared to hydro-alcoholic and dichloromethane extract group (day 3 and 7). Urinary uric acid level in aqueous extract was significantly higher as compared to hydro-alcoholic (day 1). On day 1, it was significantly higher in galo satwa as compared to hydro-alcoholic, dichloromethane extract and aqueous extract i.e all groups ($p<0.05$).

Effect on FEUA

FEUA was significantly increased in potassium oxonate induced hyperuricemic animals (1.9 ± 0.1, 1.9 ± 0.1, 2.1 ± 0.1) as compared to control (1.2 ± 0.1, 1.3 ± 0.1, 1.4 ± 0.0). Probenecid (4.2 ± 0.3, 3.2 ± 0.2, 4.3 ± 0.2) significantly increased FEUA as compared to hyperuricemic animals ($p<0.05$). FEUA was significantly higher in aqueous extract (2.6 ± 0.2, 2.8 ± 0.2) (day 1 and 7 but not on day 3) and galo satwa (2.7 ± 0.2, 2.7 ± 0.2, 3.5 ± 0.2) (day 1, 3 and 7) compared to disease control. Treatment with aqueous extract (day 1 and 7) and galo satwa (day 1, 3, 7) resulted into significant increase in FEUA as compared to hydro-alcoholic and dichloromethane

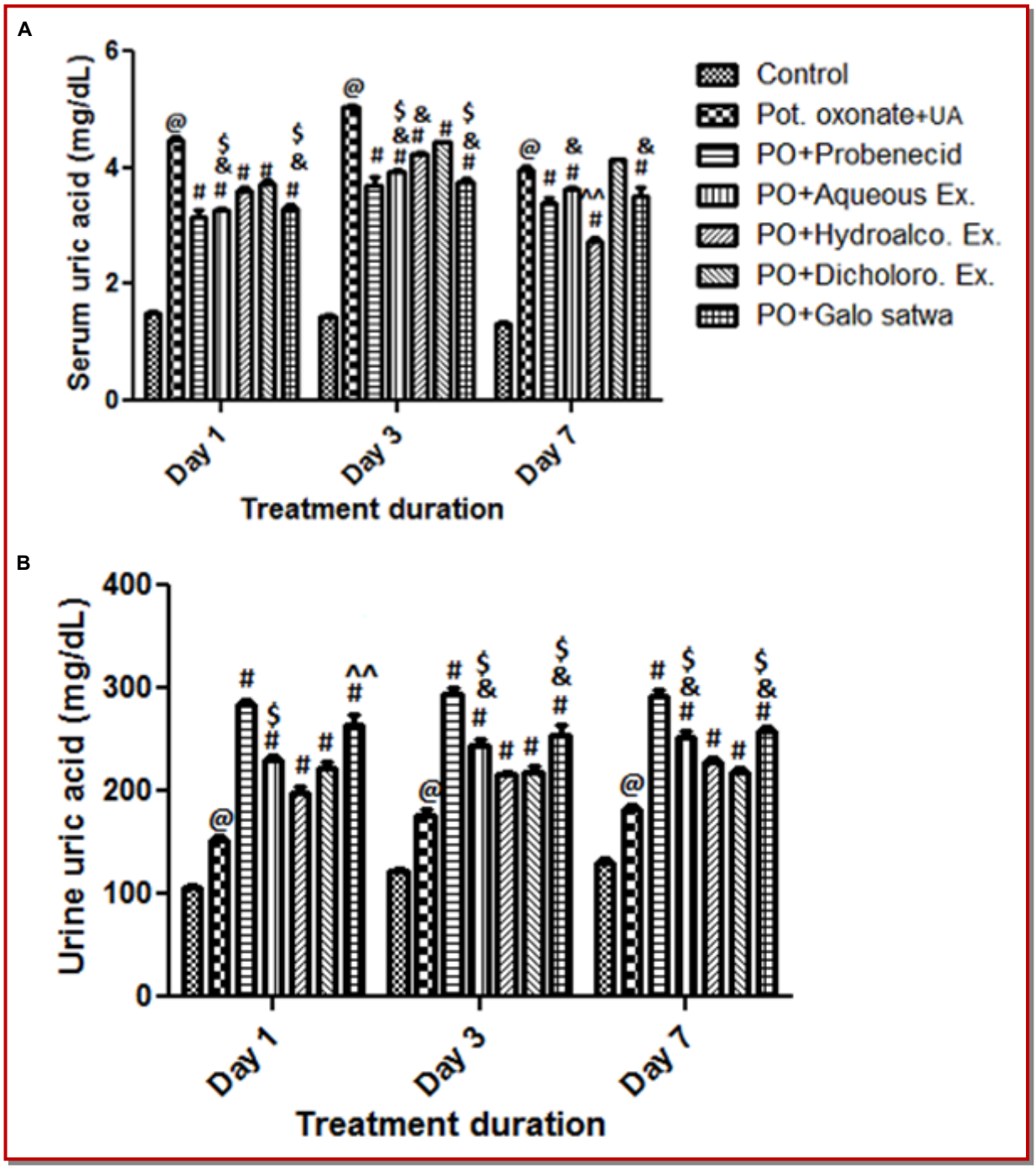

Figure 1: Effect of *T. cordifolia* extracts on serum (A) and urinary (B) uric acid levels (mg/dL)

Data expressed as mean ± SEM (n=6). One-way ANOVA followed by post Tuckey test. @ significant difference from control group, # significant difference from disease group, & significant difference from dicholomethane ex., $ significant difference from hydroalcoholic ex., ^^ significant difference from all *T. cordifolia* extracts (P<0.05)

extract (p<0.05) (Figure 2).

Effect on phenol red excretion

Concentration of phenol red was significantly higher in animals treated with probenecid (0.4 ± 0.0, 0.4 ± 0.0), aqueous extract (0.4 ± 0.0, 0.4 ± 0.0) and galo satwa (0.4 ± 0.0, 0.3 ± 0.0) as compared to controls (0.1 ± 0.0, 0.1 ± 0.0) (at 1 and 3 hours) (p<0.05). Treatment with hydroalcoholic extract (0.2 ± 0.0, 0.2 ± 0.0) and dichloromethane extract (0.2 ± 0.0, 0.1 ± 0.0) increased phenol red concentration in blood but not significantly (Figure 3).

