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Evaluation Toxicity in Ethanolic Extract of Capparis Sepiaria L. Leaf.

Manjula Ambadas Rathod[1], Pratima Mathad[2], Shivkumar Inamdar[3], Nitin Mahurkar[4]

[1]Department of Botany and Seed Technology, Sahyadri Science College, (Kuvempu University), Shivamogga-577203. Karnataka, India. Cell -8618509874,
[2]Professor, Plant Biotechnology Lab, Department of P. G. Studies and Research in Botany, Gulbarga University, Kalaburagi -585106. Karnataka, India.
[3,4]Professor, Department of Pharmacology, HKE'S, Matoshree Taradevi Rampure Institute of Pharmaceutical Sciences, Kalaburagi Karnataka India.

ABSTRACT

India is known for its rich tradition in human wealth and medicine from decades. It is also home for many valuable medicinal plants which are being used in the different system of medicines. Capparis sepiaria known as himsra in Ayurveda, is used as an active ingredient in many oils. The present study aimed to evaluate acute oral toxicity studies in the ethanolic extract of Capparis sepiaria leaf. The acute oral toxicity was carried out as per OECD guidelines and as Sabbani et. al. 2015). Results: The acute oral toxicity of ethanolic extract of Capparis sepiaria leaf was found non-toxic at 175, 250, 500, and 1000mg/kg whereas LD_{50} was found at 2000mg/kg. The present study concluded that the ethanolic extract of Capparis sepiaria leaf was non-toxic at the studied dose which can have important pharmacological actions in future herbal preparations.

HIGHLIGHTS

First ever report on folklore use the leaf of this plant for wound and bone healing.
Acute oral toxicity (Wister albino rats) Antioxidant (in vitro and in vivo).

Keywords: Acute oral toxicity. Capparis sepiaria, ethanolic extract, leaf and Wister albino rats.

INTRODUCTION

Plants still constitute one of the major sources of drugs in modern and traditional medicine worldwide (Saravana and Gopalakrishnan 2015). Large sections of the Indian population still rely on plant-based medicines as they are abundantly available, economical, and have little or no side effects. In addition to their cultural acceptability of late medicinal plants have gained global importance in the alternative healthcare system, for their proven and effective curative properties (Manjula Rathod and Pratima Mathad 2021). Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures [usually less than 24 hours] (Safety Emporium. 2006). To be described as acute toxicity, the adverse effects should occur within 14 days of the administration of the substance (The "Gold Book" 1997). Acute toxicity studies are conducted to evaluate the effects of a

single substance. Historically, a primary objective of acute toxicity testing was to determine an LD_{50} dose, Globally, there is an increase in the rate of herbal formulations consumption (Shri 2003 & Iserhienrhien and Okolie 2020). because of the belief that they are organic, harmless and effective in the treatment of diseases (Arya et. al 2012, Iserhienrhien and Okolie 2020). Paracelsus, known as the father of toxicology, has given a statement which is often quoted: "All substances are poisons; there is none which is not a poison. It is the right dose which differentiates remedy from poison" (P. Hunter 2008). Capparis sepiaria L. Belonging to the family, Capparaceae It is commonly known as kadu kattari or kanthari in Kannada and ahimsra in Sanskrit, native to India. It is a thorny, branched shrub traditionally used for the treatment of jaundice, inflammation and dysentery (Matthews, 1991). Capparis sepiaria is used as an active ingredient in many in oils. It is indicated in digestive disorders, anorexia, asthma, colds and abscess in Ayurveda. **Botanical description:** It is a prickly, evergreen much branching shrub growing to 3 to 5 meters tall with velvet hairy stem. Leaves elliptic, oblong to ovate or nearly round, 1-5cm long 1-2 cm broad often retuse, softly to hair less, leaf stalk 2-5 mm long with stipular spines. Flowers usually in corymbose at branch ends clusters of 10-30, small 5-10 mm across white, flowers February on words, fruits in April and seeds about 6 mm Medicinal uses of Capparis sepiaria are well known and the supporting scientific data available is very scanty (Thilagavathi 2018). Hence objective of the present study is to evaluate acute oral toxicity studies in the ethanolic extract of Capparis sepiaria leaf.

MATERIAL AND METHODS

Collection and identification

The plant is collected from Afzhalpur taluka, Kalaburagi district, and identified by referring flora of Gulbarga and flora of Gamble available. Also, by the digital flora of Karnataka and the plant list (World Flora Online), (Photograph of habit in Fig.1).

Fig.1 Habit of Capparis sepiaria

Preparation of plant extract

The plant material (leaf) was first washed with tap water thoroughly to remove dirt and soil deposits and dried under shade until the moisture content was completely removed; such dried leaves were powdered mechanically. Approximately 250 gm of this dried powder extracted with 100% ethanol by continuous hot percolation, using the Soxhlet apparatus. The resultant greenish-black extract was used to prepare different concentrations of doses.

