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Anti-hyperuricemic Evaluation of Swertia-chirayita and Febuxostat on Potassium Oxonate Induced Hyperuricemic Animal Model

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ABSTRACT

Introduction: Hyperuricemia is a metabolic disease characterized by an increase in serum uric acid levels. Hyperuricemia can be controlled by low purine diet or drugs like allopurinol and febuxostat; however, the incidence of adverse effects to these drugs, especially on chronic use, is a major clinical problem. Swertia chirayita; a herb from Gentianaceace family, was selected to observe it's in vivo uric acid lowering effect in white Albino Wistar rats in whom hyperuricemia was induced through potassium oxonate. Febuxostat was used as a standard.

Aims and Objectives: To observe the in vivo effect of different doses of Swertia chirayita extract (SCE) on uric acid levels and to compare its effect with the allopathic medicine febuxostat.

Place and Duration of study: A randomized controlled study was held at University of Health Sciences Lahore between June- August 2017.

Material and Methods: Forty-eight healthy adult Albino Wistar rats were randomly divided into six groups. Group I and II served as negative and positive controls respectively. Hyperuricemia was induced by injecting KO solution (01ml) intraperitoneally on 1st, 3rd and 7th day of study. Group III received febuxostat 5 mg/kg through oral gavage. Group IV, V and VI were experimental groups, treated with 100, 200 and 400 mg/kg doses of Swertia Chirayita extract respectively through oral route, 02 hours after induction by KO once daily for 07 days. Blood samples were collected to record serum uric acid levels on day zero, first, third and seventh days. (SPSS)version 20 was used for data entry and analysis, pvalue ≤ 0.05 was given statistical importance.

Results: In all experimental groups (IV, V, VI), serum uric acid levels were significantly reduced by Swertia chirayita extract (SCE) ($p \leq 0.001$) as compared to hyperuricemic rats (group II). SCE showed dose dependent SUA suppression. Results of both febuxostat and SCE showed that average SUA levels of these groups were markedly less than hyperuricemic rats (Group II) ($p < 0.001$)

Conclusion: In the light of its hypouricemic activity; we conclude that in the future, Swertia chirayita has the potential for newer therapeutic applications in this regard.

Key Words: Hyperuricemia, in-vivo, Swertia chirayita, Febuxostat.

INTRODUCTION

Hyperuricemia is characterized by increase in serum uric acid (SUA) levels ≥ 7.0 mg/dl in males and ≥ 6.0 mg/dl in females. Hyperuricemia is considered an important risk factor for gout. The prevalence of hyperuricemia is increasing globally. In a study[1], prevalence of hyperuricemia in Pakistan was found to be 39%. Gout is a most commonly occurring form of arthritis that affects quality of life[2].Hyperuricemia is as an important risk factor for gout and affects multiple other organ systems; leading to increased incidence of hypertension, metabolic syndrome, stroke, peripheral artery disease and heart failure[3]. The traditional drugs being used for gout include drugs like Allopurinol and Febuxostat, which have certain limitations regarding efficacy and adverse effects. According to Gliozzi et al[4], only 20-40% of patients on allopurinol and 45-67% on febuxostat, achieve the significantly lower level of serum uric acid. Exploring the potential of herbal medicine, which caters for both hyperuricemia and its related inflammatory arthritis can provide an excellent alternative with minimum side effects. *Swertia Chirayita* (SC) is one such prospect in this regard, Free radicals mediated oxidative damage result in

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various human ailments such as arthritis, inflammation, aging, heart diseases and cancer. *Swertia chirayita* contains many anti-oxidants phytochemicals like polyphenols, phytosterols, saponins, dietary fibers, and certain polysaccharides[5]. Moreover, it has established anti-inflammatory effect[5]. However, no study has been carried out to evaluate uric acid lowering property of SC. This study is therefore aimed to observe the hypouricemic effect of *Swertia chirayita* extract and compare it with Febuxostat to observe any significant difference.

MATERIAL AND METHODS

Study Design

Randomized control study design was adopted.

Setting

This research was done in Pharmacology & Animal Research Laboratory located in University of Health Sciences Lahore. The study was approved by IRB number UHS/REG-17/ERC/4388. Research was conducted during June- August 2017.

