



EVALUATION OF THE ACUTE TOXICITY STUDY OF METHANOLIC LEAF EXTRACT OF GOSSYPIUM HIRSUTUM IN ALBINO MICE

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ABSTRACT

The present study was carried out to evaluate the acute oral toxicity of methanol extract of *Gossypium hirsutum* leaves in male albino mice. For the acute toxicity study, mice were orally administered single dose of 100,500,1000mg/kg bw methanol extract of *G. hirsutum* and observed for behavioral changes and mortality, if any. During 14 days of study mice were observed daily for any change in their body weight, food and water consumption. At the end of 14 days mice sacrificed for hematological and biochemical analyses. The extract at highest dose of 1000mg/kg bw did not produce mortality in any of tested animals during study period. Therefore, the LD50 of this plant was estimated to be more than 1000mg/kg bw. Hematology reveals that the extract has hemolytic activity. Liver and Kidney functions were assessed by determining serum parameters like creatinine, transeaminase bilirubin, and urea. All these parameters were significantly abnormal and indicate liver and kidney dysfunctioning. The results of the study showed that the extract was categorized as *slightly toxic* and provides valuable data on the toxicity profile of plant.

INTRODUCTION

Plants and herbs have been used since ancient times to cure various ailments. By the middle of the nineteenth century, approximately 80% of all medicines were derived from herbs [1]. Herbal medicine (or) phytomedicine is recognized as the most common form of alternative medicine [2]. The world health organization (WHO) estimates that 80% of the world's population relies on these "alternative" plant based medicines especially in the developing and in the developed countries where modern medicines are predominantly used [3, 4&5]. Over the years, the use of herbs in the treatment of illnesses has been very successful and its historic usage has been useful in drug discovery development [6, 7].

Herbal remedies are considered safer and less damaging to the human body than synthetic drugs [8]. However, the lack of standardization has been a major concern regarding use of herbal medicines [9, 10]. Although herbal supplements may be considered to be safe, some are known to be toxic at high doses and others may have potentially adverse effect after prolonged use [11]. *Gossypium hirsutum* also known as upland cotton (or) Mexican cotton belongs to the family malvaceae [12]. It is the most widely planted species of cotton on the United States and is native to Central America. *Gossypium hirsutum* was named due to hairiness. *G. hirsutum* used by the traditional medicinal practitioners in the treatment of diseases such as yellow fever, influenza [13].

Leaves steeped in vinegar are applied to the forehead for headache. Cotton seed and roots have been used in treating nasal polyps, uterine fibroids and other types of cancer. Its main active ingredient, gossypol, has shown anticancer activity and is being used in china as a male contraceptive. Flowers are used to promote urination and moisten the skin. The root decoction is used for asthma, diarrhea, and dysentery; the mucilaginous tea of fresh (or) roasted seeds is used for bronchitis, diarrhea, dysentery and hemorrhage. It can also causes other harmful effects by mainly targeting the organs such as Testis with reduced sperm motility, inhibited spermatogenesis and also affects female reproductive organs and embryo development due to presence of toxic component i.e. Gossypol[14]. Despite the wide spread studies of *G. hirsutum* done by researchers, however, less emphasize has been laid on toxicological effects of this plant. Information about toxicity of this plant is very important as a baseline before exploring further to develop this plant as a new herbal medication. Preliminary phytochemical screening revealed the presence of tannins and phenol compounds, proteins, phytosterols and flavanoids. Therefore cotton leaves can be manipulated in the herbal treatment of various diseases and as a potential source of useful elements for drugs formulation. Literature survey also reveals that the *G. hirsutum* causes several toxic effects in animals due to the presence of phenolic compound, Gossypol a toxic component in animal feed. Scientific opinion of the panel on contaminants in the food chain is that Gossypol as undesirable substances in animal feed [15]. The main objective of the study was to investigate the possible acute oral toxic effects of the methanol leaf extract of the plant in albino mice using biochemical and hematological parameters.

MATERIALS AND METHODS

Plant Material

The leaves of *Gossypium hirsutum* were collected from the fields of Lam form, Guntur, A.P. It was authenticated by regional agricultural research station, Lam, Guntur.

