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# Toxicity Studies of *Abutilon crispum* and *Indigofera prostrata* Whole Plants on Wistar Rats

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ABSTRACT

*Abutilon crispum* and *Indigofera prostrata* are widely distributed Indian medicinal plants. They are extensively used to treat various diseases by Indian herbal medicine practitioners. Although, preclinical studies have been reported on these plants, the existing toxicological data is inadequate. This study aims to evaluate the acute and chronic toxicity of *Abutilon crispum* and *Indigofera prostrata* whole plants in rats. Extracts of the plants were obtained by maceration in methanol. For the acute toxicity study, a single oral dose of 2000 mg/kg of the methanol extracts of the plants were administered to the rats, and the rats were observed for 14 days for signs of toxicity or death. For the chronic toxicity study, the rats were administered 250, 500, and 1000 mg/kg of the extracts once daily for 90 days. Throughout the treatment period, the rats were observed for signs of toxicity, mortality and body weight changes. After 90 days, the animals were sacrificed and blood samples were collected for haematological and biochemical analysis, and vital organs (liver and kidneys) were used for histological examination. Acute toxicity study revealed that the methanol extracts of *Indigofera prostrata* and *Abutilon crispum* were non-toxic up to a dose of 2000 mg/kg. Similarly, in the chronic toxicity study, no significant changes were observed in the body weights, haematological and biochemical parameters of the rats. The histoarchitecture of the liver and kidneys appeared normal. Therefore, the extracts of *Abutilon crispum* and *Indigofera prostrata* whole plants are non-toxic, and is relative safe on chronic administration.

***Keywords*:** *Indigofera prostrata*, *Abutilon crispum*, Wistar rats, Toxicity.

## Introduction

Novel drugs need to be evaluated for their toxicity, before they are administered to humans. As per the Guidelines of Organization for Economic Corporation and Development (OECD), toxicity studies can be conducted primarily as acute, sub-acute and chronic toxicity studies.[1] It has been proven that toxicity studies are essential for identifying any potentially harmful consequences that may arise from using a drug.[1] As per OECD guidelines, acute toxicity is a term used to describe the harmful consequences that can arise from administering a single dose or multiple doses of a chemical orally over 24 hours. Chronic toxicity evaluation helps to identify a number of incompatible effects by offering vital information on the cumulative toxicity of a substance. Toxicological investigations are typically conducted on animals such as rats, mice, and other species, such as cats, dogs, and monkeys, in which the animals are examined for a year.[2] The objective of the current study is to evaluate the acute and chronic toxicity of *Indigofera prostrata* and *Abutilon crispum*, and to provide an up-to-date information on the toxicological profile of the plants. The data obtained from the study might be helpful in determining the exact dose that is safe for use by humans.

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## Materials and Methods

### *Plant collection and identification*

*Abutilon crispum* (L.) Medik and *Indigofera prostrata* whole plants were collected from the district of Chittoor, in the province of Andhra Pradesh, India in July 2021. The plant materials were identified and authenticated by Dr K. Madhava Chetty a retired Associate Professor of Botany at Sri Venkateshwara University in Tirupathi, India. Herbarium specimens were prepared and preserved in the herbarium with voucher numbers 1129 and 0477 for *Indigofera prostrata* and *Abutilon crispum*, respectively.

### *Plant preparation and extraction*

The whole plants of *Abutilon crispum* and *Indigofera prostrata* were shade-dried, and ground into coarse powder. The dried powdered samples (500 g each) were extracted by maceration in methanol. The extracts were filtered, and then concentrated *in vacuo* using a rotary evaporator. The concentrated extracts were further dried in a desiccator.

### *Animals*

Wistar rats weighing between 180 - 200 g were procured from VAB Biosciences, Hyderabad, Telangana, India. The animals were acclimatized to the laboratory conditions for 10 days. They were exposed to 12:12 hour dark and light cycle with a temperature range of 20-25°C. Each animal was kept in separate polypropylene cage with husk provided as bedding. The animals were well-fed and had unrestricted access to drinking water.

