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Anti-Nociceptive and Anti-Inflammatory Activity of Urito, a Poly Herbal Preparation

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ABSTRACT

Most of the drugs used at present for urolithiasis are synthetic in nature and have plenty of side effects coupled with high rates of recurrence of calculi. In this context, there are scopes for evaluation of herbs and herbal formulations in treatment of urolithiasis since they could be cheaper, have little side effects and efficacious. Plants have designed the manufacture of advanced traditional medicine structures among which are Ayurvedic, Unani and Chinese are common. There has been a rapid advancement in using phytotherapy for urolithiasis treatments in recent years and many investigators have proposed to conduct further scientific studies on its efficacy. Plant medicines are in great demand both in the developed as well as de¬veloping countries for primary health care because of their wide range of biological and medicinal activities, higher safety margin and low cost. There is a vast, yet untapped potential in these medicines so an active research into their medicinal properties is warranted. Anti-nociceptive and anti-inflammatory activity is planned to be evaluated in animal experimental models for a polyherbal product URITO.

Keywords:
Urolithiasis, antinociceptive activity, anti-inflammatory activity, polyherbal, URITO

1.Introduction

Medicinal plants and their secondary metabolites are gradually used in the treatment of diseases as a harmonizing medicine. Inflammation is a defense response of our body to harmful provocations such as allergens and/or damage to the tissues [1]. Inflammation is a host defense process that involves a complex network of cell-cell, cell-mediator and tissue interactions and occurs in response to a variety of harmful stimuli. The factors involved in an inflammatory response can be considered as endogenous (physical, chemical, mechanical, nutritional, biological) and exogenous such as immunological reactions, neurological and genetic disorders. Mostly, both the innate immune responses as well as the adaptive immune response are involved in the formation of inflammation. [2]

Reverse pharmacology [3,4,5] also known as target base drug discovery [6] is widely gaining popularity in the field of drug discovery in which a hypothesis is first made that modulation of the activity of a specific target protein will have beneficial therapeutic effects. This is gaining importance to treat most of the common diseases since the available remedies in modern medical science are known to cause unwanted side effects. This method is the most commonly used method in drug discovery today [7]. Employing reverse pharmacology techniques for the evaluation of herbal medicines is also gaining popularity nowadays, as these medicines are popular not only in developing but also in developed countries. WHO estimates that around 80% of population in developing countries relies on plant derived traditional medicines [8]. Stone formation (Urolithiasis) is a painful urologic disorder that occurs in approximately 12% of the global population with a high rate of re-occurrence [10].

The fascinating pharmacological properties of fustic and its usefulness replicated by long-term traditional use, sufficient logical assays are essential in order to assure quality and efficiency of the plant material and its preparations. Hence, decreasing side effects should be careful while scheming better-quality therapeutics for anti-nociceptive and anti-inflammatory, besides

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augmenting medicinal efficiency. The Siddha and Ayurvedic systems of treatment are being progressively familiar as an alternate approach to anti-nociceptive and anti-inflammatory.
Medicinal herbs are being used since ancient periods for the treatment of renal stones, even before the invention of modern treatments [11]. Herbal drugs are reported to be effective with minimal side effects. Herbal remedies produce anti-urolithic action by multiple mechanisms such as diuretic activity, crystallization inhibition activity, lithotriptic activity, analgesic and anti-inflammatory activities, anti-oxidant activity and anti-microbial activity. Herbs also improve the renal function and regulate oxalate metabolism which help in reducing the reoccurrence of renal calculi.URITO is a poly herbal(Aerva, Calotrops, Sarsaparrilla, Carrot, Crataeva, Alum, Rotula)

2. Materials and Methods

Anti-nociceptive and anti-inflammatory activity is planned to be evaluated in animal experimental models for a polyherbal product Urito. It has various herbal ingredients which, individually, are beneficial in Urolithiasis, but we want to evaluate whether their combined effect is safe and efficacious as well.Urito is the test drug material. It is manufactured by by Rumi Herbals Pvt. Ltd., Chennai and will be used in the study.
Albino Wistar rats weighing between 150-200 gm will be used for this study. The animals will be obtained from the animal house of Sri Lakshmi Narayana Institute of Medical sciences, Pondicherry, India. The animals will be placed in polypropylene cages with paddy husk as bedding and will be housed at a temperature of 24 ± 2º C and relative humidity of 30 – 70 %. A 12:12 light: day cycle will be followed and all the animals will be allowed to have free access to food and water.1. Acute oral toxicity studies will be performed according to OECD-423 guidelines (No. IEC/C-P/61/2014, dt 11-08-2014).
Each animal was subjected to various parameters including writhing's, changes in respiration, hypersensitivity, convulsions, lacrimation, salivation, ataxia, body temperature, spontaneous activity, and catalepsy 30 min prior to injection (baseline) and monitored for next 3-days after drug administration for any kind of behavioral, physical, and pharmacological toxic effects.

