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Subacute toxicity of Gallic acid, isolated form *Terminalia chebula* in Swiss Albino Mice

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A B S T R A C T

Gallic acid (GA) is the major secondary metabolite observed in *Terminaliachebula* and is reported to possess various pharmacological activities. The present study was designed to evaluate the subacute toxicity of GA isolated from *T. chebula* using a standardized formulation with better pharmacokinetic profile in mice. Subacute treatment with formulation optimized GA at doses of 100, 300 and 1000 mg/kg for 14 days in Swiss albino mice did not produce any signs of toxicity or mortality. No significant effect was observed on body weight, feed intake, hematological parameters and histopathology at all the dose levels tested. In conclusion, GA is found to be non- toxic up to 1000 mg/kg body weight dose in Swiss albino mice up to 14 days. Further studies are being conducted in our laboratory to further understand the safety and efficacy of GA.

Keywords: *Terminaliachebula*, Gallic acid, Mice, Subacute toxicity

A R T I C L E I N F O

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1. Introduction

Terminaliachebula, commonly known as Harithaki, is a main adjuvant in the preparation of 'Triphala'. It is used as alaxative in chronic constipation and also as a rejuvenator ofthe body. *T. chebula*has been recently reported to possess various biological properties like, antioxidant, antibacterial, antifungal, antidiabetic, anticancer, antiulcer, wound healing etc [1]. The chemical constituents presentedin the plant extract are mainly tannins, chebulic acid, chebulinic acid, chebulagic acid, ellagic acid, gallic acid etc. [2-10]. Gallic acid (GA) is the major secondary metabolite observed in *T. chebula*during isolation and it is 18% in the aqueous extract. GA has been recently recognized as possessing anti-allergic, anti-inflammatory, anti-mutagenic and anti-carcinogenic activities. In our recent article we presented the gallic acid (GA) extraction, isolation and identification from *T. chebula*and its antiviral activity against HIV strain 92HT599 [2]. GA has shown good inhibition against 92HT599 strain with IC50 value at0.49 µM. Further, we have also reported the pharmacokinetics of GA using different formulations in rats and mice [3]. GA showed good pharmacokinetics in mice when formulated in 10 % cyclodextrin. Previous studies have shown the no observe adverse effect level (NOAEL) of GA in mice and rats [11-12]. However, these studies did not use the standardized formulation and did not report the pharmacokinetic levels achieved in the study or with the formulation used. Hence in the present study we have performed subacute toxicity of GA using a standardized formulation with better pharmacokinetic profile in mice.

2. Materials and Methods

Isolation of GA from *Terminaliachebula*

The dried fruits of *Terminaliachebula* are powdered in an electrical grinder. 250 gm of the powdered fruit was taken in hexane (1000 mL) and stirred for 24 h at room temperature and the hexane layer was decanted to remove fatty acids, chlorophylls and the lipids. Remaining crude residue was extracted with 95% ethanol (1000 mL) for 48 h at room temperature. The solution was filtered and the residue was extracted two more times each with 95% ethanol (500 mL) for 48 hr at RT and the extracts were filtered under suction pump. The combined extracts were concentrated under reduced pressure using a rotary evaporator at 50-55°C to obtain crude residue is 148 gm. This residue was tested for organic compounds by using high performance liquid chromatography (HPLC) and Liquid chromatography–mass spectrometry (LCMS). Analysis of LCMS and HPLC confirmed the fruit extract of *Terminaliachebula* contains phenolic compounds as major constituents. The crude extract of *Terminaliachebula* shows 18 % of gallic acid by HPLC and the molecular weight was confirmed by LC-MS. The pure gallic acid was isolated following column chromatographic technique from the crude extract of *Terminaliachebula* by using 100-200 mesh silica gel.

Formulation

Gallic acid (extracted from *Terminalia chebula* fruits) 100, 300 and 1000 mg/kg doses were formulated in vehicle (10% β cyclodextrin and 90% water) by trituration method.

Experimental Animals and Housing Conditions

Male Swiss albino mice weighing 30-35g were used for study. Animals were free access to food & water. They were maintained under standard laboratory conditions which included 12-hour light-dark cycle, humidity 50-60% and temperature of 23±2 degrees centigrade. Animals are allowed for a one week of acclimatization period prior to the study.