### *Acute oral toxicity study*

The methanol extracts of *Abutilon crispum* and *Indigofera prostrata* were used for oral acute toxicity investigation following OECD test guideline 425 as previously described.[3] The animals were fasted for three

hours prior to extracts administration but had free access to water. The rats were administered a single dose of 2000 mg/kg b.wt of methanol extracts through oral gavage for a period of 14 days. The animals were observed for any morphological and behavioral changes, including touchiness, tremors, salivation, spasms, torpidity, and loose bowels if any and deaths during the study period.[4]

*Chronic oral toxicity study*

Chronic toxicity study of the extracts was assessed following the OECD guideline 407. The rats were divided into seven groups. Group I was used as the control and was administered distilled water only. Groups II to VII animals were considered the test groups, and were administered the extracts as follows: Group II were administered 250 mg/kg of *Abutilon crispum* extract (MEAC) orally, Group III were administered 500 mg/kg of *Abutilon crispum* extract orally, Group IV received 1000 mg/kg of *Abutilon crispum* extract orally, Group V received 250 mg/kg of *Indigofera prostrata* extract (MEIP) orally, Group VI received 500 mg/kg of *Indigofera prostrata* extract (MEIP) orally. Group VII animals received 1000 mg/kg of *Indigofera prostrata* extract (MEIP) orally. The animals were administered the extracts once daily for the 90-days.[5] Throughout the study period, the body weight of each animal in the groups was measured in the beginning and at the end of the study. After ninety days, blood samples were collected from the rats in each group using a retro orbital plexus capillary tube and placed into Eppendorf tubes with an anticoagulant. The serum was maintained at 40$^{o}$C and was used to estimate several serum biochemical parameters, such as total protein, glucose, albumin, creatinine, serum glutamate-pyruvate transaminase (SGPT), serum glutamate-oxaloacetate transaminase (SGOT), bilirubin, and electrolytes (calcium, chloride, and phosphorus). Blood samples were also collected in trisodium citrate coated tubes, and used for hematological analysis. After blood sample collection, the animals were euthanized using phenobarbital infusion (120 mg/kg b.wt). Internal organs, including kidneys, liver, heart, brain, and lungs were harvested. The relative organ weight was determined, and histological analysis was done.

*Biochemical analysis*

*Serum biochemical analysis was estimated using semi-automated analyzer.*

*Measurement of serum glucose*

Serum glucose was measured using Trinder method, where chemicals from a reagent kit are added to the test sample, and a standard glucose solution. Absorbance of the reaction product was measured at 505 nm against a reagent blank.[6,7]

*Measurement of serum electrolytes*

Serum electrolytes including calcium, chloride, and phosphorus were determined following standard procedures.

*Measurement of serum creatinine*

Serum creatinine concentration was estimated using the reagent kit obtained from AGD biomedicals. The absorbance of the test and standard was measured at 520 nm.

*Determination of serum total protein concentration*

The serum total protein concentration was determined using the end point assay method with the help of reagent kit obtained from Span Diagnostics Ltd. Absorbance of the resulting solution for both the test and standard was measured at 578 nm against a reagent blank.

*Determination of serum albumin concentration*

The serum albumin concentration was determined using the bromocresol green end point technique. The absorbance of both the test and standard was measured at 630 nm against a reagent blank. The results were recorded in g/dL.

*Determination of serum globulin concentration*

Serum globulin concentration was estimated using the following formula:

Globulins = Total protein - Albumin. The values were given in g/dL.

*Determination of serum bilirubin concentration*

Serum bilirubin concentration was measured using the Malloy and Evelyn method.[8] To two test tubes, each containing 1.8 mL of distilled water and 0.2 mL of test serum were added 0.6 mL of diazo reagent. To a blank test tube was added 0.5 mL HCl (1.5%). Thereafter, 2.5 mL of methanol was added to both test and blank. The cylinders were then placed on ice for 30 minutes, after which the absorbance was measured at 540 nm. A standard calibration curve of bilirubin was prepared. The bilirubin concentration was expressed in mg/dL.

*Measurement of serum transaminases (GOT and GPT)*

Serum transaminases (GOT and GPT) were estimated according to Reitman and Frankel technique using the chemicals obtained from Span Diagnostics Ltd.[9] Absorbance of the reaction mixture was measured at 505 nm against a reagent blank. Values were presented as $UL^{-1}$.