Sample extraction: The leaves of *G. hirsutum* were collected, shade dried and powdered to fine form. Twenty five grams of this powder was packed in "SOXHLET APPARATUS" using non absorbent cotton. Extraction was carried out with 200ml of methanol. The resultant alcoholic extract was collected, filtered and concentrated in a china dish. The obtained extract was subjected to the identification of constituents by preliminary phytochemical screening and acute toxicity studies.

Experimental Animals

Adult healthy male albino mice (10-12 weeks) weighing 20-30gm were used for the acute toxicity study are housed in plastic cages under standard environmental conditions. All mice were acclimatized at an ambient temperature of $25\pm 2^{\circ}\text{C}$, with a 12hrs light-dark cycle for at least 7 days prior to the experiment. All the animals were provided with food and water ad libitum. The experiment was conducted in accordance with the internationally acceptable guidelines for evaluating the safety and efficacy of herbal medicines (WHO2000:OECD2008) and were approved by Institutional Animal Ethical Committee (IAEC).

Sample preparation

The dried methanol extract was suspended using 1% carboxy methyl cellulose as a vehicle. The suspension was stored at 4°C for further usage.

Acute toxicity study

In order to evaluate acute oral toxicity 4 groups of 6 mice each are used in the experiment. The control group received the vehicle (1% CMC in distilled water), and 3 test groups received single dose of methanol extract of plant in a dose of 100, 500, 1000mg/body weight orally using gavage needle. The dose was selected after several screenings on mice. The experimental animals were deprived of food for 24 hrs prior to extract administration. They were monitored continuously for 3hrs thereafter for any signs of toxicity such as reduction in locomotion, aggressiveness, reaction to stimuli (tail pinch, noise), social

interactions, aspects of feces and mortality. Dead animals in each group are counted within 48hrs following the administration of the extract. The surviving animals are monitored daily for 14 days on the changes in skin, fur, respiration, eye, mucus membrane (nasal), body weight, and food and water consumptions.

Cage Side Observation

In this study, animals were observed individually and special attention was given during the first 4hrs and thereafter, every 12hrs daily, for a total of 14days. All observations were systemically recorded, with individual records being maintained for each animal. The cage –side observation included evaluation of skin and fur, eyes, respiratory effects, autonomic effects such as salivation, diarrhea, urination and the central nerve effects including tremors and convulsions, changes in the level of activity, reactivity to handling (or) sensory stimuli and altered strength.

Body weight Measurement

Individual body weights were measured and recorded prior to the administration of extracts and daily for 2weeks throughout the experimental periods

Food and water Consumption

The amount of food and water was measured daily from the quantity of food and water supplied and the amount remaining after 24hrs for 2weeks of the study period.

Haematological Analysis

For this analysis blood samples were collected prior to the end at the end of experimental period. All the mice were anesthetized using chloroform and the samples were collected via cardiac puncture (inferior venacava puncture) in plastic test tubes containing EDTA anti coagulant. Hematology analyses were performed on whole blood using the automatic hematology system ADVIA 60 open tube (Bayer corporation, USA) to evaluate the following parameters: erythrocytes count (RBC), hemoglobin(HGB), total leukocyte count (WBC), and differential

leukocyte count (Polymorphs, Lymphocytes, Eosinophils, Monocytes).

Biochemical analysis

At the end of acute toxicity study (14 days) all the animals were anesthetized using chloroform and bled via inferior venacava puncture. The samples were collected in plastic tubes and allowed to stand for complete clotting. The clotted blood sample were centrifuged at 3000rpm for 15min and serum samples were aspirated off and frozen at -80°C. Serum sample were analysed for determination of urea, creatinine, glucose, total cholesterol, alanine amino transferase (ALT), total bilirubin (BIL).

Statistical analysis

The results were expressed as mean \pm standard deviation. One way analysis of variance (ANOVA) was employed for b/w and within group's comparison. A mean difference was considered significant at $P < 0.05$ level

RESULTS

The oral administration of *G. hirsutum* leaves extract in doses ranging from 100-1000mg/kg bw showed very low level of activity (slow movement), slow breathing (dyspnea) and decreased intake of food and water. No mortality was observed in any of the groups and these results indicating that the median lethal dose (LD50) is higher than 1000mg/kg bw for male albino mice. Significant decrease in body weight of treatment groups compared to the control group, results shown in graph-1. The results of the hematological and biochemical analyses are summarized in Tables 1, 2. Hematological and serum biochemical results showed statistically significant ($p > 0.05$) differences in all the treatment groups.

