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Acute Oral Toxicity of Ethanolic Leaf Extract of Garcinia indica in Albino Rats

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ABSTRACT:-
Garcinia indica (kokum) is a herbal drug used in the treatment of ulcers, inflammations, infections, etc.The Acute Oral Toxicity study of Ethanolic Extract of Garcinia indica leaf (EEGI) was carried out as per OECD guideline 425: Acute Oral Toxicity: Up and Down Procedure to check the safe and tolerable dose. EEGI was prepared by using Soxhlet Apparatus. The toxicity studies were conducted as per OECD 425. Limit test was done by administering 2000mg/kg dose orally to the treatment group and adverse effects and mortality was recorded for 14 days. These factors were compared with the control group. Then, the animals were sacrificed and 6 vital organs (heart, kidney, liver, spleen, ovary and lungs) were sent for histopathological examination. Both the control and treated groups revealed no toxic effects like changes in skin, fur and eyes, salivation, diarrhea, inflammation, etc. The histopathological results did not reveal any abnormalities in the organs. The results revealed that the oral administration of EEGI did not produce any significant toxic effect on rats. Hence, the drug was found to be safe and it can be used for the further therapeutic activity.

KEYWORDS: Garcinia indica, Acute Oral Toxicity, Ethanolic extract.

I. INTRODUCTION

Herbal drugs contribute a major share in all the officially recognized systems of health in India. They are rightfully existed side by side with Allopathy and are not in 'the domain of obscurity'. Plant derived medicines are used in all civilizations and cultures and hence, plants have always played a key role in health care systems worldwide. Herbal medicines are extensively practised in the prevention, diagnosis and treatment of various illnesses.

Toxicology is the 'science of poisons' which is the study of harmful poisonous effects of drugs with emphasis on detection, prevention and treatment of poisonings. Almost any substance can be harmful at some doses but, at the same time the levels of its safe usage or its degree of its safety is recognised.

Garcinia indica (Clusiaceae) is a slender evergreen tree with dropping branches; its leaves are ovate and are dark green in colour. The plant is found in the tropical rain forest of western Ghats, from Konkan to Mysore, Coorg and Wyand. It flowers in November- February and fruits ripen in April-May.[1] The fruits of Garcinia indica are used in home remedies in case of heat stroke and infections. Other uses are to relieve sunstroke, dysentery, mucous diarrhea, antihelmentic, antiulcer, anti-tumor and it is also a good liver tonic. Active constituents of Garcinia indica are anthocyanins, fatty acids, hydroxycitric acid, garcinol, isogarcinol, etc.[2]

The present study aims to determine the toxicity of ethanolic extract of G. indica leaves using an acute oral toxicity test as per Organization for Economic Cooperation and Development (OECD) guideline 425.

II. MATERIALS AND METHODS

COLLECTION OF PLANT:

The plant, G.indica was procured from Konkan , Ratnagiri District, Maharashtra. The plant was identified and authenticated from the Department of Botany, Andheri West, Mumbai with voucher number:- #:ampp167191674.

PREPARATION OF EXTRACT

The leaves of G.indica were washed with tap water and were shade dried for 6-7 days. After drying, the leaves were grinded through blender and converted in to a fine powder. The powder was extracted by ethanol using Soxhlet Apparatus.[2] The extracts were collected and stored in a desicators until used for further studies.

II. ACUTE TOXICITY STUDY

PREPARATION AND GROUPING OF EXPERIMENTAL ANIMALS:

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Healthy adult female albino rats of Wistar strain weighing 150-180 g were procured from Bombay Veterniary College, Parel and according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) New Delhi India, the experimental protocols were approved by Institutional Animal Ethics Committee (IAEC), Bharati Vidyapeeth College of Pharmacy (BVCP/IAEC/01/2019).

Non-pregnant female rats were selected because they are slightly more sensitive than male rats. The animals were kept in polypropylene cages. Room temperature and humidity was maintained at 25^{o} C – 27^{o} C and 55-56 % respectively with a light dark cycle of 12 hours.[7] Clean paddy corn cob bedding was provided to the animals. The animals were fed with commercially available feed and unlimited supply of filtered drinking water.

PROCEDURE:

The animals were divided randomly into two groups i.e Control and Treatment. Each group consisted 6 animals. The animals were acclimatized for 7 days before the study. The animals were fasted overnight with free access to water only. Limit test was conducted by administering 2000 mg/kg dose of ethanolic extract of Garcinia indica orally. The animals were kept under observation for 30 mins and later for 4 hours. As no toxic symptoms were observed. 3 more animals were given the same dose. The animals in the control group were administered with 1% CMC solution. The control and treatment groups were observed further for 14 days to make note on various symptoms like hair loss, change in skin colour, shivering, salivation, etc. Weights of the animals were observed. On the 14^{th} day the rats were sacrificed and their six vital organs (lungs, kidney, liver, heart, ovaries and spleen) were sent for histopathological evaluation.[7]

IV. RESULTS AND DISCUSSION

1. BODY WEIGHT

The body weights of the animals were calculated and recorded in table no.1. there were no significant changes in the body weight. However, all the animals had normal increase in body weight without drastic difference between both control and treated groups. Thus, the administration of G.indica leaf extract does not affect the growth of animals.