*Measurement of serum alkaline phosphate (ALP)*

ALP was evaluated according to the method of Kind and King.[9] The absorbance of the test and standard was measured at 640 nm in comparison to the reagent blank.

*Haematological analysis*

The samples were subjected to haematological analysis using automated haematology analyzer. The parameters evaluated include; white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hgb) concentration, platelets count, and mean cell volume.

*Histopathological analysis*

The histopathological examination of sections of the liver and kidneys was done according to standard procedures.

*Statistical analysis*

Data were reported as mean ± standard error of mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) and Dunnet-t test using SPSS statistical programme. Statistical significant differences between means was considered at P-value $\leq 0.05$.

## Results and Discussion

People have been using herbal formulations for the treatment of diseases since ancient times because they were thought to be safe, effective and to have few adverse effects. To determine the effective dose of herbal formulations and understand the potential consequences of using these herbal remedies, information regarding their oral toxicity is essential.[10] A realistic analysis of acute and chronic toxicity studies of herbal medicines or herbal formulations may provide knowledge that would increase public confidence on the safe use of these herbal products, and also increase their use in pharmaceutical formulations. Evaluating the toxicological effects of extracts is a crucial initial step in assessing the potential dangers of any medicinal plant meant for use by humans or animals.[11]

In the present investigation, Wistar rats were administered extracts of *Abutilon crispum* and *Indigofera prostrata* whole plants, and their acute and chronic effects were monitored. For the acute oral toxicity test, the rats showed no symptoms of toxicity, and all the rats survived for the 14-days treatment period. The median lethal dose ($LD_{50}$) was found to be greater than 2000 mg/kg body weight and all the evidences indicate that *Abutilon crispum* and *Indigofera prostrata* methanol extracts are safe at 2000 mg/kg. Overall results reveal the non-toxic nature of *Abutilon crispum* and *Indigofera prostrata* methanol extracts.

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For the chronic toxicity tests, the methanol extracts of both plants at 250, 500, and 1000 mg/kg b.wt. appear essentially non-toxic. However, in situations where some plant components like flavonoids are used in the therapy of chronic conditions like diabetes, cancer and others, there is the need to be cautious of the chronic effects of these components on body weight, and vital organs.

*Effect of extracts on body weight of rats*

The changes in body weight in each experimental rats in all the groups following the chronic administration of *Abutilon crispum* and *Indigofera prostrata* whole plant extracts are shown in Table 1. The average body weight of the rats in the treatment groups and the control group did not differ significantly. This finding demonstrated that the Indian medicinal herbs; *Indigofera prostrata* and *Abutilon crispum* methanol extracts had very little or no influence on animal growth.

*Effect of extracts on organ weight*

The relative organ weight of the rats in the treatment and control groups at the end of the study are presented in Table 2. Vital organs, including the heart, kidneys, liver and lungs were found to show no significant changes in weight compared to the control. This indicate that administration of the extracts results in no adverse effects, meaning that the medicinal plants *Indigofera prostrata* and *Abutilon crispum* may be safe on chronic use.

**Table 1:** Effect of methanol extracts of *Abutilon crispum* and *Indigofera prostrata* whole plant on body weight (90 days)

| Weight (g) | Group I (Control) | *Abutilon crispum* | | | *Indigofera prostrata* | | |
|---|---|---|---|---|---|---|---|
| | | Group II (250 mg/kg) | Group III (500 mg/kg) | Group IV (1000 mg/kg) | Group V (250 mg/kg) | Group- VI (500 mg/kg) | Group- VII (1000 mg/kg) |
| Initial | 172.34 | 171.21 | 175.66 | 175.99 | 174.21 | 175.58 | 177.97 |
| Final | 255.63 | 244.52 | 256.73 | 264.77 | 224.12 | 222.86 | 202.74 |

Values are mean ± SEM (n = 6)

**Table 2:** Effect of methanol extract of Abutilon crispum and Indigofera prostrata whole plant on organ weight (90days).

