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# TOXICITY STUDIES OF *BOUGAINVILLEA GLABRA* AND *MUCUNA PRURIENS*

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**Keywords:**

*Bougainvillea glabra*, *Mucuna pruriens*, Biochemical parameters, Sub-chronic toxicity, SGOT, RBC, Creatinine

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**ABSTRACT:** The present study was aimed to evaluate the acute toxicity study and subchronic toxicity study with methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* on Wistar albino rats. The acute dose was administered at 2000 mg/kg body weight on 1st day and observed for 14 days, while chronic dose was given at 250, 500, and 1000 mg/kg b.w. for 90 days. In subchronic toxicity study, hematological (Platelet count, RBC, WBC, hemoglobin, MCV & MCH) and biochemical studies (SGOT, SGPT, ALP, creatinine, urea & bilirubin) were carried out from plasma and serum after completion of 90 days treatment period. During the treatment period, the rats were observed for toxicity symptoms and, in addition to mortality & bodyweight alterations, were documented. In the result of acute toxicity study, methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* were found to be non-toxic at a dose of 2000 mg/kg b.w. Similarly, no significant difference was observed in hematological analysis and biochemical parameters from control groups and test animals in the subchronic toxicity study. The findings suggested that methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* were well tolerated both in acute and subchronic toxicity studies.

**INTRODUCTION:** Plant medications, widely known as herbal medications, have been conventionally used in the management of various diseases, where it is widely practiced in the Malaysian communities. Preparations from herbs and herbal sources have sustained to obtain importance from the public because of the strong belief that these products are safe for the treatment of ailments [1].

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Toxicity associated with herbal preparations alerted a number of international & national restraining establishments to advance and introduce a various set of indicators for evaluating, observation and eliminating the associated toxicity with products of the herbal source. Acute toxicity evaluation includes the dose measurement, which kills 50% of the animal groups tested.

While sub-chronic toxicity examination encompasses the effects of the test compound determination upon the long run management. Thus, toxicity studies on herbal plants to do in order to upturn the confidence in their safety to humans, predominantly for use in the progress of pharmaceuticals [2]. Acute as well as subchronic toxicity tests, will evaluate the expansion of new

medicines with repetitive tests of toxicity distributed by the pharmaceutical companies [2]. In view of emerging, enhanced earliest medicines which measure economical, safe, cheap and user-friendly or mostly drug targets of plant-based were used [3]. An effort to extract maximum information regarding the toxicity study of drugs from a minimum number of study animals is highly encouraged. Toxicity testing of plant extracts/ phytoconstituents always plays a crucial part in the improvement of new drugs. Keeping this in focus, the study undertaken was to estimate the acute and subchronic toxicities of *Bougainvillea glabra* and *Mucuna pruriens* methanol extracts on some natural and metabolic constraints in animals.

## MATERIALS AND METHODS:

**Collection and Authentication of Plant Material:** The whole plant of *Bougainvillea glabra* & *Mucuna pruriens* were collected individually in the month of July from the botanical garden, Hanamkonda, Warangal district, Telangana, India. The plant materials were taxonomically recognized by Dr. V. S. Raju, Professor, Botany Department, Plant Systematics Lab, Kakatiya University, Warangal District, Telangana, India. A voucher specimen of *Bougainvillea glabra* (4610) and *Mucuna pruriens* (4612) was deposited in the herbarium.

**Extraction:** Powder of *Bougainvillea glabra* and *Mucuna pruriens* were extracted individually with methanol by the Soxhlet method of extraction continuously. The solvent was removed by a rotary vacuum evaporator; the remaining mass of extract was concentrated and dried. The extracts were stored in a desiccator for further studies.

**Experimental Animals:** Albino rats of Wistar strain (200-250 gm) were procured from Ghosh enterprises Kolkata, India. All the rats were housed in polypropylene enclosures and conserved in a suitable atmosphere (28-32 °C) with 12-12 h of light and dark cycle.

Every day the entire groups of animals were treated with regular diet *ad libitum* and had free admittance to regular water. The protocol was accepted by the Institutional Animal Ethical Committee organized for the purpose [CPCSEA Registration no. 1287/ PO/Re/S/09/CPCSEA].