Sub-acute toxicity study

The toxicity study as carried out using male Swiss albino mice weighing 30-35g.After acclimatization period, animals were randomly divided into following five groups containing six animals in each group. Group I- Normal control, Group-II Vehicle control– received vehicle orally 10ml/kg. Group III, Group-IV and Group-V animals were orally treated with Gallic acid at the dose of 100, 300 and 1000 mg/kg/10ml respectively for 14 days.

Observations

During the experimental period, body weight, feed intake, toxic manifestations and mortality were monitored daily.

Hematological, biochemical and Tissue analysis

On 15^{th} day (After 14^{th} day dosing) animals were anaesthetized with isoflurane anesthesia and blood was collected from retro orbital sinus in EDTA containing tube. Blood is used for the analysis of hematological parameters like hemoglobin, red blood cell count, white blood cell count, platelets, reticulocyte, neutrophils, eosinophils, lymphocytes, monocytes and ESR etc. After blood collection animals were humanely scarified by CO_2 asphyxiation and gross pathology of vital organ like liver, lungs, kidney, heart, brain and spleen werecarried out and stored in 10% buffered formalin for histopathological analysis.

Histopathology: After fixation, tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of ~5 microns thickness were obtained using a microtome. The deparaffinized sections were stained with hematoxylin and eosin (H&E) and observed under a light microscope. Representative two samples from in each group are used for histopathological study

Statistical analysis

All values are expressed as mean ± standard error of the mean (SEM). The Graphs were generated using Graph-Pad Prism (Version 5, Graph Pad Software, La Jolla, CA). Statistical analysis was performed by two-way ANOVA followed by Bonferroni multiple comparison test. Results were considered statistically significant at $p<0.05$.

3. Results and Discussion

Clinical signs and mortality

There were no clinical signs were observed in all treatment groups and all the animals were survived throughout the experimental period.

Body weight changes:

Increase in body weight was observed in all the treatment group animals. However there was no significant difference in body weight between the vehicle control group and the treatment groups.

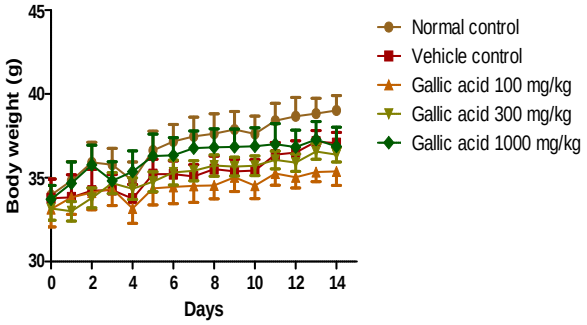

Figure 1: Effect of GA on body weight in Swiss albino mice

Food consumption

Cumulative feed intake of the all the groups were found to be similar. No significant differences were observed in food consumption between the vehicle control and treatment groups (data not shown).

Hematology/ blood biochemistry

In the treatment groups, there is no significant differences were noted in any hematological parameter compared to vehicle control group.

Histopathology:

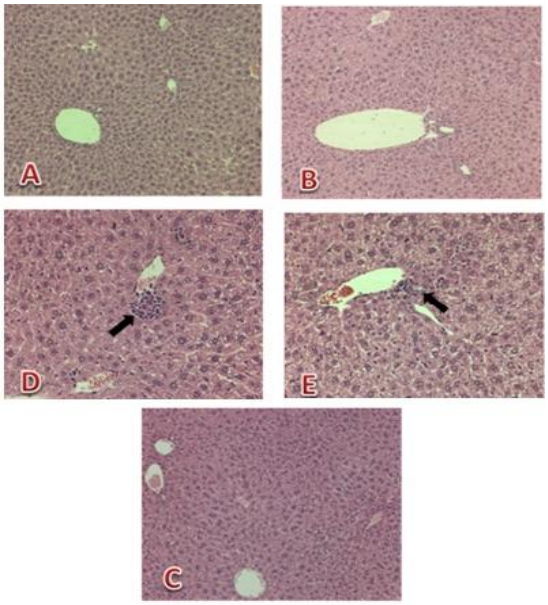

Figure 2: Light micrographs of liver sections of the groups. A-Normal control, B-Vehicle control, C-Gallic acid- 100 mg/kg, D- Gallic acid -300mg/kg and E- Gallic acid -1000mg/kg.