| Organ weight (g) | Group I (Control) | *Abutilon crispum* | | | *Indigofera prostrata* | | |
|---|---|---|---|---|---|---|---|
| | | Group-II (250 mg/kg) | Group-III (500 mg/kg) | Group IV (1000 mg/kg) | Group V (250 mg/kg) | Group VI (500 mg/kg) | Group VII (1000 mg/kg) |
| Liver | 3.14 ± 0.03 | 3.20 ± 0.35 | 3.24 ± 0.02 | 3.33 ± 0.13 | 3.16 ± 0.51 | 3.25 ± 0.16 | 3.35 ± 0.13 |
| Lungs | 0.84 ± 0.13 | 0.85 ± 0.15 | 0.87 ± 0.13 | 0.84 ± 0.12 | 0.91 ± 0.5 | 0.94± 0.18 | 0.97 ± 0.14 |
| Heart | 0.47 ± 0.1 | 0.36 ± 0.05 | 0.41 ± 0.02 | 0.44 ± 0.02 | 0.48 ± 0.06 | 0.53 ± 0.03 | 0.54 ± 0.02 |
| Kidneys | 0.63 ± 0.01 | 0.65 ± 0.04 | 0.61 ± 0.03 | 0.65 ± 0.06 | 0.67 ± 0.07 | 0.93 ± 0.05 | 0.99 ± 0.06 |

*Effect of extracts on serum biochemical parameters*

The results of serum biochemical parameters evaluated are presented in Figures 1 and 2. After the 90-day treatment period, the serum biochemical parameters (Bilirubin, SGOPT, ALP and SGPT) of the rats in the treatment group did not show any significant difference from that of the control group. These biochemical parameters are often used as indices for assessing liver function.[12] In addition, there was no appreciable variation in serum levels of albumin, globulin and total protein between the animals in the treatment groups and that of the control. The current test did not demonstrate hypoproteinemia, a usual finding in liver injury. Finally, the serum creatinine levels in both the treatment and control groups were within the normal range. The functioning of the kidneys, and the renal system is usually assessed from the serum creatinine levels.[13,14] Estimation of the renal function parameters like urea, creatinine and serum electrolytes give useful information on drug-induced renal toxicities.[15] The results of serum creatinine obtained from this study indicate that *Abutilon crispum* and *Indigofera prostrata* extract have no toxic effect on the kidneys.

*Effect of extracts on haematological parameters*

The effect of the extracts on hematological indices are shown in Figures 3 and 4. There were no noticeable alteration in the haematological parameters (hemoglobin, platelets, RBC, WBC and mean cell volume) of the animals treated with the plants extracts when compared to the control animals. Any chemical, medication, or plant extract administered to experimental animals may result in hemorrhage or interruption of blood cell synthesis; a reduction in RBC synthesis is associated with anemia.[16,17] The results of the study indicate that the methanol extracts of *Abutilon crispum* and *Indigofera prostrata* whole plant is relatively safe, would not cause any toxic effect on the hematopoietic system.

*Histopathological effect of the extracts*

Histopathological analysis of the vital organs (liver and kidneys) of the rats treated with the methanol extracts of *Abutilon crispum* and *Indigofera prostrata* whole plant showed no changes in the histoarchitecture of rats when compared to the rats in the control group. Photomicrographs of the liver and kidney sections of rats treated with methanol extracts of *Abutilon crispum* and *Indigofera prostrata* are shown in Figures 5 and 6. From the results, it is clear that the extracts of both plants have no toxic effect on the organs investigated.