The rats were kept under regular circumstances in an animal house as per the guidelines of Committee for the purpose of control and supervision on experiments on animals (CPCSEA).

**Acute Toxicity Study:** Study of methanol extracts of *Bougainvillea glabra* (MEBG) and *Mucuna pruriens* (MEMP) was performed individually in rats by using OECD guideline 425. Pretreatment of a distinct dose of methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens*, the rats were starved for 3 h. A particular oral dose, individual methanol extract 2000 mg/kg of both plants were administered using oral gavage to the rats. The animals which survived were observed for an additional 2 weeks for respiratory, piloerection, as well as lachrymatory and locomotors actions [4].

**Sub Chronic Toxicity Study:** Forty two animals (either sex) were used for the study. Rats were distributed into seven groups of 6 rats (3 males and 3 females) in every group.

Group, I served as control and received distilled water (2 mL/kg, p. o). Group II, III, and IV were treated as tested groups and received the methanol extract of *Bougainvillea glabra* (MEBG) (250, 500, and 1000 mg/kg body weight correspondingly). Likewise, groups, V, VI, and VII gave 250, 500, and 1000 mg/kg of methanol extract of *Mucuna pruriens* (MEMP) for 90 consecutive days. At the end of the experiment, other parameters like body weight and organ weight were analyzed [4].

**Analysis of Hematological Parameters and Biochemical Factors:** At the termination of the experimental period, chloroform anesthesia was given to animals and sacrificed.

The cardiac puncture technique in rats was applied to collate blood samples into tubes with EDTA for measuring hematological parameters. Blood was also collected in centrifuge tubes for the estimation of biochemical parameters.

**Body Weight and Organ Weight:** Each rat was weighed and recorded weekly throughout the study period, and the percent weight change for each rat at the termination of each study was calculated.

% Weight change = (Difference between interval body weight and initial body weight × 100 / Initial weight of the body

Rats were euthanized, blood was collected later internal organs were removed and weighed to calculate the relative organ weights followed by observation.

Row = (Organ weight) × 100 / (Bodyweight)

**Hematological Parameters:** Hemoglobin (Hb) content was estimated using hemoglobin meter. Platelet count, white blood cells (WBC), and total red blood cells (RBC) were estimated by hemo-cytometer. Mean cell volume (MCV) was calculated depending on the volume of the average red cell in the sample. Mean cell hemoglobin was calculated from hemoglobin and RBC. Based on average Hb concentration in the RBC, mean corpuscular hemoglobin concentration (MCHC) was calculated. Hematocrit was calculated from RBC count and MCV.

**Biochemical Parameters:** Centrifuging of blood samples collected in tubes without anticoagulant at 3000 rpm for a few mins was done. Analysis of separated serum for various clinical parameters such as alkaline phosphatase (ALP), SGOT, and SGPT based on standard techniques was made. Creatinine, urea, uric acid, and total bilirubin were analyzed by known methods. The total amount of protein was determined by standard methods.

**Estimation of SGOT and SGPT:** SGOT and SGPT were estimated by standard method [5]. The samples were incubated for five min at 37 °C. With sodium phosphate buffer, the volume was adjusted to 1.0 mL by 0.1 mL serum. The composition was heated for half an hour for SGPT and SGOT, correspondingly.

A half mL of 2, 4-DNP was poured into the solution and kept aside for half an hour at 25 °C. Lastly, the color was established by adding 5 mL of sodium hydroxide, and the obtained product was observed at 505 nm, IU/L.

**Estimation of ALP:** ALP was estimated by Kind and King [6]. To 1 mL of a substrate, 0.1 M buffer 1.5 mL was poured & at 37 °C warmed for a few min. One mL of folins phenol reagent was poured. After centrifuging the content, the supernatant was removed. Absorbance was noted at 640 nm after adding two mL of sodium carbonate solution and $MgCl_2$ with 0.1 M to the supernatant.

**Estimation of Creatinine:** To each test tube, 1 mL of picric acid and serum was mixed. Sodium hydroxide was added. A result of the display of a semi-auto analyzer was recorded [7].