DETERMINATION OF ACUTE ORAL TOXICITY

Target animal: Target animal: Healthy young adult Wister albino rats were brought from Shree Venkateshwara Enterprises, Bangalore**.** Healthy young adult Wister albino male rats, weighing 150-180gm at the start of the experiment. The present study was approved by the Institutional Animal Ethics Committee of 1948/Po/Re/S/17/CPCSEA, Dated 23/02/2017, HKE'S, Matoshree Taradevi Institute of Pharmaceutical Sciences, Kalaburagi Karnataka.

The rats were divided into three groups (n=4) as follows:

Group I: Control (Distilled water)

Group II: Ethanolic extract of Capparis sepiaria leaf

A total of four were systematically selected for each Group-I control and Group-II, Ethanolic extract of Capparis sepiaria. The rats were kept in their respective cages for about 15 days acclimate to the laboratory conditions before the start of activity. The animals were housed individually in clean polypropylene cages. Room temperature and humidity were maintained at 25°C to ±30°C and 45-55% respectively with a light-dark cycle of 12h (light from 06:00 AM to 06:00 PM). Clean paddy husk bedding was provided to the animals. The animals were fed with commercially available standard ad libitum pellets and filtered drinking water.

Procedure: Acute oral toxicity study was carried out following the procedure described by (Sabbani 2015 and as per OECD 425 guideline). The Acute oral toxicity study was conducted using the limit dose test of up and down procedure according to OECD/OCDE Test Guidelines on Acute Oral Toxicity (OECD Test Guidelines 425) (AOT), 2001), at a limit dose of 2000 mg/Kg. Prior to dosing, animals were fasted overnight before being weighed and all the extracts were orally administered in a single dose using a Tuberculin tube (The volume given was not more than 2ml/100gm body weight). Following the period of fasting, the fasted body weight of each animal was determined and the dose was calculated according to the body weight. After the ethanolic leaf extract was administered, food was with-held for a further 3-4 hours. Control animals were administered with normal distilled water. Single animals were dosed in sequence usually at 24-hour intervals. Using the default progression factor, doses were selected from the sequence 175mg/Kg, 250mg/Kg, 500mg/Kg, 1000mg/Kg and 2000mg/Kg. Because no estimate of the substance's lethality was available, dosing was initiated at 175mg/Kg till 2000mg/Kg as recommended in OECD Guidelines 425.

LD_{50} value

As per Acute Oral Toxicity (Guideline 425) the LD_{50} value of ethanolic extract of Capparis sepiaria leaf was calculated.

Mortality:

Mortality was calculated by observing animal behaviour at different test doses of ethanolic extract of Capparis sepiaria leaf.

Wellness parameters

Animals were observed continuously during the first 30 min after dosing and periodically for the next 24 hours, 48 hours 7 days and then up to 14 days and results were recorded for each animal. Observations included changes in skin fur, eye colour, alertness, hyperactivity, grooming, torch responses, lacrimation, urination, sleep, and mortality. Changes in wellness parameters were compared with that of control animals.

Body weight

The body weight of individual animals was recorded before the administration of ethanolic extract of leaf on first day and last day of the experiment, Changes in the weight of individual animals were calculated and compared with that of the control animals

STATISTICAL ANALYSIS

The results were expressed as mean ± Standard error mean [Significant value P < 0.001***, P < 0.01**and P<0.05**] using one-way ANOVA (Graph Pad Instat3) and Microsoft Excel.

RESULTS

In the present study the ethanolic extract of Capparis sepiaria leaf was subjected to acute oral toxicity. Pharmacological activities of medicinal plants helpful for the establishment of crude drugs in the form of medicines. As the medicinal plants are the richest source of secondary metabolites, which directly involved in defence mechanism of human body and responsible for many pharmacological activities, hence the Search of herbal medicine alternative to modern medicine increasing day by day as modern medicine have more side effects on human and animal health.

LD_{50} value

As per calculations from Acute Oral Toxicity (Guideline 425) the LD 50 value of ethanolic extract of leaf was found to be at 2000 mg/kg body weight.

Mortality

No mortality was observed at 175, 250, 500 and 1000 mg/kg body weight but 2000mg/kg of ethanolic extract of Capparis sepiaria leaf showed mortality.

Body weight:

The body weight of the test animals was calculated and are recorded. There were no significant changes in body weight. However, all animals exhibited a normal increment in body weight without drastic differences between both control and treated groups. The body weights of rats were increased after the oral administration of ethanolic extract of Capparis sepiaria leaf, thus it indicates that the oral administration of 175 to 1000mg/kg does not affect the growth of the rats [Table -1]. Some photographs of taking body weight of rat were represented in Fig. (2 and 3).