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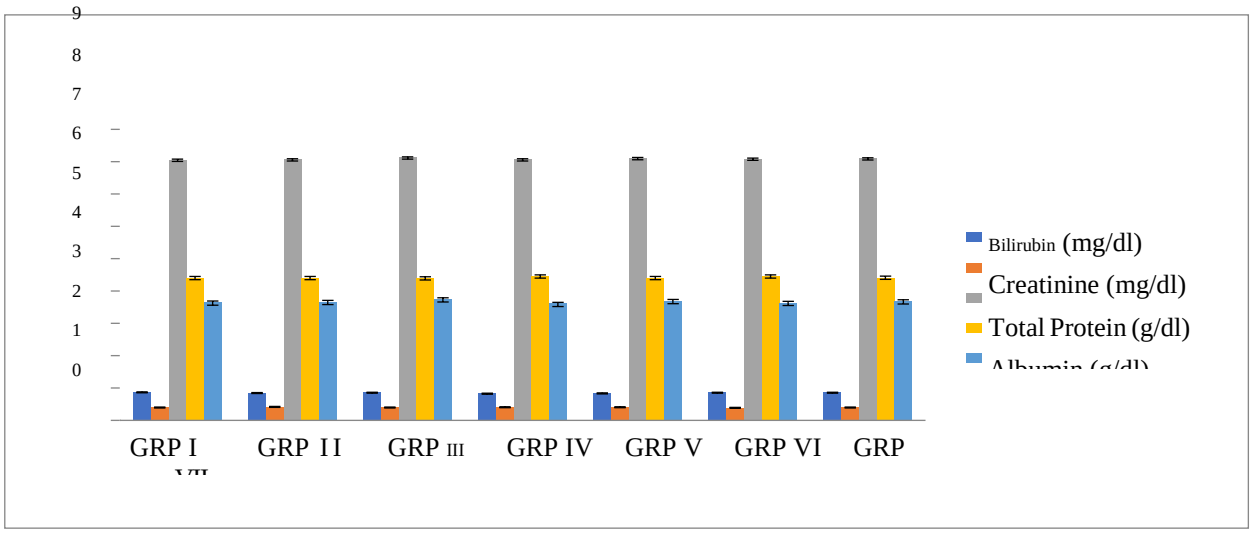


**Figure 1:** Effect of methanol extracts of *Abutilon crispum* and *Indigofera prostrata* whole plant on serum biochemical parameters

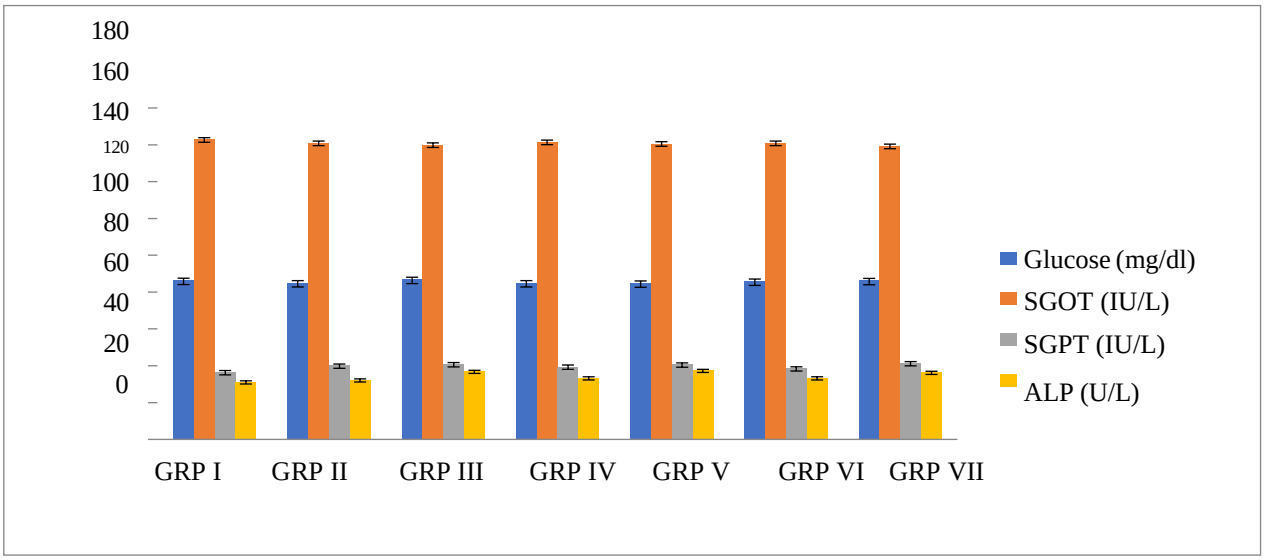


**Figure 2:** Effect of methanol extracts of *Abutilon crispum* and *Indigofera prostrata* whole plant on serum biochemical parameters