The new shoots of *Swertia chirayita* were taken from Imran Pharma (PVT) Ltd (ISO9002000/Certified), Johar Town Lahore. Specimen was submitted to the Department of Botany University of the Punjab Lahore for identification. Method mentioned by Lodhi etal[6] was adopted with some modifications. Plant shoot was washed with plain water to removed duts particles. Plant was spread on a paper and allowed to dry for a fortnight in Laboratory. Material was finely ground. Half kilogram of plant material was soaked in 5-litre ethanol (95%) for 48 hrs with occasional stirring. Beaker was covered with a tin foil to avoid evaporation of ethanol. The specimen was processed through filter paper. Extract came out to be light brown and shiny.

The Filtrate was evaporated by Rotary evaporator (Hei-Vap HL, Heidolph, Germany) at 37˚C. The concentrate was freeze dried by lyophilizer (Alpha 1-2 LD plus Germany). Essence was marked and kept safe at-20˚C. Percentage yield was 5% w/w.

Sampling Technique

Simple random sampling was used for animal grouping (n=8)

Selection of Experimental Animals

Forty-eight adult, healthy, male Wistar rats weighing (150-190 mg) were randomly selected from animal house U.H.S Lahore and divided in six groups by simple random sampling. There were eight rats in each group (n=8). Experimental animals were kept in the Animal House of U.H.S Lahore for one week to acclimatize, at controlled room temperature (22 to 24˚C) and humidity (45−65%). Light and dark cycle of 12/12 hour was maintained. Rodent chow and water ad libitum was served[8]. Blood sampling was carried out on day zero.

Induction of Hyperuricemia

Oxonic acid inhibits uricase enzyme essential for uric acid metabolism in lower primates. Potassium oxonate (KO) (250 mg/kg) was weighed. Weighed amount of KO was dissolved in 0.9% normal saline and vortexed (Vortex Mixer, VELP Scientifica, Italy). The solution was opaque. Potassium oxonate solution was filtered through micro-filter. Concentration of KO was adjusted so that I ml of solution has desired amount of drug (250 mg/kg).

Hyperuricemia was induced by injecting KO solution (01ml) intraperitoneally on 1st, 3rd and 7th day of study. Strict aseptic measures were observed. Blood sample was taken from tail vein 2 hrs later. SUA levels between 2-3 mg/dl were considered as normal[9]. Animals having serum uric acid (SUA) more than 4 mg/dl were enrolled for the study[10].

Preparation of Test Samples

Concentrations (100, 200 and 400 mg/kg) of *Swertia chirayita* extract (SCE) and febuxostat 5mg/kg were prepared according to body weight. Weighed amount of test compound were dissolved in 100 µl of DMSO and vortexed (Vortex Mixer, VELP Scientifica, Italy) to ensure thorough mixing. One milliliter of *Swertia chirayita* extract test solution was given daily by feeding tube (no.6) for seven days[11].

Experimental Groups

Six groups (n=8) of experimental animals were experimented on for 1 week

Group I (Negative control) n=8

No drug intervention was done. Vehicle (Distilled water and 100 µl dimethyl sulfoxide) was given orally through feeding tube (no.6) for seven days.

Group II (Positive control) n=8

Intraperitoneal injection of 1 ml of KO was given for induction on day 1, 3 and 7

Group III (Standard group) n=8

Febuxostat 5 mg/kg was given orally to group III ratsby feeding tube (no.6), 2 hrs after induction by KO. Febuxostat was given daily for a week[12].

Group IV, V, VI (Low, medium and high dose) n=8

Swertia chirayita plant extract 100,200 and 400 mg/kg respectively were given orally by feeding tube (no.6) to hyperuricemic animals for 7 days[13].

Blood Sampling and Uric Acid Assay

Blood sample and uric acid analysis was undertaken on 0, 1st, 3rd& 7th day of study from tail vein.

Statistics: Statistical Package of Social Sciences (SPSS)version 20 was used for analysis. Results were expressed as Mean ± Standard deviation. One-way analysis of variance (ANOVA) was performed to measureuric acid levels between experimental groups. P-value≤0.05 was given statistical importance. Post hoc Tukeys test was carried out to compare differences among experimental groups.

