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Antihyperuricemic activity of gum of *Calophyllum inophyllum* ultra high dilutions in potassium oxonate induced wister albino rats

Shifa Shaffique*[,1,+], SaeedAhmad[2], Tayyeba Rehman[3], Haseeb Anwar[1], Hafiz Muhammad Asif[3], Ghulam Hussain[1] & Waheed Mumtaz Abbasi[4]

[1]Department of Physiology, Faculty of Life Sciences, Government College University, Faisalabad 63100

[2]Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

[3]University College of Conventional Medicine, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

[4]Medical and Health Division, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

E-mail:[+]shifa.shafiquee@gmail.com

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Hyperuricemia is a biochemical abnormality and it affects the quality of life. Many medicinal substances are used in lowering the uric acid. The study designed to find hypouricemic effects ofultrahigh dilutions, the gum of *Calophyllum inophyllum, Acid benzoicum* in oxonate of potassium induced hyperuricemic wistar albinomodel.

The experiment consist of 11 groups of albino rats. All the groups were treated with oxonate of potassium except normal control. Healthy group of animals received only distilled water, hyperuricemic control group were given alcohol mixed in distilled water. A standard allopathic medical substance Allopurinol and *Acid benzoicum* ultra high dilutions were given in time dependent manner (single dose/day) in different experimental groups. Blood samples were collected by rat tail vein bleeding. The uric acid and creatinine levels of serum were analyzed by using standard measuring kits. The Student's *t*-test was used for statistical analysis of difference between the groups $p \leq 0.05$ was reflected significant.

Oral intake of *Acid benzoicum* ultrahigh dilutions reduceduric acid levels of hyperuricemic wistar albino rats in time dependent mode. At 3rd day and 7th day administration of *Acid benzoicum* ultrahigh dilutions decreased the level of uric acid more ominously as compared to one day administration. However, allopurinol a standard allopathic drug normalized the uric acid level in all experimental groups.

The current work showed significant hypouricemic effects of *Acid benzoicum* ultrahigh dilutions in hyperuricemic wistar albino model. However, clear conclusion of hypouricemic activity of *Acid benzoicum* needed repetition of experimental work.

Keywords: Antihyperuricemia, *Acid benzoicum*, Animal model, Potassium oxonate

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Hyperuricemia is a common biochemical abnormality globally with raised level of uric acid (UA) up to 6.8 mg/dL[1]. It may be caused by increased production or lesser excretion of uric acid. Worldwide prevalence of available non-steroidal drug for the treatment of Hyperurecemia (Gout) and the drug has fewer side effects such as allergic reactions which limit their use in patients. Therefore it is the need to introduce new hypouricemic agents[2,3]. The old literature of homeopathy showed that *Acid benzoicum* (gum of *Calophyllum inophyllum*) is an organopathic remedy for amelioration of gouty complaints due to hyperuricemia. It excretes the uric acid crystals through urine[4]. The primary objective of experiment was to find hypouricemic effects of *Acid benzoicum* ultrahigh dilutions on uric acid in potassium oxonate induced hyperuricemia Wister albino rat model.

The research hypothesis is evaluated for application ion and refinement) of laboratory animals and procedures by Pharmacy Research Ethics committee (PREC), The Islamia University Bahawalpur prior to the start of experimental work (approval no. Ref No. 41-2016/PREC dated 23-08-2016.). After application of 3R's, rat model was selected. The whole manuscript complied to the ARRIVE guidelines[5].

The Wistar albino rats (150-250 g) were placed in polycarbonate cages (47 × 34 × 18 in^2) and were alienated into 11 different groups and each group consisted of 4 animals. Sample size was calculated through resource equation method and was sufficient

*Corresponding author

for statistical analysis[6]. The animals were treated and assessed in the order given in Fig. 1. Oxonate of Potassium (250 mg/kg) was used to induce the hyperuricemia in rats, as described previously[7]. All the medicines including allopurinol, ultra high dilutions of homeopathic medicines, and succussed alcohol were given orally once daily at 10 am - 11 am. Nine groups were orally administered with allopurinol 10 mg/kg, *Acid benzoicum* 1 min, *Acid benzoicum* 30 c for 1, 3 and 7 days respectively. Two groups, normal control and hyperuricemic control were given distilled water and succussed alcohol 70% (vehicle of used homeopathic medicines as per company mentioned) respectively. Homeopathic ultrahigh dilutions and 70% succussed alcohol were mixed (2 drops) in 1 cc distilled water for oral administration. After one hour administration of medicine on day 1st, 3rd and 7th, blood samples were collected from rats by tail vein bleeding. Blood was centrifuged at 3000 rpm for 5 min to get serum. Serum was preserved at -20°C until analyzed[1]. The level of uric acid and creatinine levels in serum were analyzed by using Microlab 300. Uric acid liquicolor kit and Creatinine liquicolor kit (human diagnostic worldwide) were used respectively. Results obtained by this activity were analyzed by SPSS software. The Data was expressed in the form of mean ± standard error of mean (SEM). The $p \leq 0.05$ (Two tailed values) was considered significant.