Table -1. Effect of ethanolic extract of Capparis sepiaria L. on Body weight of test animals (in Gm)

Group	Body weight of rats in (Gm) Before and after treatment for each plant (Mean ±SEM)							
	0 Days	14 Days	0 Days	14 Days	0 Days	14 Days	0 Days	14 Days
	175 mg/kg	175 mg/kg	250 mg/kg	250 mg/kg	500 mg/kg	500 mg/kg	1000 mg/kg	1000 mg/kg
Control	190±02	220±01	160±00	200±0	160±02	220±00	170±0	230±00

	0.001**	0.001*	0.001***	0 0.001*** **	0.05**	0.001***	0 0.001*** *	0.001*** *
C sepiaria	170±01 0.001*** *	240±00 0.001*** *	170±02 0.05**	240±00 0.001*** **	170±02 0.05**	250±00 0.001**	170±01 0.05**	260±02 0.001*

MEAN = SEM and P < 0.001* , 0.001** and P<0.05****

Wellness parameters

Animals were observed continuously during the first 30 minutes after dosing and observed periodically for the next 24 hours, 48hours 7 days and then up to 14 days of test dose 175, 250, 500, 1000 and 2000mg/kg of body weight and compared with the control group and observations were recorded for each animal [Table-2], Some photographs of observing wellness parameter of rat were represented in Fig. (2, 3 and 4).

Fig. 2. **Fig. 3.** **Fig. ..**

(Observing wellness parameters)

Table -1. Effect of eth ext. of Capparis sepiaria L. Leaf on wellness parameters at different test dose

Observations	**175 mg/kg**				**250mg/kg**				**500 mg/kg**				**1000mg/kg**				**2000mg/kg**			
Duration	**Hours**		**Days**		**Hours**		**Days**		**Hours Days**				**Hours**		**Days**		**Hours**		Days	
	24	48	7	14	24	48	7	14	24	48	7	14	24	48	7	14	24	48	7	14
Capparis sepiaria L.																				
Skin fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**p**			
Hyperactivity	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	**p**			
Eye color	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				
Alertness	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				

Grooming	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	**P**			
Tremors	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**P**			
Torch response	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	**P**			
Lacrimation	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B				
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N				
Mortality	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	**M**			
Control																				
Skin fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**			
Hyperactivity	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	**A B**			
Eye color	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**			
Alertness	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**			
Grooming	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	**A B**			
Tremors	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**			
Torch response	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	**A B**			
Lacrimation	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	**A B**			
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	**N**			
Mortality	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	A B	**A B**			

N- Normal, P-Present, AB- Absent, M- Mortality

Fig.5.

Fig.6.

(Taking body weight of rat)

Some images of acute oral toxicity studies

Discussion

Phytotherapy is gaining popularity as WHO encourages the appropriate ethnomedicinal use and signifies safety evaluation of herbal medicines (WHO 1993, Vaghasiya 2011). The earlier results of Capparis sepiaria indicated that the plant possess numerous biologically active compounds which could serve as potential source of drugs in herbal medicine (Rajesh et al. 2010, Piacente and Pizza1997). The ethanolic extract of Capparis sepiaria leaf was reported to possess flavonoids 570.0±0.00, phenols 480.0±0.01, tannins 476.0±0.00, alkaloids 381.1±0.00, terpenoids 0.321±0.02 and glycosides 0.180±0.00 mg/100gm respectively (Manjula A Rathod, Pratima Mathad, Shivkumar Inamdar and Nitin Mahurkar 2025) The phytochemicals are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. Further there is a need to establish the studies scientifically on the toxicity and adverse effect of these remedies. Therefore, additional acute oral toxicity study is crucially needed to identify the range of doses that could be used subsequently, but also to reveal the possible clinical signs elicited by the substances under investigation. It is a useful parameter for investigating the therapeutic index of drugs and xenobiotics (Rang, Dale and Ritter 2001). In the present study the acute oral toxicity in ethanolic extract of Capparis sepiaria leaf was non-toxic at 175, 250, 500 and1000mg/kg however LD_{50} was found at 2000mg/kg of body weight. Earlier toxicity studies on ethanolic extract of Capparis sepiaria leaf were proved to be safe up to 300 to 5000mg/kg of body weight in mice (Rajesh et. al 2010). The animals body weight is also an important factor evaluating the toxicity of a substance (Jahn and Günzel1997). The reduction in body weight and internal organ weight can be a simple and sensitive index of toxicity after exposure to a toxic substance (Raza et al. & Teo et al. 2002). Changes in organ weight have long been accepted as indicators of test-induced changes, which are often associated with treatment-related effects (Sellers et al. 2007 & Ferreira et al 2014). Our study as there is increase in the body weight after the administration of ethanolic extract of Capparis sepiaria leaf indicates it has no toxic effects on test animals. According to (Raina et al. 2015) and (Cajuday and Pocsidio 2010) the weights of the organs are markers of pathological and physiological wellness status of animals. Changes in organ weights are hallmarks of toxicity in experimental animals, which are determined by toxicity tests (Hilaly et. al. 2004). As use of medicinal plants increases, experimental screening of the toxicity of these plants is essential to assure the safety and efficacy of those natural sources (Johnson and Pelter 1996, Sabbani 2015).

Conclusion

The present study concluded that the ethanolic extract of Capparis sepiaria was non-toxic at the studied doses which can have important pharmacological actions in future herbal preparations. Since Capparis sepiaria was a known plant in Ayurveda, has to be explored more for its therapeutic uses.

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