RESULTS

Effect of *Swertia Chirayita* extract on serum uric acid levels in hyperuricemic rats.

The effect of different doses of SCE (100, 200 and 400mg/kg) and febuxostat in hyperuricemic animal model is shown (Table-1, Fig-1). In negative control group, (Group 1) mean SUA was 2.28 ± 0.1 (Mean + S.D) on day zero. Levels ranged from 2.1 to 2.6 mg/dl. In negative control group (Group 1) SUA remained almost same throughout the experiment ($p > 0.05$) (Table-1). On day zero, mean SUA levels in all experimental groups had nonsignificant difference ($p > 005$). Potassium oxonate was used for induction of hyperuricemia in all groups except negative control (Group 1) and it significantly raised SUA levels in positive control group (Group II) ($p < 0.001$). It showed 100% induction on 7th day.Results of both febuxostat and SCE groups showed that average SUA levels of these groups were markedly decreased than positive control group (Table-1). SCE demonstrated dose dependent SUA suppression.

Groups	0 Day (mg/dl)	Day-1 (mg/dl)	Day-3 (mg/dl)	Day-7 (mg/dl)
Group I (Negative control)	2.28±0.1	2.31± 0.17	2.27± 0.1	2.25± 0.2
Group II (Positive Control)	2.20±0.1	5.98± 1.7#	9.16± 1.3#	10.05± 1.4#
GroupIII (Febuxostat)	2.30±0.2	3.95± 1.0	2.92± 0.6*	1.31± 0.5*
Group IV SCE(100 mg/kg)	2.35±0.2	5.08± 1.1	4.71± 1.2*	4.02± 0.8*
GroupV SCE(200mg/kg)	2.33±0.1	4.83± 0.8	4.33± 0.7*	3.47± 0.4*
GroupVI SCE(400mg/kg)	2.36±0.2	4.26± 0.5	3.6±0.5*	2.39± 0.5*

Table-1: Effect of *Swertia chirayita* Extracts on uric acid levels in hyperuricemic animal model.

Results are expressed as Mean ± Standard Deviation (n=8), where # indicates p value < 0.001 as compared to group 1 and * indicates p value < 0.001 as compared to group II.

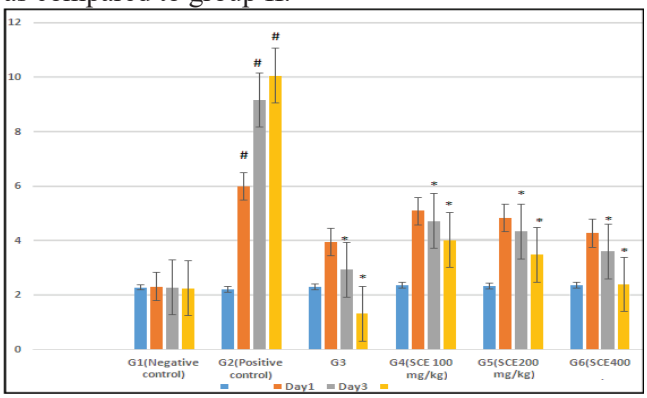

Fig-1: Effect of *Swertia chirayita* extract on serum uric acid levels in hyperuricemic rats

Results are expressed as Mean±Standard Deviation (n=8), where indicates p-value < 0.001 as compared to group Iand *indicates pvalue <0.001as compared to group II.

DISCUSSION

Hyperuricemia puts significant health burden on society. In future hyperuricemia will be the second common metabolic disease after type-2 diabetes[14]. Elevated uric acid can trigger gout as well as other comorbidities like diabetes, hypertension, heart diseases and metabolic syndrome[15]. Currently numerous drugs are available to modulate the uric acid levels. Allopurinol was available for hyper uricemia from the last four decades. In 2009, FDA approved febuxostat as a non-purine, potent and selective XOI[16]. Unlike allopurinol, febuxostat lacks structural resemblance to nitrogenous bases. Therefore, it has no drug interaction with the enzymes involved in purines and pyrimidines metabolism[17]. We selected febuxostat as a standard in our animal model because according to available data febuxostat has not been used as a positive control in hyperuricemic experimental studies. More attention has been given to natural products during the past decades in treating hyper uricemia. Chemical compounds like febuxostat carry drawback of different adverse effects on chronic use like headache, liver dysfunction, dizziness, vomiting and abdominal pain[17]. *Swertia chirayita* was chosen as an experimental plant in this case, as it is already in widespread use in subcontinent as a home remedy for flatulence, indigestion, malarial fever, laxative, anthelmintic,