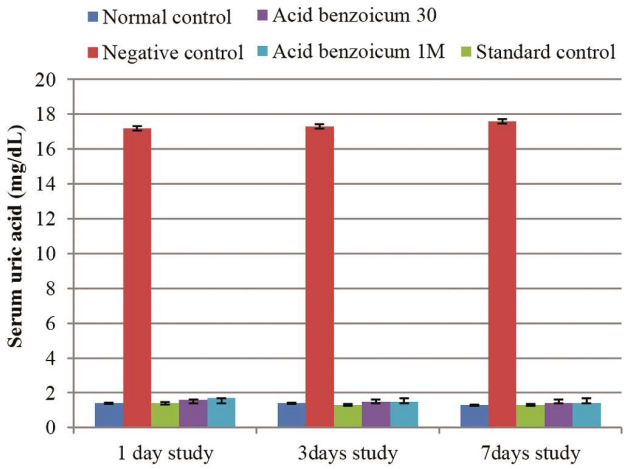

Fig. 1 — Effects of *Acid benzoicum* 30 c, 1M and allopurinol on serum UA levels on day 1, 3, 7 in rats

The baseline characteristics of different experimental groups were mentioned in Table 1. Fig. 1 clearly demonstrates the statistically significant antihyperuriemic effects of *Acid benzoicum* 30 c and 1 min in time dependent manner. There were no adverse event (immobility, loss of weight, an inability to eat or drink or death of animals) observed during course of study. Serum creatinine levels were also reduced in time dependent manner. (Table 2)

The present study evaluated the effects of *Acid benzoicum* 30 c and *Acid benzoicum* 1 min hyperuricemia in potassium oxonate induced hyperuricemic model of rats. A rapid increase in levels of uric acid in serum in all the groups compared to normal control group indicating that the hyperuricemia rat model was effectively established. This finding matched to the previous studies reporting hyperuricemia induced by potassium oxonate intra peritoneal in rats and mice[1,7-9]. Hyperuricemia triggered when there are disturbances in uric acid regulating pathways. Potassium oxonate causes hyperuricemia by inhibition of uricase or by blocking of an electrogenic

Table 1 Baseline Characteristics of different groups

Baseline Characteristics	Baseline data of different groups.				
	Normal control	Hyperuricemic control	Standard control (1, 3, 7 day study groups)	*Acid benzoicum* 30c (1, 3, 7 day study groups)	*Acid benzoicum* 1M (1, 3, 7 day study groups)
Body weight (g) (Mean ±S.E.M)	206 ±4	203 ±7	206 ±4	201 ±3	201 ±2
Serum uric acid (Mean ± S.E.M)	1.3±0.07	1.5±0.06	1.4±0.07	1.4±0.08	1.4±0.05

Table 2 Effects of Creatinine level in wistar albino rats administered with *Acid benzoicum*

Treatment	Dosage	Serum creatinine level (mg/dL)		
		1 day study	3day study	7 day study
Normal control		0.3±.07	0.2±.06	0.2±.05
Negative control		2.3± 0.6##	2.4±0.21##	2.7±1.4##
Standard control	10 mg/kg	0.4±.07**	0.3±.02**	0.2±0.1**
Acid benzoicum 30C	5 drops in 10cc distilled water	0.5±0.06*##	0.3±0.08**	0.3±0.2**
Acid benzoicum 1M	5 drops in 10cc distilled water	0.6±0.05*##	0.4±0.13**	0.4±0.13**

urate transporter activity in renal proximal tubule that controls serum UA levels[10-12]. Antihperuricemic drugs reduced hyperuricemia by inhibiting xanthine oxidase and action on renal or extra-renal urate transporters. In the present study, orally administered allopurinol (10 mg/kg) significantly decreased potassium oxonate induced hyperuricemia in rats. It has action on a urate transporter and Cl/urate transporter and inhibit xanthine oxidase[8]. Results matched to the several studies presented the reduction of hyperuricemia with allopurinol at same dosage[1,13,14]. According to previous research, potassium oxonate induced hyperuricemia in rats may be due to either blocking of UAT activity or inhibition of uricase activity. One day study depicts the antihyperuicemic potential of allopurinol, *Acid benzoicum* 30 c and 1 min in potassium oxonate induced rat model that received medicines after one hour of intra peritoneal injection of potassium oxonate. The rats of 3 day and 7 day study groups received homeopathic medicines as well as allopurinol for consecutive 2 or 6 days respectively and then received single potassium oxonate intra peritoneal injection before final dose of medicines. Potassium oxonate is a hyperuricemia inducing agent, but when rats previously treated with allopurinol, *Acid benzoicum* 30 c and 1 min received potassium oxonate, serum uric acid levels remained normal in all the groups. Hahnemann, founder of homeopathy, described in aphorism 43 that when two similar diseases meet together in an organism, it resulted in cure[15]. During one day study, *Acid benzoicum* administration reduced serum uric acid level. However, single dose of homeopathic medicine administration can't turn increased serum UA to normal levels. A homeopathic medicine gently annihilates similar acute disease with slight homeopathic aggravation (aphorism 154, 155)[16]. The initial elevated serum UA levels may be due to homeopathic aggravation.

Homeopathic medicines could act as prophylactic when administered to healthy individuals having risk of disease. *Arsenicum album* and *Veratrum album* are the two well-known homeopathic medicines used as a prophylactic of cholera. It might be possible that homeopathic medicines administered in 3 day or 7 day study groups produce prophylactic effect in rats that inhibit the action of potassium oxonate in those rats. Moreover, a homeopathic drug proving (HDP) is the application of a substance in nontoxic dilutions to healthy individuals. The tested material causes mental, physical or psychological symptoms that can be reversed[17]. The specific effects of homeopathic medicines are of non-molecular origin, yet provide powerful clinically effective biological activities[18]. The potencies selected in current study were medium (30 c) and higher (1 min). Both potencies showed similar hypouricemic potential in rats. It has been assumed that highly diluted substances transfer biological activity to cells by electromagnetic fields[19]. In conclusion, the current study indicated antihyperuricemic potential of *Acid benzoicum* 30 c and 1 min in oxonate of potassium induced hyperuricemia model of wistar albino rats model in time dependent manner.

Competing interests

None of the authors have any competing interests.

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