Animals Designs

A	Species	Albino Wistar Rats
B	Age / Weight	Rat 150-200 gms
C	Gender	Both Sex
D	Numbers to be used Rat	160
E	Number of days each animal will be housed	15 days

Anti- Nociceptive studies:

Acetic acid induced abdominal constrictions: Method described by Koster et al (1959**)** [12]will be used. 0.6% of freshly prepared acetic acid in a dose of 10 ml/kg will be injected intra-peritoneally, following which the number of abdominal constrictions will be recorded for 15 minutes. A significant decrease in this response in test group will be considered as anti-nociceptive effect.

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Results will be expressed as absolute value in tabulated format and percentage inhibition of abdominal constriction will be calculated

$$\% \text{ inhibition} = \frac{\text{Treated group response} - \text{Control group response}}{\text{Control group response}}$$

Eddy Hot Plate: Method described by Eddy and Leimbach (1953) will be used [13]Animals will be placed on a hot plate maintained at 55 ± 0.5ºC. Maximum contact period of 15 seconds will be permitted to avoid any thermal injury to the paw of animals. Time taken by the animal to lick its hind paws or to jump from the plate will be recorded as the reaction time. A significant increase in this reaction time in the treatment group will be considered as an anti-nociceptive response.

Haffner's Tail Clip (Mechanical) Method: Method described by Bianchi and Franschine (1954)will be used [14].A sleeved bulldog clamp will be used to apply mechanical pressure at the base of the tail of the animal. A maximum application period of 15 seconds will be used to avoid any mechanical trauma to the tail of the animal. The time taken by the animal to attempt to dislodge the clamp by biting will be recorded as the reaction time. A significant increase in this reaction time in the treatment group will be considered as an anti-nociceptive response.

Anti-Inflammatory studies:

Carrageenaninduced paw edema: Method described by Winter et al (1962) will be used. [15]A 1% w/v suspension of carrageenan will be prepared freshly in normal saline and injected into subplantar region of left hind paw (0.1 ml). In control group animals, only vehicle will be injected. Test drug will be administered orally, according to body weight, one hour before (depending on the expected peak effect) carrageenan challenge. A mark will be made at the ankle joint of each rodent. Paw volume up to the ankle joint will be measured in drug treated and control groups before and 3 hours after carrageenan challenge using a plethysmograph filled with mercury. Edema will found out and % reduction in edema will be calculated using the following formula:

$$\% \text{ reduction in edema} = \frac{\text{Mean edema in control group} - \text{mean edema in drug treated group}}{\text{Mean edema in control group}}$$

A significant percent of reduction in edema will be considered as an anti-inflammatory response.

Statistical Analysis:

The results will be represented as mean ± SEM. The data's will be analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's "t" test. P value <0.05 will be considered as significant.

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3. Results and Discussion

Anti-nociceptive activity is normally mediated by one of the two following pathways namely opioid pathway and by stimulating the opioid receptors Cyclo-oxygenase pathway, by inhibiting this pathway. The antinociceptive activity was agreed out in a wide panel of rodent models of acute pain; such as, the acetic acid-induced writhing, tail clip, tail immersion and formalin tests. These methods are classical nociception models used to screen prospective antinociceptive compounds or polyherbal extracts. Antinociceptive valuation using the acetic acid induced writhing test showed that oral administration of herbal extract created a statistically important reserve of writhes compared to the control. This is a sign of the peripheral analgesic activity of the extract, subsequently any cause that lowers the writhing number demonstrates analgesia by inhibiting prostaglandin synthesis, a peripheral mechanism of pain inhibition. [16,17].