Based on summary of histopathological observation, no major toxic reaction were observed (necrotic and inflammatory changes) in spleen, lung, kidney, heart, and brain in GA treated with 100mg/kg, 300mg/kg & 1000 mg/kg when compared to vehicle control and normal control group. However following mild changes were observed. In liver mild foci of inflammation noticed in the centrilobular region in one animal from GA treated with 300mg/kg and two animals from GA treated with 1000mg/kg. In Spleen GA treated with 1000mg/kg showed mild proliferation of lymphatic follicles. However in the low dose treatment animals no significant changes were observed. In lungs mild alveolar inflammation noticed in vehicle control and normal control group but the entire drug treated group did not showed any inflammatory changes. In heart also mild myocardial inflammation noticed in vehicle control, control group and GA treated with 1000mg/kg but low dose groups did not showed any such lesions. No reactive changes noticed in the kidney and brain of vehicle control, control group and the all the treatment group animals. Except mild reactive changes in the lungs and liver (is spontaneous nature appeared both vehicle control and normal control group) there is no drug related toxic changes in the any organ.

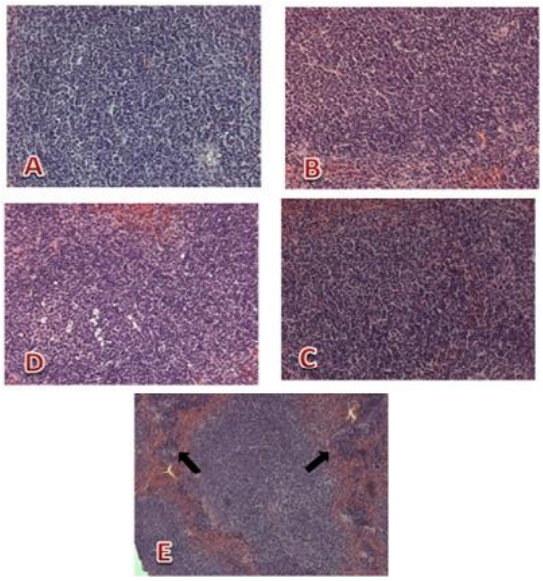

Figure 3: Light micrographs of spleen sections of the groups. A-Normal control, B-Vehicle control, C-Gallic acid- 100 mg/kg, D- Gallic acid -300 mg/kg and E- Gallic acid- 1000 mg/kg.

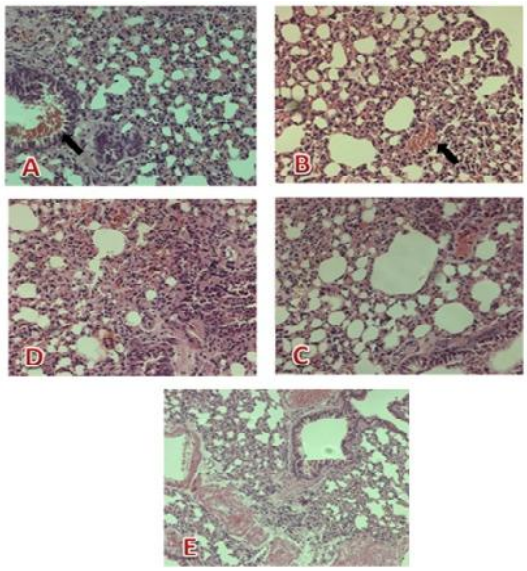

Figure 4: Light micrographs of lungs sections of the groups. A-Normal control, B-Vehicle control, C-Gallic acid- 100 mg/kg, D- Gallic acid -300 mg/kg and E- Gallic acid- 1000 mg/kg.