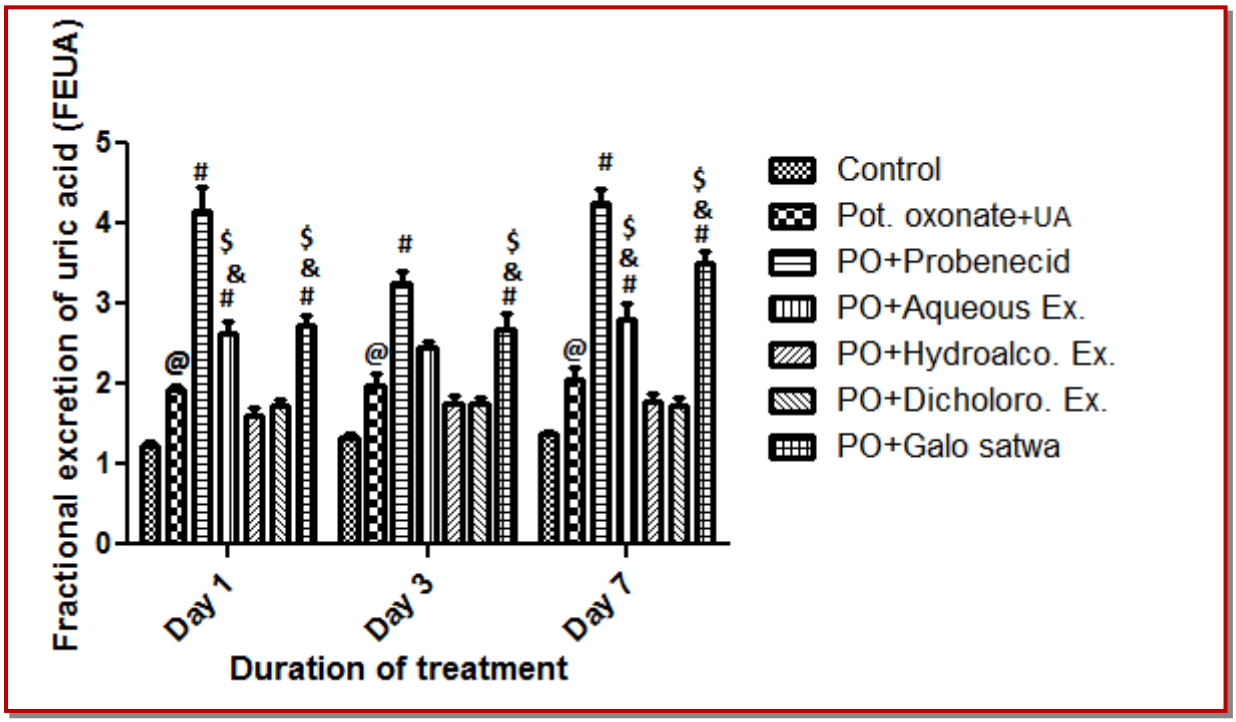

Figure 2: Effect of *T. cordifolia* extracts on FEUA

Data expressed as mean ± SEM (n=6). One-way ANOVA followed by post Tuckey test; @significant difference from control group, #significant difference from disease group, & significant difference from dicholomethane ex., $ significant difference from hydro-alcoholic ex. ($p<0.05$)