The tail clip and immersion methods were used to assess the chief mechanism of analgesic activity of the extract. The extract showed low activity in these two models of nociception. It is well-known that centrally acting analgesic drugs elevate the pain threshold of mice related to pressure and heat. Thermal tests are more sensitive to opioid-μ receptors, and non-thermal tests are more sensitive to opioid-k receptors [18].

Carrageenan induced inflammation is useful in sensing orally active anti-inflammatory agents; consequently, it has a significant predictive value for anti-inflammatory agents acting through mediators of acute inflammation [19]. The progress of oedema induced by carrageenan injection creates a biphasic event. In the first stage, during the first hour, histamine, serotonin and bradykinin are the mediators involved, while prostaglandins are implicated in the second phase (3-5 h) [20].The extract showed a modest inhibitory effect at the early phase but pointedly inhibited the paw oedema in late phase of inflammation. The effect of the extract in this model may be endorsed to the inhibition of the release of pro-inflammatory mediators of acute inflammation, especially prostaglandins. In this context, extract play a key role as protective factors against the carrageenan-induced acute inflammation. This finding corroborates the anti-inflammatory activity of unsaturated fatty acids, as linoleic and linolenic acids, which significantly inhibited the edema induced by PGE2, LTB4, arachidonic acid and carrageenan

Histamine, serotonin, bradykinin and prostaglandins are established mediators of acute phase of inflammation causing increase in vascular permeability and vasodilatation [21,22]. These inflammatory mediators are released in the body endogenously and contribute to the various phases of paw edema [21] seen in animal experimental models. Considering that the use of analgesic and anti-inflammatory drugs exerts a wide range of side effects [23], there is currently a strong interest in developing new therapeutic agents from natural products [24].

In summary, comparing the results obtained in the antinociceptive activity models; the ethanolic extract of was quite forceful in acetic acid writhing test, thus, indicating peripheral antinociception. Likewise, the highly significant inhibitory effect of the extract on the nociceptive reaction in the late phase of the formalin test advises that the extract could suppress inflammatory nociception. Moreover, in promise with the results from the antinociceptive tests, the extract also prompted anti-inflammatory effects. The results achieved in this study proven the antinociceptive and anti-inflammatory actions for the extract. However, the mechanism of these actions is uncertain, and the active chemical compounds responsible of the antinociceptive and anti-inflammatory activities of the extract remains to be elucidated. In this study, the anti-inflammatory effects of Urito by assessing the magnitude of inhibition of paw edema induced by these mediators and comparing it to the magnitude of inhibition seen with carrageenan induced edema.

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Ethical approval: The study was approved by the Institutional Ethics Committee

4. Conflict of Interest

The authors declare no conflict of interest.

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Table 1. Effect of Urito, poly herbal extract on tail clip induced pain in mice

Treatment	Dose (mg/kg)	Pre-treatment reaction latency (s)	Post-treatment reaction latency (s)	% Inhibition
Control	10 ml/kg	4.1 ± 0.2	1.9 ± 0.2	-
Urito, poly herbal Extract	50	2.3 ± 0.2	4.8 ± 0.5	24.2
	100	2.8 ± 0.4	6.0 ± 1.0[a]	21.4
	200	3.8 ± 1.6	4.3 ± 0.2	3.8

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Morphine	10	2.4 ± 0.5	8.9 ± 1.5b	50.8

Values are mean ± SEM, (n=6). ap < 0.05, bp < 0.001 compared to control (One-way analysis of variance followed by Tukey's multiple comparison test).

Table 2. Effect of Urito, poly herbal extract on formalin-induced pain in mice

Treatment	Dose (mg/kg)	Early phase(0-5 min)	% Inhibition	Late phase(0-5 min)	% Inhibition
Control	10 ml/kg	71.3 ± 8.9	-	91.8 ± 9.8	-
Urito, poly herbal Extract	50	43.6 ± 3.9a	38.9	48.3 ± 5.0b	47.4
	100	46.7 ± 5.0c	34.5	41.3 ± 6.5b	55.0
	200	24.7 ± 2.9b	65.4	15.2 ± 6.5b	83.5
Morphine	10	00.0 0.0b	100	00.0 ± 0.0b	100.0

Values are mean ± SEM, (n=6). ap < 0.01, bp < 0.001, cp < 0.05 compared to control (One-way analysis of variance followed by Tukey's multiple comparison test).

Table 3: Effect of Urito, poly herbal Extract on Carrageenan-induced inflammation in rats.