antidiarrheal and skin diseases[18]. Research was conducted for safety evaluation of *Swertia chirayita* in mice and no significant toxicity was observed[19]. Phytochemical analysis of *SC* revealed presence of flavonoids, xanthones, secoiridoids, alkaloids, terpenoids, iridoids, chiratin, stearic acid, ophelic acid, oleic acid, and palmiticacid[20]. Flavanoids and polyphenolic components extracted from different plants possessed XOI activity. XO inhibitory effect of active components like flavonoids, terpenoids and alkaloids has been reported[21]. Taking into account the presence of flavonoids, alkaloids, xanthones, terpenoids and iridoides in SC, we can say that this XOI effect of *SCE* may be due to presence of the reactive compounds. Primary objective of our study was, to observe the *in vivo* dose dependent effect of ethanolic extract of *Swertia chirayita* on xanthine oxidase inhibitory assay. Additionally, its uric acid lowering effect was compared with that of febuxostat at a range of doses. Febuxostat decreased SUA levels drastically as compared to positive control group. SUA levels fell to 87% at the end of the experiment (seven times less); that is highly significant with p value < 0.001 (Table 1). Group IV, V and VI were treated with low, medium and high doses respectively.

Decrease in SUA levels in hyperuricemic animal model was in a dose dependent manner. Maximum efficacy was observed at high dose of 400mg/kg. SUA levels reduced in-group 1V as compared to positive control group. The percentage reduction on 1st, 3rd and 7th day was noted as 15%, 48% and 60% respectively. So there was a significant decrease in uric acid levels at the end of experiment (p value < 0.001))(Table I). Treatment of group V with medium dose (200mg/kg) of plant extract also resulted in fall in SUA levels. When compared with positive control group values are statistically significant (p value < 0.001) (Table-1). On the 1st day, the percentage decrease was 19%. Levels were further reduced on 3rd and 7th day to 52% and 65% respectively. High dose (400mg/kg) of *SCE* also decreased uric acid levels as compared to positive control group. There was a 29% reduction on day 1, followed by 60% on day 3 and 76 % (five folds less) on day seven. The decrease in SUA is highly significant statistically (p-value <0.001)(Table-1). Overall, there was a reduction in serum uric acid levels in a dose dependent manner in plant extract treated groups. Serum uric acid levels decreased more with high as compared to low dose. Ziya-ur-rahman et al[22] used potassium oxonate in an experimental model to induce hyperuricemia and the methodology adopted was same as ours. SUA reduction with standard drug in-group III was 41% as compared to 87% in our model. The difference in result could be due to use of allopurinol instead of febuxostat as standard. Moreover, allopurinol was given for only three selected days while we administered febuxostat daily for a week. Same doses of plant extracts were used. There was dose dependent reduction in uric acid levels like *SCE.* The percentage reduction was 16%, 25% and 47% respectively. Low percentage reduction as compared to our research can relate to presence of more active phytochemicals in our plant and use of *SCE* daily for 7 days instead of 3 days in this study. Haidari et al[12] conducted a study on similar experimental model. Potassium oxonate used in a dose of 250mg/kg on 1st, 3rd and 7th days raised SUA levels significantly to 53% as compared to negative control group. Allopurinol (5mg/kg) was used as standard and caused 55% reduction of SUA as compared to 87% by febuxostat. As reported by many researchers this difference can be explained based on superior and more potent effect of febuxostat to allopurinol. Exploring new options for the increasingly prevalent hyperuricemic condition which is interlinked with multiple other metabolic disturbances should be further encouraged and their clinical safety and efficacy should be observed in experimental models.

CONCLUSION

This study proved our hypothesis that SCE has uric acid lowering effect in hyperuricemic animal model. *Swertia chirayita* has the potential for newer therapeutic applications in this regard. It will be a useful contribution to the antihyperuricemic drugs scientific study as no acute toxicity and change in animal behavior was observed.

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