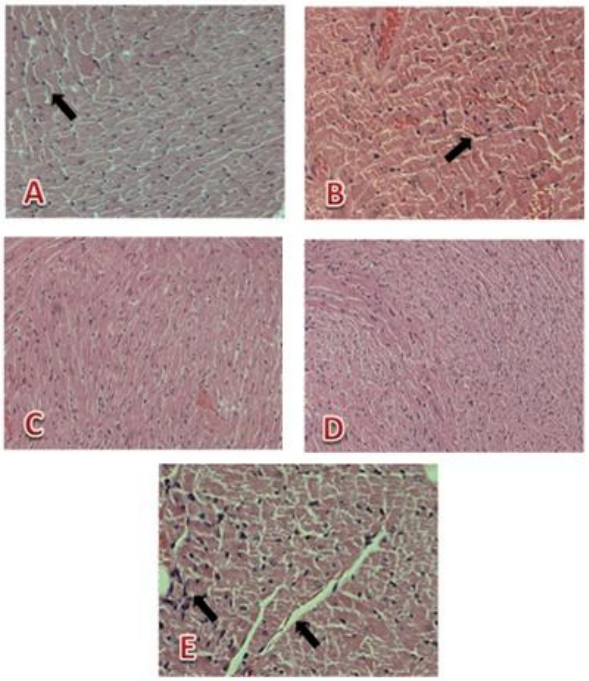

Figure 5: Light micrographs of heart sections of the groups. A-Normal control, B-Vehicle control, C-Gallic acid- 100 mg/kg, D- Gallic acid -300 mg/kg and E- Gallic acid- 1000 mg/kg.

Discussion:

In our previous study, GA has shown good anti- HIV activity against various strains *in vitro*[2]. GA has also been reported to induce cell death in cancer cells [10]. Even though studies were performed to understand the toxicity profile of GA in rodents [11-12], none of them used the optimized formulation with better pharmacokinetic profile. Previously we have established that the pharmacokinetic profile of GA is better in vehicle (10% β- cyclodextrin and 90% water) [3]. Hence in the present study we performed sub-acute toxicity study of GA using this formulation. In the present study, GA at all the doses tested did not show any significant changes in the body weight, feed intake and hematological parameters over 14 days of treatment. The absence of toxic effect even at 100 mg/kg clearly shows the non-toxic nature of GA. These observations were consistent with previous reports [11-12]. Even with the optimized formulation for GA in our study, there was no significant toxicological observation were found. Further we performed histopathology observations of major organs, here except mild reactive changes in the lungs and liver (is spontaneous nature appeared both vehicle control and normal control group) there wasno drug related toxic changes in any organs were observed. These results clearly demonstrated the very non- toxic nature of GA even up to kg dose for 14 days.

Table 1: Hematological parameters after 14 days oral treatment with Gallic acid

Parameters	Normal control	Vehicle control	GA-100 mg/kg	GA-300 mg/kg	GA-1000 mg/kg
Hemoglobin	13.56±0.04	14.23±0.50	13.10±1.15	13.66±0.18	13.03±0.2
Total Red Blood Cell	4.56±0.04	4.83±0.12	4.35±0.19	4.65±0.27	4.70±0.34
Total White Blood Cell	8367 ±315	9933±780	10333±2195	10450 ±654	10400±1204
Neutrophils	39.00±3.12	47.00±1.10	44.33±3.45	43.16±2.79	43.00±1.59
Lymphocytes	45.66±1.12	46.66±1.05	44.00±2.03	49.33±1.48	46.66±1.05
Eosinophils	10.66±3.04	6.66±1.71	9.00±1.32	9.00±1.67	10.33±0.56
Monocytes	1.66±0.21	1.80±0.18	1.50±0.32	2.00±0.0	2.00±0.0
Basophils	NIL	NIL	NIL	NIL	NIL
Platelets count	5.86±0.29	5.85±0.44	7.08±0.63	7.00±0.35	7.33±1.04

4. Conclusion

In conclusion, GA is found to be non- toxic up to 1000 mg/kg body weight dose in Swiss albino mice up to 14 days. Further studies are being conducted in our laboratory to further understand the safety and efficacy of GA.

5. Acknowledgements

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