Treatment	Dose (mg/kg)	Increase in paw circumference (cm)				
		1 h	2 h	3 h	4 h	5 h
Control	10 ml/ kg	0.3 ± 0.0	0.4 ± 0.0	0.45 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Urito, poly herbal Extract	50	0.3 ± 0.0 (24.2)	0.3 ± 0.0 (21.1)	0.4 ± 0.0 (22.2)	0.3 ± 0.1^a (46.2)	0.2 ± 0.0^b (55.8)
	100	0.1 ± 0.0^b (69.7)	0.2 ± 0.0^c (55.3)	0.2 ± 0.0^b (66.7)	0.1 ± 0.0^b (75.0)	0.1 ± 0.0^b (78.9)
	200	0.3 ± 0.0 (24.2)	0.2 ± 0.1^a (39.5)	0.2 ± 0.2^a (48.9)	0.3 ± 0.0^a (40.4)	0.3 ± 0.0^c (46.2)
Indomethacin	10	0.3 ± 0.0 (15.2)	0.3 ± 0.0 (26.3)	0.3 ± 0.0^a (44.4)	0.3 ± 0.0 (36.5)	0.3 ± 0.0^c (46.2)

Values are mean ± SEM, (n=6). ap < 0.05, bp < 0.001, cp < 0.01 vs. control (One-way analysis of variance). Values in parenthesis are % inhibition.

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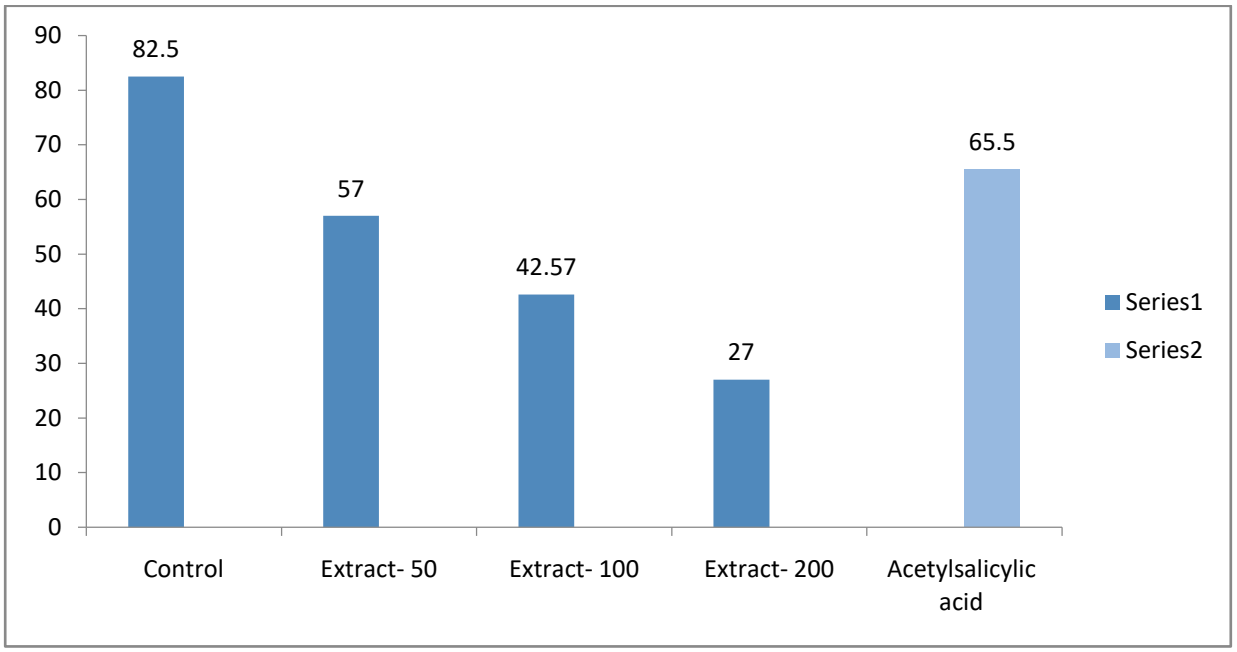
Figure 1 – Effect of on Urito, poly herbal Extract acetic acid-induced writhing in mice.



Extracts mg/Kg. Values are mean ± SEM (n = 6). ***p < 0.001 compared to control (One-way analysis of variance).