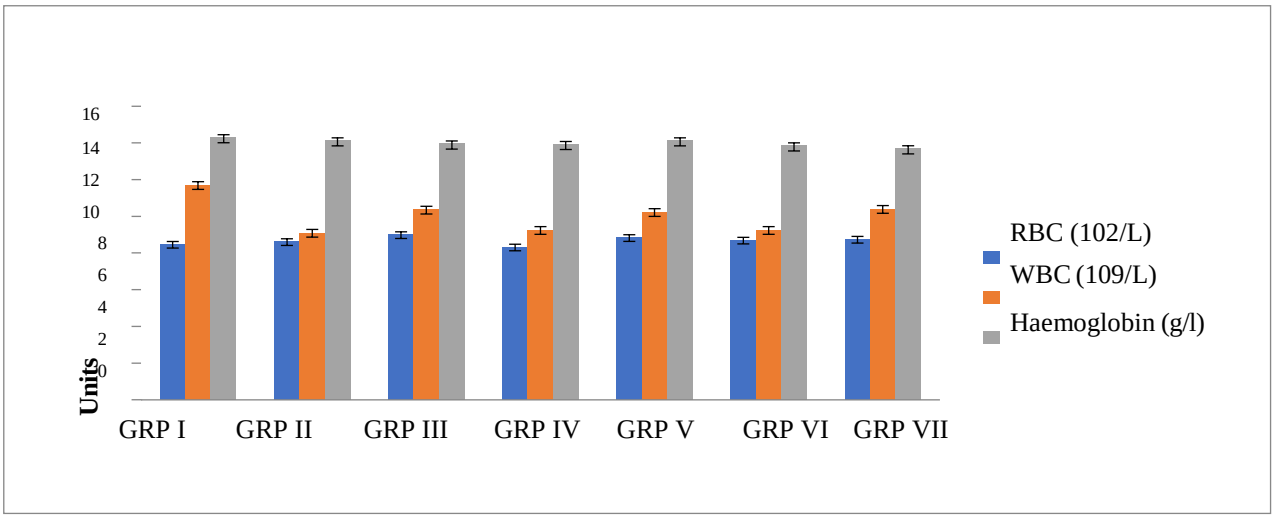


**Figure 3:** Effect of methanol extracts of *Abutilon crispum* and *Indigofera prostrata* whole plant on haematological parameters

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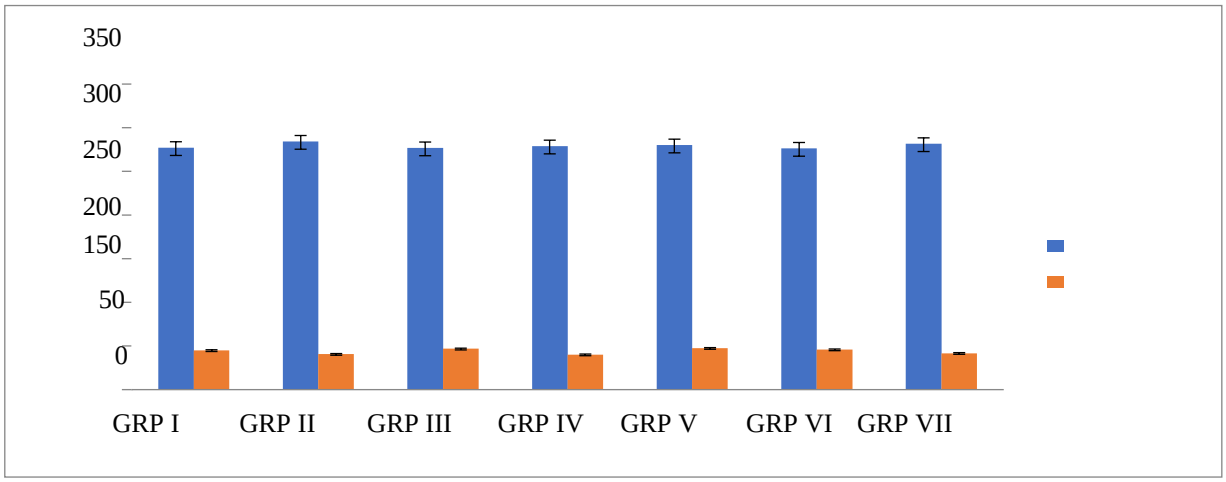


**Figure 4:** Effect of methanol extracts of *Abutilon crispum* and *Indigofera prostrata* whole plant on hematological parameters