**Estimation of Urea:** 1 mL of urea was added to the serum sample & kept in a semi auto analyzer. The readings on the monitor were recorded at the end of assay [8].

**Estimation of Uric Acid:** To test tubes, each 1 mL urizyme buffer (polyhalogenated benzoic acid in tris buffer at pH 7.5 + 0.05), 0.1 mL of urizyme reagent (4-aminoantipyrine, peroxidase, uricase) and 0.025 mL of serum was added and incubated for 5 min at 37 °C. At 510 nm, absorbance was recorded.

**Estimation of Total Bilirubin:** It was performed by Malloy and Evelyn [9] method. 0.5 mL of diazo reagent, half mL of 1.5% HCl and 2.0 mL of distilled water were added to the serum sample. Finally, it was cooled in ice after the addition of methanol 2.5 mL, and then at 540 nm, absorbance was recorded.

**Estimation of Total Protein:** Alkaline copper sulphate 2 mL was added to serum solution 0.3 mL. Then Folin ciocalteau solution 0.2 mL was added to all the tubes and warmed for 20 min. at 660 nm absorbance was recorded. For standard, bovine serum albumin was selected [10].

**Histopathology Study:** Light microscopic study of internal organs processed by hematoxylin stain and eosin stain was made.

**Statistical Data:** All values were expressed statistically by mean + standard error of the mean (SEM), ANOVA, which is a one-way analysis of variance, continued by Dunnett's test and mean ± standard error of the mean (SEM).

## RESULTS:

**Study of Acute Toxicity:** In this study, the administration of methanol extracts of *Bougainvillea glabra* (MEBG) and *Mucuna pruriens* (MEMP) at 2000 mg/kg did not produce any mortality and symptoms in 14 days of toxicity study in rats. No symptoms of toxicity like lacrimation, respiration, changes in locomotion and piloerection, were observed in rats.

**Study of Sub-chronic Toxicity:**

**Body Weight:** No significant differences in body weights were detected for 90 days. The variation in the body weight and organ weights at the end of treatment were displayed in **Tables 1** and **2**. A slight increase in body weight was observed in rats, which received 1000 mg/kg dose of *Bougainvillea glabra* methanol extract. Similarly, rats treated with *Mucuna pruriens* methanol extract exhibited weight gain during the study. The results exhibited a slight variation in body mass between methanol extract of *Bougainvillea glabra* (MEBG), and methanol extract of *Mucuna pruriens* (MEMP) treated and controlled animals with time.

**TABLE 1: EFFECT OF METHANOL EXTRACTS OF *BOUGAINVILLEA GLABRA* AND *MUCUNA PRURIENS* ON BODY WEIGHT 90 DAYS**

| Weight (gm) | Group-I (Control) | 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 | 172.43 | 171.12 | 174.78 | 176.87 | 173.18 | 174.33 | 176.39 |
| Final | 254.87 | 243.12 | 257.43 | 263.12 | 223.77 | 221.54 | 201.76 |

Values are Mean ± SEM (n=6)

**Organ Weight:** Long term administration of methanol extracts of both the plants individually did not cause any significant alterations in test animals **Table 2**.

Slight changes in the relative weights of the liver and spleen were observed by the administration of *Bougainvillea glabra* and *Mucuna pruriens* extracts. Nevertheless, a slight increase in weight of lungs was observed at a dose of 1000 mg/kg of methanol extract of *Bougainvillea glabra* and *Mucuna pruriens* in 90 days study period. Similarly, the administration of methanol extract of *Bougainvillea glabra* and *Mucuna pruriens* exhibited minor weight change in kidneys and heart in treated rats.

However, the above-mentioned changes were not considered toxicologically important as there were no significant variations were witnessed in hematological as well as serum biochemical examinations.