DISCUSSION

Herbal medicine is universally popular in primary health care, particularly in developing countries. The wide usage of these so-called “natural remedies” (or) “medicinal herbs” is a result of the fact that the general public believes them to be safe without compromising health effects [16].



Figure 1- Plant and leaves of Gossypium hirsutum

Table 1 Phytochemical screening of methanol extract of leaves

Phytoconstituents	result
Carbohydrates	+
Tannins & phenol compounds	+
Proteins	+
Alkaloids	-
Phytosterols	+
Cardiac glycosides	-
Saponins	-
Flavonoids	+

+ = present - = absent

Graph1. Body weight changes in mice in acute toxicity study.

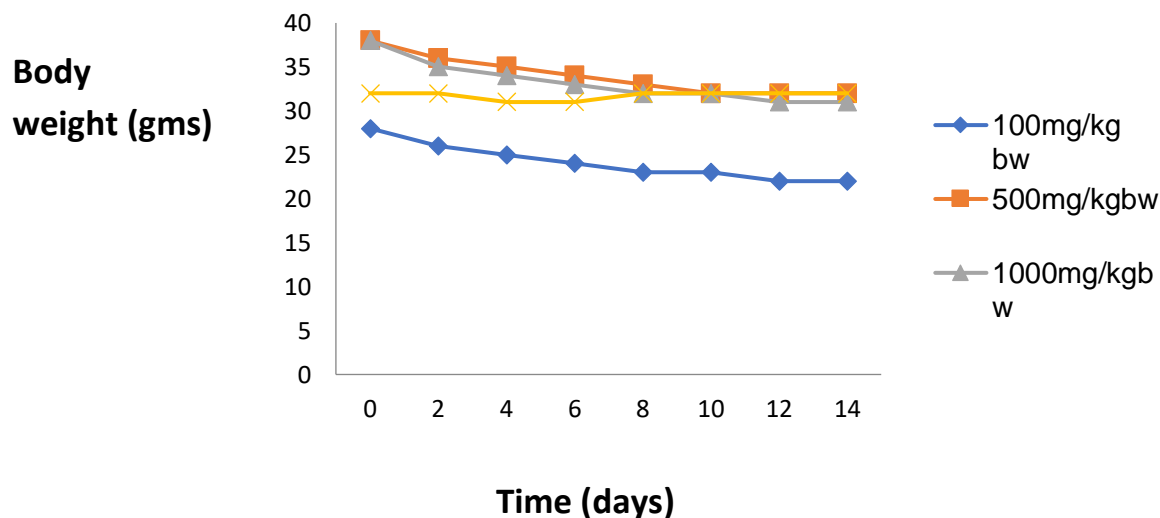


Table1. Hematological results from mice in acute toxicity study.

Parameters	Control	100mg/kg	500mg/kg	1000mg/kg
Hb (g/dl)	14.0±0.2	11.7±0.38	10.28±0.099	8±0.32
TRBC (mill/cumm)	4.5±0.10	3.9±0.17	3.5±0.16	3.2±0.12
Total leucocyte count (cells/cumm)	6000±1.63	5700±0.53	4900±0.57	4600±1.20
Differential leucocyte count				
Polymorphs (%)	34±1.53	43±1.73	54±0.90	60±0.89
Lymphocytes (%)	58±2.12	47±4.23	41±2.83	32±3.54
Eosinophils (%)	04±1.41	4.3±1.83	4.6±1.14	05±1.53
Monocytes (%)	04±2.54	4.2±2.83	4.7±2.24	05±2.21

Values are expressed as mean \pm SD, n=6(number of animals per group).p<0.05 was considered significant. All the values were significantly differ from control at p<0.05.

Table 2. Serum biochemical parameters from mice in acute toxicity study.

Parameters	control	100mg/kg	500mg/kg	1000mg/kg
ALT (U/L)	158 \pm 1.71	187 \pm 8.36	236 \pm 2.51	298 \pm 3.87
Urea (mg/dL)	40.0 \pm 0.86	276 \pm 0.35	339 \pm 0.35	481.2 \pm 0.48
Glucose (mg/dL)	465 \pm 5.98	327 \pm 0.10	259 \pm 0.15	191.5 \pm 1.08
Creatinine (mg/dL)	1.6 \pm 0.36	1.37 \pm 0.58	1.12 \pm 0.91	1.0 \pm 0.70
Cholesterol (mg/dL)	114 \pm 0.35	106 \pm 0.81	95 \pm 0.39	90.8 \pm 0.53
Total bilirubin (U/L)	0.7 \pm 0.34	14 \pm 3.5	28 \pm 4.5	36.2 \pm 6.6

Values are expressed as mean \pm SD, n=6(number of animals per group).p<0.05 was considered significant. All the values were significantly differ from control at p<0.05.