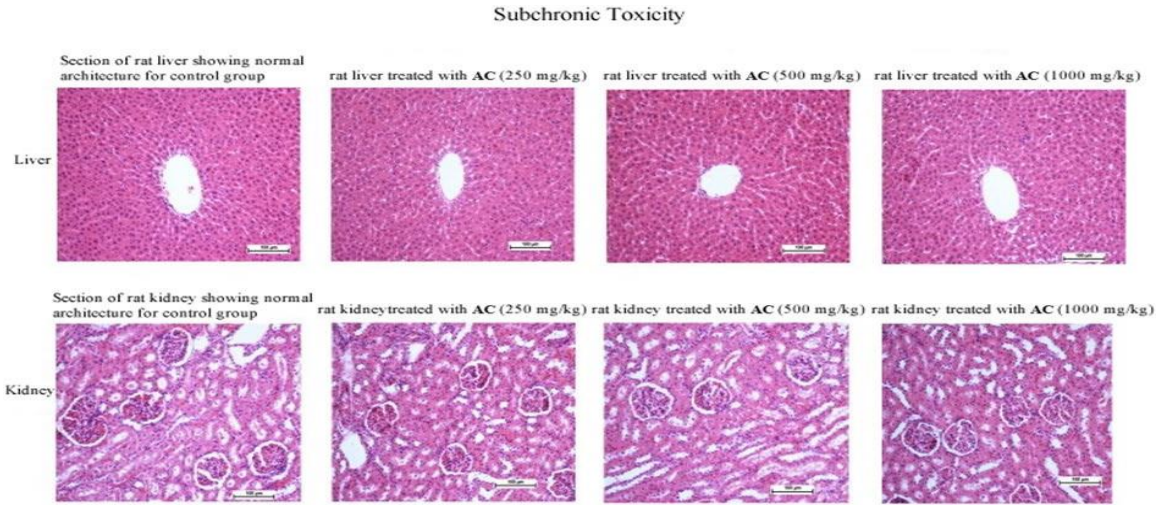


**Figure 5:** Photomicrographs of liver and kidney sections after treatment with methanol extracts of *Abutilon crispum* whole plant for 90 days. (AC = *Abutilon crispum*)

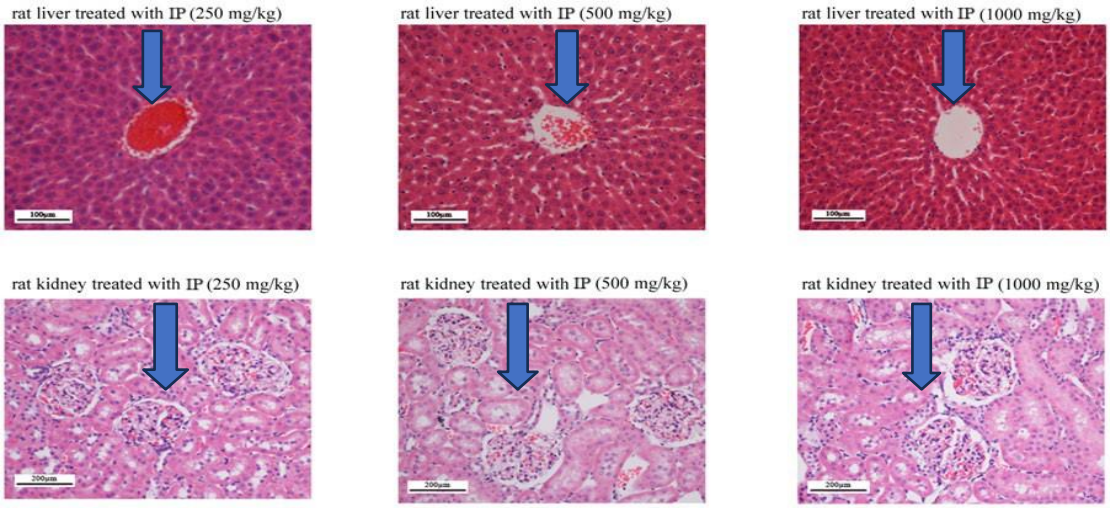


**Figure 6:** Photomicrographs of liver and kidney sections after treatment with methanol extract of *Indigofera prostrata* whole plant for 90 days. (IP = *Indigofera prostrata*)