**TABLE 2: EFFECT OF METHANOL EXTRACTS OF *BOUGAINVILLEA GLABRA* AND *MUCUNA PRURIENS* ON ORGANS WEIGHT 90 DAYS**

| Organ | Group-I control | 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 | 3.13 ± 0.02 | 3.15 ± 0.22 | 3.23 ± 0.01 | 3.31 ± 0.11 | 3.17 ± 0.31 | 3.24 ± 0.14 | 3.34 ± 0.13 |
| Lungs | 0.83 ± 0.12 | 0.84 ± 0.12 | 0.86 ± 0.12 | 0.83 ± 0.15 | 0.90 ± 0.12 | 0.95 ± 0.19 | 0.96 ± 0.15 |
| Heart | 0.46 ± 0.13 | 0.35 ± 0.06 | 0.41 ± 0.02 | 0.43 ± 0.02 | 0.48 ± 0.06 | 0.53 ± 0.03 | 0.54 ± 0.02 |
| Spleen | 0.65 ± 0.12 | 0.67 ± 0.12 | 0.69 ± 0.12 | 0.75 ± 0.15 | 0.61 ± 0.16 | 0.66 ± 0.14 | 0.59 ± 0.12 |
| Kidneys | 0.63 ± 0.01 | 0.64 ± 0.02 | 0.60 ± 0.02 | 0.62 ± 0.05 | 0.67 ± 0.07 | 0.93 ± 0.05 | 0.99 ± 0.06 |

Values are Mean ± SEM (n=6)

**Hematological Parameters:** The outcomes exhibited that both the plant extracts did not exhibit a slight change in parameters such as hemoglobin, RBC, MCH, MCV, PLC, MCHC, and hematocrit of animals treated when associated to control groups. A methanol extract of *Bougainvillea glabra* revealed a dose-dependent increase in WBC and platelet count. Overall the result indicated that hematological parameters remain unaffected by the treatment of rats with methanol extracts of both the plants during the experimental period **Fig. 1** and **2**.

**Serum Biochemical Parameters:** The results of subchronic toxicity study for serum biochemical parameters were revealed in **Fig. 3** and **4**. The results of serum biochemical parameters indicate that MEBG & MEMP shown minor changes in the levels of ALP, SGPT, total bilirubin, and SGOT. A slight decrease in uric acid levels in rats treated with methanol extract of *Bougainvillea glabra* and *Mucuna pruriens* was observed when related to the control group.

Correspondingly, animals treated with methanol extract of *Bougainvillea glabra* and *Mucuna pruriens* for 90 days showed a slight increase in total protein level.

Similarly, when equated to the control group a slight change in the intensities of creatinine and urea were observed in *Bougainvillea glabra* and *Mucuna pruriens*.

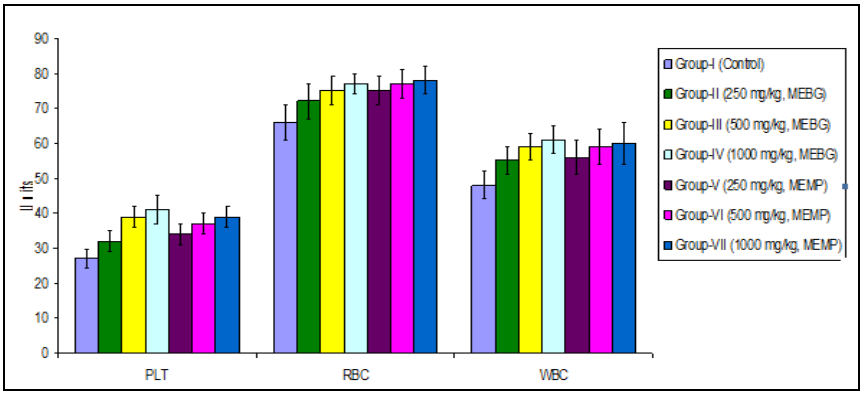


**FIG. 1: HEMATOLOGICAL PARAMETERS (PLT, RBC AND WBC) IN EXPERIMENTAL RATS AFTER 90 DAYS TREATMENT.** Values are Mean ± S. D.

