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EVALUATION OF ACUTE TOXICOLOGICAL AND PHARMACOLOGICAL ACTIVITY OF LEAVES OF *RUTA CHALEPENSIS ON* LABORATORY ANIMALS

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ABSTRACT

Objective: *Ruta chalepensis* is belongs to the family of Rutaceae is locally known as Tena'adam contains extremely wide variety of aromatic plants, among them rich is the genus Ruta. It is ornamental and medicinal plant rich used in the treatment of inflammation, ulcer, hypotension, reproductive disorders and menstrual problems. Therefore, present study was focused on preliminary phytochemical analysis of different extract (methanol, acetone, and aqueous) and analgesic activity evaluated by Mice. **Methods:** The Analgesic Activity was studied by Tail immersion method diclofenac sodium 10mg/kg and acetic acid induced (0.2ml of 1% acetic acid and 0.25ml of 3% acetic acid) module acute toxicity study and preliminary phytochemical screening was also studied to evaluate the toxicity. **Results:** No toxicity profile was observed in rats after oral administration of the leaf extract of methanol, acetone, and aqueous of *Ruta chalepensis* dose of 2000mg/kg. There was significant ($p<0.001$) reduction in biochemical parameters with respect to control. Phytochemical screening of the fruit extract revealed the presence of tannins, alkaloids, flavonoids and Cardiac Glycosides. **Conclusion:** Generally this research data indicates *Ruta chalepensis* has both analgesic effect and acute toxicity on mice at specific dose due to the presence of active ingredients like tannins, alkaloids, glycosides and flavonoids. *Ruta chalepensis* therefore has acute toxicity at dose of 2000mg/kg when taken orally. The study validates its traditional use in analgesic treatment depend on doses below 500mg/kg.

KEYWORDS

Ruta chalepensis, Photochemical screening, Toxicity and Analgesic activity.

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INTRODUCTION

Ruta chalepensis is originally indigenous to the Mediterranean region, Canary Islands, Cape Verde Islands, Sudan, Ethiopia, and Somalia for cooking and medicinal purposes. The family Rutaceae is used in traditional system of medicine worldwide[1-3]. *Ruta chalepensis* a source of diverse classes of natural products with biological activities including

antifungal, antioxidant, depressant and anti-inflammatory activities. In folk medicine, Ruta is credited with a long list of medicinal uses including emmenagogue, antihelmintic, antiinflammatory, spasmolytic effects antitumoral, analgesic and antidepressant. It has pleiotropic pharmacological properties, attributed to the high content of alkaloids, glycosides, flavonoids, and saponins found in the leaves of the plant[4,5]. In Ethiopia, the majority of peoples that lives in rural areas and the poor people in urban areas mainly on traditional medicines to meet their primary health care needs. Even if their culture and attitude contributed to their usage of traditional medicine, they have no scientifically proved know how. As a result, most of the people may get exposed to unnecessary health problem due to the unfortunate custom of the traditional medicinal plants. The danger of losing valuable information is thus high considering the increasing cultural change, mobility and displacement of communities due to several factors. This *Ruta chalepensis* is used for the treatment of muscular pain, injuries, sprains, eye strain, joint and bone pain, arthritis, rheumatism, toothache, tennis elbow, back pain and headache. The current study to conduct for phytochemical analysis, acute toxicity, and analgesic activity of different solvent extracts of *Ruta chalepensis*[6-9].

METHODS

Collection and identification of plant materials

Plants were collected from different areas of Gamo Gofa Zone particularly from shele and Arbaminch towns Based on the ethno medicinal survey information obtained from local inhabitants, plants was found most important for the treatment of stomach-ache in the study area. This plant is ‘‘Tena Adam’ (*Ruta chalepensis*) each specimen was labeled, numbered, annotated with the date of collection, the locality and their medicinal uses and their approximate dosages of administration were recorded. The specimens were identified at Arbaminch University biology department by experienced plant botanist.

Extraction of the Study Plant Materials

The fresh leaves were collected, washed fussily and dried under shaded room at temperature of (25°C) for 15 days. The dried leaves were prepared into powder using dry grinder. The powdered leaves to concede through sieve number 40 and stored in air tight containers. 500g of powdered leaves were extracted with maceration process for 48 hours with occasional stirred for every 30 min for 6 hours, the solvents in order of increasing polarity, Acetone, methanol and water. The solution was filtered using a Whatman filter paper and the solutions were evaporated by ruta. The extract was evaporated at 40°C. The dried crude extract was dissolved in dimethyl sulfoxide (DMSO) and used for further analysis from each extract.