Table 1: Effect of ethanol leaf extract of Garcinia indica on the body weight of rats at 2,000 mg/kg dose after 14 days.

Group	Body Weights	
	Before Treatment	After Treatment
Control	182 ± 7.5	186 ± 6.36
Treated	182 ± 7.2	186 ± 6.36

2. GENERAL SIGNS AND BEHAVIOURAL OBSERVATIONS

No significant changes were observed in skin fur, pupil, salivation, alertness, diarrhea, urination, sleep, tremors, gripping, etc of the treated as well as the control group animals.

Table 2: Observations for the limit test at 2,000 mg/kg body wt of ethanolic leaf extract of Garcinia indica.

Observations	30 mins		4 hours		8 hours		24 hours		48 hours		Day 7		Day 14	
	C	GI	C	GI	C	GI	C	GI	C	GI	C	GI	C	GI
Skin fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Skin Colour	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Pupil	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lacrimation	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Urination	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Diarrhea	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB

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Alertness	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hyper-activity	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Gripping	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tremors	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB	AB
Sleep	N	N	N	N	N	N	N	N	N	N	N	N	N	N

C- control, GI- Garcinia indica leaf extract
N- Normal, AB- Absent

3. MORTALITY

No mortality was exhibited at 2000mg/kg body weight dose of G. indica ethanolic extracts and resulted were tabulated in Table no. 3

Table-3: Mortality record for ethanolic extract of Garcinia indica leaf for 14 days.

Group	Control	Ethanolic leaf extract of Garcinia indica
Hour 1	NIL	NIL
Hour 2	NIL	NIL
Hour 3	NIL	NIL
Hour 4	NIL	NIL
Hour 24	NIL	NIL
Day 2	NIL	NIL
Day 3	NIL	NIL
Day 4	NIL	NIL
Day 5	NIL	NIL
Day 6	NIL	NIL
Day 7	NIL	NIL
Day 8	NIL	NIL
Day 9	NIL	NIL
Day 10	NIL	NIL
Day 11	NIL	NIL
Day 12	NIL	NIL
Day 13	NIL	NIL
Day 14	NIL	NIL

4. HISTOPATHOLOGICAL ANALYSIS

The microscopic images showed no differences between the control and treated groups. The microscopic examination revealed that, all the organs (heart, lungs, kidney, liver, spleen, ovaries) from the treated animals did not show any toxic effects when observed under the microscope using 100x magnification power.

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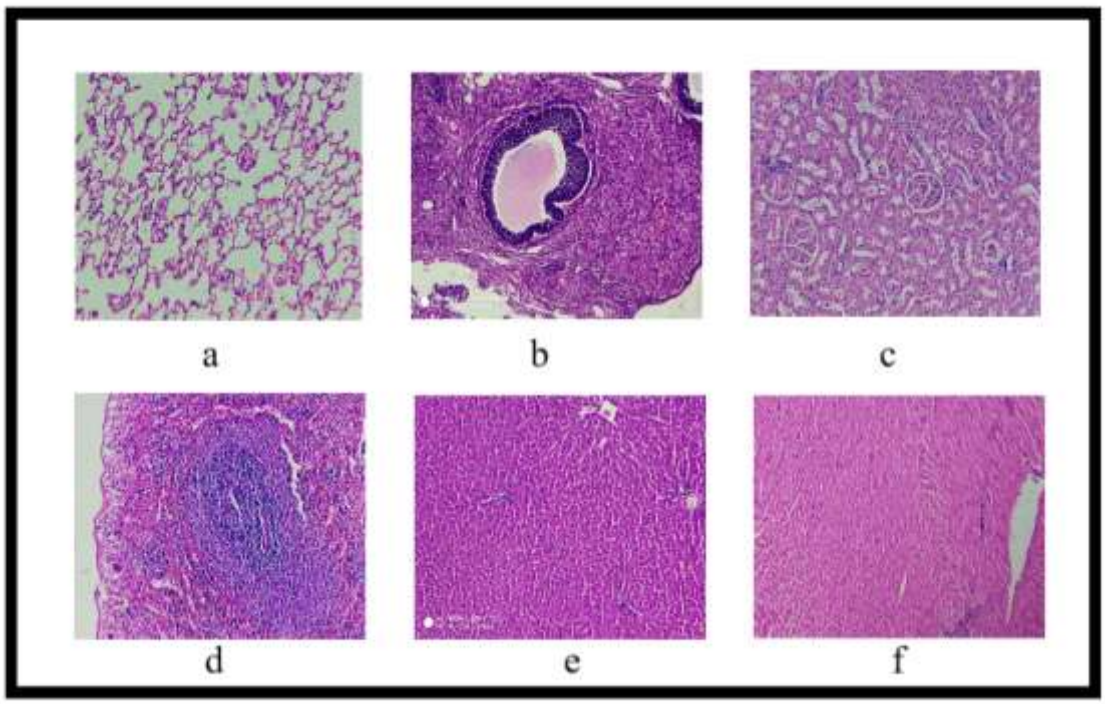