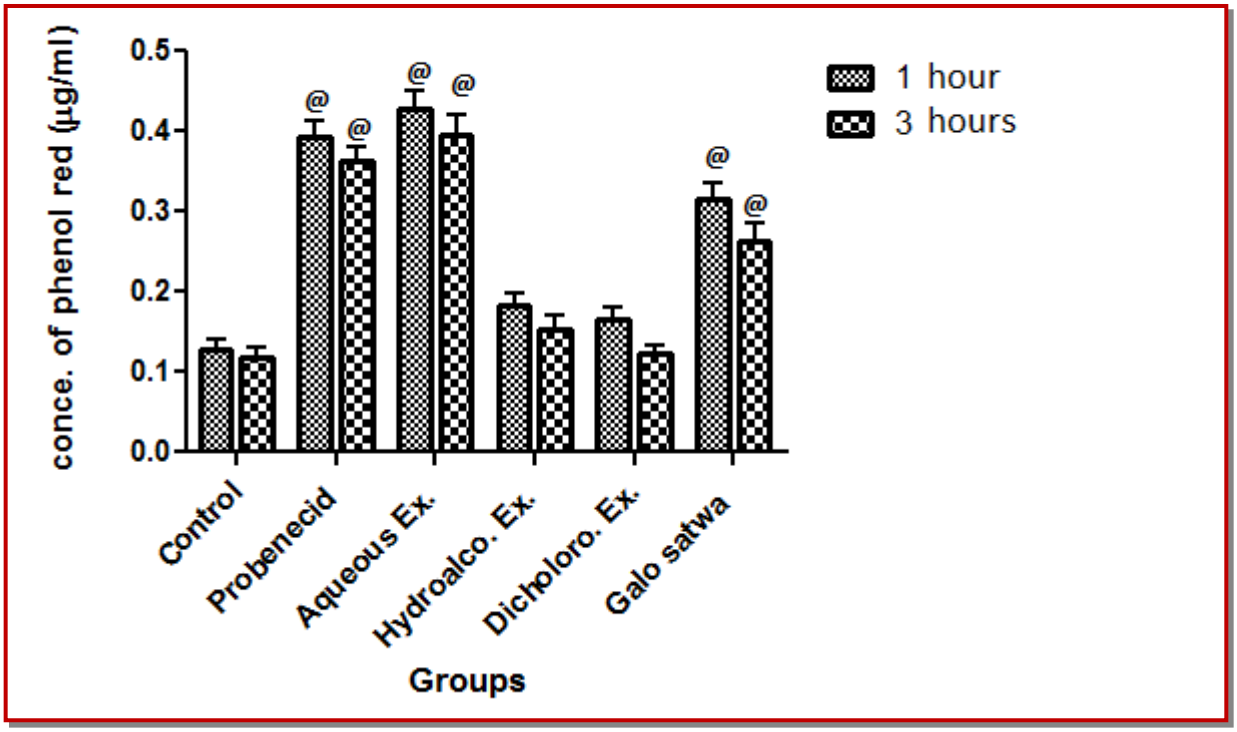

Figure 3: Effect of *T. cordifolia* extracts on phenol red excretion (µg/mL)

Data expressed as mean ± SEM (n=6). One-way ANOVA followed by post Tuckey test; @significant difference from control group ($p<0.05$)

Discussion

In hyperuricemic animals, serum uric acid level was significantly increased as compared to control. Probenecid and all the extracts significantly decreased serum uric acid level. Urinary uric acid level was significantly increased in hyperuricemic animals as well as animals treated with probenecid or either of the extracts. Urinary uric acid level was significantly higher in animals treated with galo satwa and aqueous extract as compared to hydro-alcoholic and dichloromethane extract treated groups. There was no significant difference in uric acid excretion between galo satwa and aqueous extract treated animals. Fractional excretion of uric acid was significantly increased in hyperuricemic animals as compared to normal controls. Probenecid, aqueous extract and galo satwa significantly increased FEUA as compared to hyperuricemic group while hydro-alcoholic and dichloromethane extract did not. In phenol red excretion model, probenecid, aqueous extract and galo satwa treatment significantly increased plasma concentration of phenol red in blood. Concentration of phenol red was higher in hydro-alcoholic and dichloromethane extract group but not statistically significant.

Uricosuric like probenecid increased urinary uric acid level and decreased elevated serum uric acid level in oxonated induced hyperuricemia (Yonetani and Iwaki, 1983). The higher dose of potassium oxonate may result in a much higher increase in urinary uric acid level overshadowing the uricosuric effects being investigated (Sugino and Shimada, 1995). Yu et al., (2006) also reported increased excretion of uric acid level in hyperuricemic animals. Our study showed similar results. Fractional excretion of UA was not increased in hydro-alcoholic and dichloromethane extract