Phytochemical screening test

The most common Phyto-chemicals (secondary metabolites) such as alkaloids, glycosides, flavonoids, tannins, saponins and phenolics present in powdered forms of the study medicinal plants were analyzed[10].

Tail immersion method

The tail immersion method was used to evaluate the analgesic activity. Tail immersion was conducted as described by Aydin *et al*[11]. The painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water Mice reacting to hot water at 55degree Celsius were observed using parameters withdrawing tails from water. Mice was treated with normal saline, diclofenac, acetone extract and aqueous extract of *Ruta chalepensis*. The time of withdrawal was the following. The result of tail immersion test in mice is presented in Table No.3.

Acetic acid induced writhing

This test was done using the method described by Collier *et al*[12]. The effect of *Ruta chalepensis* extract on the acetic acid- induced abdominal constrictions in mice is presented in Table No.4. Mice were used in groups of four per dose of plant extract, standard drugs, physiological saline. The animals were kept individually in cages before the commencement of the experiment. Control mice were pre-treated with physiological saline and after

15 min each mouse was injected intra peritoneally with 0.2ml of 1% acetic acid. 5 min after the administration of acetic acid, the animals were observed and writhes were counted for 30 min. The experiment was repeated using other groups of animals which were pre-treated for 15 min with graded doses of plant extract, paracetamol prior to injecting them with 0.25ml of 3% acetic acid. This was considered a positive analgesic response and the percentage inhibition of writhing was calculated[12].

Procurement of experimental animals

Animals were selected as per the OECD guidelines. Healthy young and nulliporous, non-pregnant Adult Swiss albino mice of either sex weighing 20-25g and aged 6-8 weeks old were selected, because literature survey of lethal dose 50% test shows that usually there is little difference in sensitivity between sexes, but generally females were found slightly more sensitive, obtained from Ethiopian Health and Nutritional Research Institute (EHNRI) and were used Arbaminch university for the study The animals were fed with standard pellet diet and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under the alternate cycle of 12 hrs of darkness and light. The animals were acclimatized to the laboratory conditions for 1 week before starting the experiment. The animals fasted for at least 12 hrs before the onset of each activity. This experiment was conducted in accordance with the internationally accepted laboratory animal care and use guideline

Experimental Design

The mice were divided into the 4 groups of each containing six mice.

Group I

Control mice, which fed normal diet and water.

Group II

Mice treated with diclofenac sodium (10mg/kg)

Group III

Mice treated with *RC* (200mg/kg, oral.)

Group IV

Mice treated with *RC* (400mg/kg, oral.)

Statistical analysis

The experimental data were expressed as mean ± SEM. Statistical analysis was carried out by one way analysis of variance. A level for $p < 0.05$ was considered to be statistically significant

RESULTS AND DISCUSSION

Animals were observed individually for 48 hrs after dosing at the first 30 minutes, periodically and during the first 24 hrs, with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days. Additional observations were also made if the animals continue to display signs of toxicity. Observations included were changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity, and behavior pattern. Observations were also made and checked for tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. Results were tabulated in Table No.1.

Preliminary phytochemical screening

Data obtained from the phytochemical analysis of *Ruta chalepensis* indicated the presence of the following chemical components: tannis, cardiac glycosides, flavonoids, saponis, and phenolic compounds.

***In Vitro* in acute toxicity**

Major organs such as lungs, liver, kidneys, and heart were observed after the 14 days of vivo observation for *in vitro* toxicity these organ were not affected (such like swelling, inflammation etc).

Discussion

The result's of the study indicate that *Ruta chalepensis* has acute toxicity and analgesic effect due to chemical components found in crude extracts such as alkaloids, flavonoids, cardiac glycosides and tannins. The extract of *Ruta chalepensis* not produced death or signs of toxicity even at the dose of 2000mg/kg which suggests that the extract was no toxic at lethal dose in the rat. The guideline 423 of the Organization for Economic Cooperation and Development (OECD) establishes that substances with an LD50 < 5mg/kg are highly toxic, whereas LD50 values from 5 to 50mg/kg are very toxic,