Figure 1:Histopathological images of six vital organs at 100x. a) lungs b) ovary c) kidney d) spleen e) liver f) heart

According to World Health Organisation, 78-80% of the rural population rely on traditional medicine and nowadays it has become universally popular, particularly in developing countries. The use of herbal drugs in primary healthcare has become beneficial because of natural source. However, these bioactive products from medicinal plants are recognised to be safe and thus, are extensively used as medicines. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Therefore, there is a need to establish the studies scientifically on the toxicity and adverse effect of these remedies. So, Acute Oral Toxicity study is needed to identify the range of doses and also helps to reveal the clinical signs elicited by the substances under investigation.

Hence, the present study was conducted to study toxicity of ethanolic extract of G.indica leaves using OECD guideline 425. In this study, the Wistar albino rats were used to observe the toxic effects of ethanol leaf extract of G.indica. Based on the literature, the oral route administration is the most convenient and commonly used. Since, the crude extracts are administered orally, the animals should be fasted prior to taking the dose because food and other chemicals in the digestive tract may affect the reactions of the compound.

In this study, the animals in the control and treated groups were administered with vehicles and crude leaf extract respectively. Then, the animals were monitored daily until day fourteen for any toxic signs and mortality. Any clinical symptom is one of the major important observations to indicate the toxicity effects on organs in the treated groups. During the 14 days of period acute toxicity evaluation rats which are orally administered with single dose 2000mg/kg of ethanolic leaf extract of G.indica showed no observable symptoms of neither toxicity or deaths. All the animals gained weight and displayed no significant changes in behaviour. The physical features such as skin, fur and eyes were found to be normal and even as the body weight of the rats showed as increase, indicates that the administration of the crude extract has no toxicity on the growth of animals. The food intake and water consumption was not affected by the administration of G.indica and did not induce appetite suppression and had no deleterious effects.

V. CONCLUSION:

The study reckoned that the ethanolic leaf extract of this plant did not cause any signs of toxicity were observed in the animals treated with extract at dose 2000mg/kg thus, establishing its safety in use. The histology examination showed that no changes in the organs of both control and treated groups. Hence, G.indica can be used as medicinal agent in known dosage for various activities as mentioned above.

CONFLICT OF INTERESTS:

The author declares no conflict of interest.

REFERENCES:

[1]. Jagtap P, Bhise K, Prakya V, A Phytopharmacological Review on Garcinia Indica, International Journal Of Herbal Medicine, 2015.

[2]. C. Tharachand,C. Immanuel Selvaraj and Z. Abraham, Comparative Evaluation of Anthelmintic and Antibacterial Activities in Leaves and Fruits of Garcinia cambogia (Gaertn.) Desr. and Garcinia indica (Dupetit-Thouars),Choisy, An International Journal, 2015; Vol 58, 379-386.

[3]. Khatib NA, Patil PA, Evaluation of Garcina indica Whole Fruit Extracts For Hypoglycemic Potential in Streptozotocin Induced Hyperglycemic Rats. Research J. Pharm. and Tech, June 2011; 4(6).

[4]. Kirana H, Srinivasan BP, Aqueous Extract of Garcinia indica Choisy Restores Glutathione in Type 2 Diabetic Rats, J Young Pharma, Vol 2(3), 265-268.

[5]. Shrereen S, A Review on the Molecule: Hydroxycitric acid, World Journal of Pharmaceutical sciences, Volume 7 (2), 393-418.

[6]. R.R.K.W.Munaweera, Garcinia in Diabetic and Fat control, www.researchgate.net/publication/323614265.

[7]. https://www.oecd.org (Organization for Economic Cooperation and Development guideline 425).

[8]. Mcmanus JGA, Mowry RW, Staining Methods:- Histological and Histochemical; Harper and Row; New York, USA, 1984.

[9]. Kumar M P, Suba V et al, Acute and Subchronic Oral Toxicity Assessment of the Ethanolic Extract of the root of Oncoba spinosa (Flacourtiaceae) in Rodents, Tropical Journal of Pharmaceutical Research, October 2015; 1849-1855.

[10]. Khadke SS, Pachauri DR, Mahajan SD, An Acute Oral Toxicity Study of Gnidia glauca (Fresen.) Gilg. in Albino Rats as per OECD Guideline 425, International Journal of PharmTech Research, April-June 2011; Vol. 3(2), 787-791.

[11]. Hazarikaa I, Sundarib PS , Madhua D et al, Acute oral toxicity evaluation of extracts of Hydrocotyle sibthorpioides in wister albino rats as per OECD 425, Toxicological Reports, 2019; Volume 6, 321-328.

[12]. Dharmalingam S, Natesan G, Evaluation of acute toxicity of the methanolic extract of Tanacetum parthenium L. in albino wistar rats, Journal of Scientific and Innovative Research, 2017; volume 6(3), 113-115.