## Conclusion

The methanol extracts of *Indigofera prostrata* and *Abutilon crispum* did not show any sign or symptom of toxicity, or death. Throughout the 90-day treatment period, there were no significant changes in the body weights, organ weights, biochemical parameters, and hematological indices of the rats. In addition, the extracts demonstrated potential organ protection as revealed by histological examination. Overall findings suggest that oral administration of *Abutilon crispum* and *Indigofera prostrata* methanol extracts is relatively safe.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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## References

1. Arome D and Chinedu E. The importance of toxicity testing. J Pharm BioSci. 2013; 4:146-148.
2. Gandhare B, Kavimani S, Rajkapoor B. Acute and subacute toxicity study of methanolic extract of *Ceiba pentandra* (Linn.) Gaertn. on rats. J Sci Res. 2013; 5(2):315-324.
3. Saleem U, Amin S, Ahmad B, Azeem H, Anwar F, Mary S. Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb. roots in albino mice as per OCED 425 TG. Toxicol Rep. 2017; 4:580-585.
4. Charles LP and Bhaskar RK. Acute and sub-chronic toxicological studies on methanolic stem extract of *Acalypha indica* Linn in albino wistar rats. Int J Pharm Pharm Sci. 2014; 6(9):560-563.
5. Vilash V, Suja SR, Latha PG, Shine VJ, Rajasekhran S. Chronic oral toxicity studies of crude ethanolic extract and ethanolic fraction of *Pellionia heyneana* wedd. leaf in Wistar rats. Int J Pharm Pharm Sci. 2016; 8(8):306-312.
6. Soon YY and Tan BK, Evaluation of the hypoglycemic and anti-oxidant activities of *Morinda officinalis* in streptozotocin-induced diabetic rats. Singapore Med J 2002; 43(2):077-085.
7. Trinder P. Determination of glucose-by-glucose oxidase method. Ann Clin Biochem. 1969; 6:24-26.
8. Malloy HT and Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biochem. 1937; 119:481-490.
9. Raja S and Ravindranadh K. Acute and subchronic toxicity studies of *Limnophila heterophylla* and *Michelia champaca*. Int J Res Pharm Pharmacother. 2017; 6(3):348-357.
10. Eran BA, Noah S, Lee HG, Kamer M, Suha O, Elad S. Potential risks associated with traditional herbal medicine use in cancer care: A study of middle eastern oncology health care professionals. Cancer. 2016; 122:598-610.
11. Kwan YP, Ibrahim D, Yeng C, Subramaniam S, Sreenivasan S. Acute and subchronic toxicity study of *Euphorbia hirta L*. methanol extract in rats. Hindawi Publishing Corporation; Biomed Res. Int. 2013; 1-14 p.
12. Yu J, Wang Y, Qian H, Zhao Y, Liu B, Fu C. Polyprenols from *Taxus chinensis var. mairei* prevent the development of ccl4-induced liver fibrosis in rats. J Ethnopharmacol. 2012; 142(1):151-160.
13. Krishna RG and Sundararajan R. Toxicity studies of *Bougainvillea glabra* and *Mucuna pruriens*. Int J Pharm Sci Res. 2020; 11(10):1000-1008.
14. Zhiqiang J, Min L, Zhe Q, Yuanyuan Z, Shutong Y, Anshan S. Toxic effects of zearalenone on oxidative stress, inflammatory cytokines, biochemical and pathological changes induced by this toxin in the kidney of pregnant rats. Environ Toxicol Pharmacol. 2014; 37(2):580-591.
15. Idakwoji PA, Oguche M, Sule FA, Oniwon WO, Shaibu IE, Edegbo E, Onoja AO, Ukwubile II. *In vitro* antidiabetic activity and sub-chronic toxicity profile of ethanol extract of *Tephrosia bracteolata* leaves. Trop J Nat Prod Res. 2024; 8(4): 012-7019.
16. Onyeyilli PA, Iwuoha CL, Akinniya JA. Chronic toxicity study of *Ficus platyphtlla blume* in rats. West Afr J Pharmacol Drug Res. 1998; 14:27-30.
17. Thomas A and Radhakrishnan EK. Acute and subacute oral toxicity studies of hydroalcoholic extract of *Terminalia arjuna* (Roxb.) bark in rodents. Trop J Nat Prod Res. 2023; 7(7):3351-3359.