Since there is a lack of proven scientific studies on the toxicity and adverse effects of these remedies, further investigations are vitally needed and apply for *G. hirsutum*. *G.hirsutum* used by the traditional medical practitioners in the treatment of diseases such as yellow fever, influenza [13]. Gossypol is a toxic component that is produced for defense by the plant and causes deleterious effects to no ruminants. The utilization of cotton plant and its by-products is limited due to the presence of gossypol in various parts. There were reports in the print media that sheep died in Telangana region of Andhra Pradesh after grazing on leaves and pods of harvested Bt cotton plant residue in fields [17]. Scientific opinion that Gossypol as undesirable substance in animal feed [15]. The main target organ of gossypol toxicity following repeated spermatogenesis and depressed sperm counts. Suppressed spermatogenesis on humans is partly irreversible. Gossypol also affects female reproductive organs and embryo development. Because of several harmful effects makes these studies important. In the current study, the methanol extract has been used since the previous study has shown that methanol extract possess higher content of phenolic compound i.e. gossypol. In the 14th day of acute toxicity evaluation mice treated with *G.hirsutum* extract at a dose of 100, 500, 1000mg/ kg bw showed no mortality, this may be due to less gossypol content in *G. hirsutum* than other gossypium species [18]. Thus the results obtained in the study suggested that LD50 of *G.hirsutum* is higher than 1000mg/kg as no lethality was found in the experimental period. In case of cage side observation treatment group showed

very low level of activity (slow movement), slow breathing (dyspnea) and significant changes in food & water consumption i.e. loss of appetite this is evident by decrease in body weight. In general, increase (or) decrease in the body weights of animals can be used as an indicator of adverse effects of drugs and chemicals [19]. In the present toxicity study, *G.hirsutum* leaves extract showed decrease in body weight of mice at any dose throughout the treatment period. The hematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in humans and animals [20]. Blood parameters are relevant indicators for risk evaluation. Olson etal (2000), reported that changes in the hematological system have a higher predictive value for human toxicity when the data are extrapolated from animal studies [21]. In case of hematology decrease in concentration of %Hb, TRBC, TLC was observed. In case of DLC, increase in concentration of eosiniphills, polymorphs, monocytes and decrease in lymphocytes were observed. These results will indicates that *G.hirsutum* leaves extract have hemolytic activity. The kidneys and liver are two major organs that play roles in detoxification. A number of cases of renal and hepatic toxicity have been reported following the use of phytotherapeutic products [22, 23, 24&25] . Measurements of urea and creatinine levels in the blood are usually performed to evaluate kidney functions [26]. The most common parameters used to assess liver function are ALT, AST and ALP (). Biochemical analyses showed significant increase of ALT, bilirubin, urea and decreased

levels of cholesterol, glucose, creatinine indicates impairment to hepatic functions, damage of liver cells, kidney dysfunctions, impaired carbohydrate metabolism.

CONCLUSION

The present studies demonstrate that *G. hirsutum* leaves extract will produce anorexia (loss of appetite), severe haemolysis after single oral dose of administration. The highest dose of extract also induces acute severe hepatotoxicity and mild nephrotoxicity but there is no mortality was observed because of low gossypol content in *G. hirsutum* among all gossypium species. This indicates that the extract has a LD 50 value higher than 1000mg/kg bw. This study serves as a guide for selection of plants for further phytochemical work on the isolation and identification of the active compounds. It also provides valuable data on the toxicity profile of plant that should be essential for further study. Further studies may focus on chronic toxicity in order to evaluate its long term effects.

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Abbreviations: Hb, Hemoglobin; RBC, Red blood cell(or) erythrocytes; WBC, Leukocyte count; DLC, Differential leucocytes count; ALT, Alanine amino transferase; EDTA, Ethylenediamine tetra acetic acid; OECD, organization of economic committee and development; IAEC, Institutional animal ethics committee.

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