treated groups. This simply can be explained by function of urine creatinine and urine volume excreted by each animal. Most of the uricosurics inhibit excretion of phenol red and increase its blood level which is an indirect indication of uricosuric activity. Probenecid, aqueous extract and galo satwa increased phenol red concentration in blood but not hydro-alcoholic extract and dichloromethane extract. This indicates inhibition of organic anion transport in renal brush border responsible for excretion of phenol red and uric acid by aqueous extract and galo satwa.

There are several different carriers with overlapping specificities. The classical view of a single carrier transporting many different organic acids is probably an oversimplification (Tanner et al., 1979). Four complementary DNAs have recently been cloned which are expressed as proteins transport urate. Few proteins have been localized to the basolateral membrane of proximal tubular cells and others in apical membrane of proximal tubular cells (Rafey et al., 2003).

Conclusion

Extracts of *T. cordifolia* show potent uricosuric action. Uricosuric activity of hydro-alcoholic and dichloro methane extract may be attributed to other transporter involved in secretion of uric acid and probably extracts may be working in different pattern. Out of all four, aqueous extract and galo satwa were found more efficacious. Both of these contain higher amount of polysaccharide and galo satwa is the pure starch of *T. cordifolia.* They may used for treatment of hyperuricemia and prophylactic treatment of gout.

Financial Issue

Self-funded

Ethical Issue

All the animals were maintained in accordance with Committee for the purpose of Control and Supervision of Experiments on Animals. The protocol (KBIPER/ 2009/131) was approved by Institutional Animal Ethics Committee of K. B. Institute of Pharmaceutical Education and Research, Gandhinagar.

Conflict of Interest

Authors declare no conflict of interest

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