LD50 values from 50 to 300mg/kg are toxic, LD50 values from 300 to 2000mg/kg are dangerous, and LD50 values higher than 2000mg/kg are not dangerous. *Ruta chalepensis* acetone, methanol, aqueous extracts shows LD50 =2000mg/kg p.o. in mice. The acetic acid-induced writhing reflex and tail immersion models were used to evaluate the analgesic activity of *Ruta chalepensis* since tests of analgesic drugs commonly measure nociception and involves the reaction of animals to painful stimuli. The stimulus may be thermal (tail immersion) and chemical. Acetic acid-induced writhing reflex is a model of visceral pain which is highly useful for screening analgesic drugs and several chemicals acetic acid could induce writhing reflex in laboratory animals. Intraperitoneal injection of 1% glacial acetic acid produced abdominal writhing in this experiment. Acetic acid produces writhing reflex in animals by activating the chemo sensitive nociceptors Also, it has been noted that the level of analgesia in acetic acid-induced models is indicated by the percent reduction in the number of abdominal constrictions[13,14]. In this experiment, the reference drug and *Ruta chalepensis* extract at 200 and 400mg/kg significantly decreased the mean number of abdominal constrictions or writhes which was dose dependent. The analgesic effect of *Ruta chalepensis* seen in this experiment may be mediated through peripheral pain mechanism and or through suppression of prostaglandin pathway since it has been observed that any agent that decreases the number of writhing will demonstrate analgesia preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition[15,16].

In the tail immersion, the extract showed a significant increase at the dose of 200mg/kg when compared to the negative group .the extract at the doses of 200 and 400mg/kg significantly increased the pain reaction time and the extract at the dose of 400mg/kg had a better analgesic effect than other groups which was unexpected but may be the maximum dose beyond which the analgesic activity of the extract will no longer be increased in this model. The tail immersion models have been used to study centrally acting analgesics. In these models, sensory nerves sensitise the nociceptors and the involvement of endogenous substances such as prostaglandins are minimized.

Table No.1: Acute toxicity study of methanolic extract of leaves of *Ruta chalepensis* based on OECD guidelines 423

S.No	Number of animals	Dose in mg/kg	Report
1	3	5	No death
2	3	50	No death
3	3	300	No death
4	3	2000	No death

Table No.2: Effect of *in vivo* acute toxicity of *Ruta chalepensis* on mice observed

S.No	Mice by gram	Extractions	Dose	Observation up to death
1	Mice (41gm)	Water	20.5mg	Circulatory increase Sleep Lethargy Tremors Induce breathing
2	Mice (43gm)	Methanol	21.5mg	Less movement Tremors Convulsions Respiration decrease salivation Body hair flat CNS depressed
3	Mice (48gm)	Acetone	24mg	Hair rise increasing heart bit unable to move freely, Respiratory increases. Depressing

Analgesic activity

Tail immersion method

Table No.3: The effect of *Ruta chalepensis* on tail immersion method in mice

S.No	Group	Dose mg/kg	Mean latency(s) before and after drug administration(s)					
			0min	30 min	60 min	90 min	120min	150min
1	Group I (Control)	5ml	2.3±0.08	2.46±0.07	2.41±0.08	2.37±0.12	2.38±0.12	2.34±0.08
2	Group II (Diclofenac Sodium)	10	2.4±0.08	4.6±0.15**	6.3±0.13**	5.6±0.15**	4.8±0.15**	3.7±1.05**
3	Group III (200)	200	2.4±0.11	4.6±0.15*	6.3±0.06**	5.9±0.03**	4.9±0.04**	2.8±0.12*
4	Group IV (400)	400	2.32±0.06	5.3±0.11**	7.6±0.12**	8.05±0.14**	6.3±0.18**	4.3±0.08**

Values are in mean ±SEM; (n=6) *p<0.05, ** p<0.01 vs control

Acetic acid induced writhing

Table No.4: Effect of *Ruta chalepensis* on acetic acid-induced writhing reflex in mice

Groups	Dose mg/kg	0 min	15 min	30 min	45min	60 min
Saline control)	10	No effect	No	Abdominal stretching	Two times writhing	Three times writhing
Diclofenac	500	No effect	No	No	Abdominal stretching	One times writhing
Acetone	200	No effect	One times writhing	Abdominal stretching with writhing	No writhing	No writhing and abdominal stretching
Aqueous	400	No effect	Abdominal stretching and one times writhing	Abdominal stretching	No writhing	No writhing and abdominal stretching

Figure No.1: Leaves of *Ruta chalepensis L*

CONCLUSION

Generally this research data indicates *Ruta chalepensis* has both analgesic effect and acute toxicity on mice at specific dose due to the presence of active ingredients like tannins, alkaloids, glycosides and flavonoids. *Ruta chalepensis* therefore has acute toxicity at dose of 2000mg/kg when taken orally. The study validates its traditional use in analgesic treatment depend on doses below 500mg/kg.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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