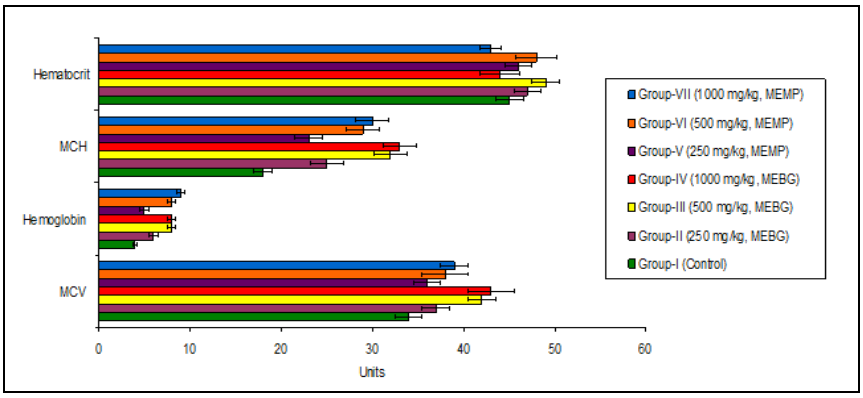


**FIG. 2: HEMATOLOGICAL PARAMETERS (MCV, HEMOGLOBIN, MCH AND HEMATOCRIT) IN EXPERIMENTAL RATS AFTER 90 DAYS TREATMENT.** Values are Mean ± S. D. of N=6.

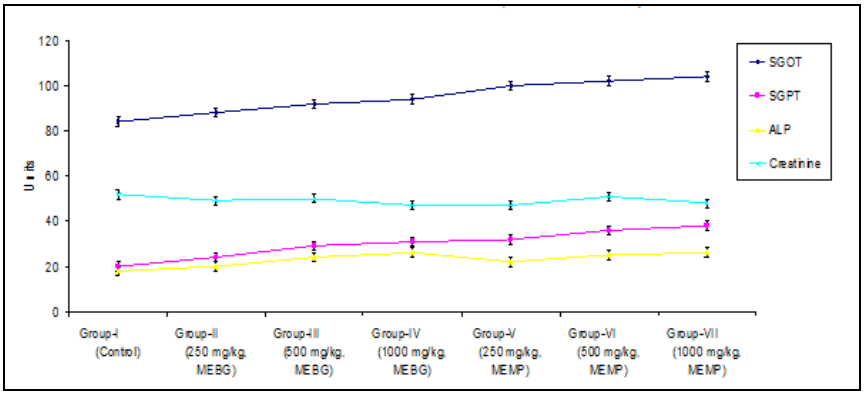


**FIG. 3: BIOCHEMICAL PARAMETERS (SGOT, SGPT, ALP AND CRE) IN EXPERIMENTAL RATS AFTER 90 DAYS TREATMENT.** The data represents the Mean ± S. D. for each group of rats.

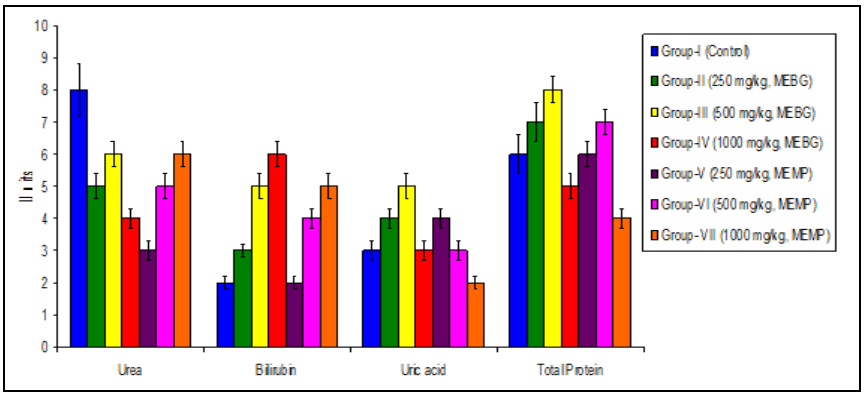


**FIG. 4: BIOCHEMICAL PARAMETERS (UREA, BILIRUBIN, URIC ACID AND TOTAL PROTEIN) IN EXPERIMENTAL RATS AFTER 90 DAYS TREATMENT.** Values are Mean ± S. D. (N=6)

**Histopathology Study:** The harvested organs had not shown any treatment associated histopathological variations in extract-treated and controlled rats. The cellular architecture was maintained in the tissues of treatment and controlled group animals of subchronic toxicity study. Photomicrographs of liver and kidney sections after treatment with methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* were shown in **Fig. 5** and **6**.

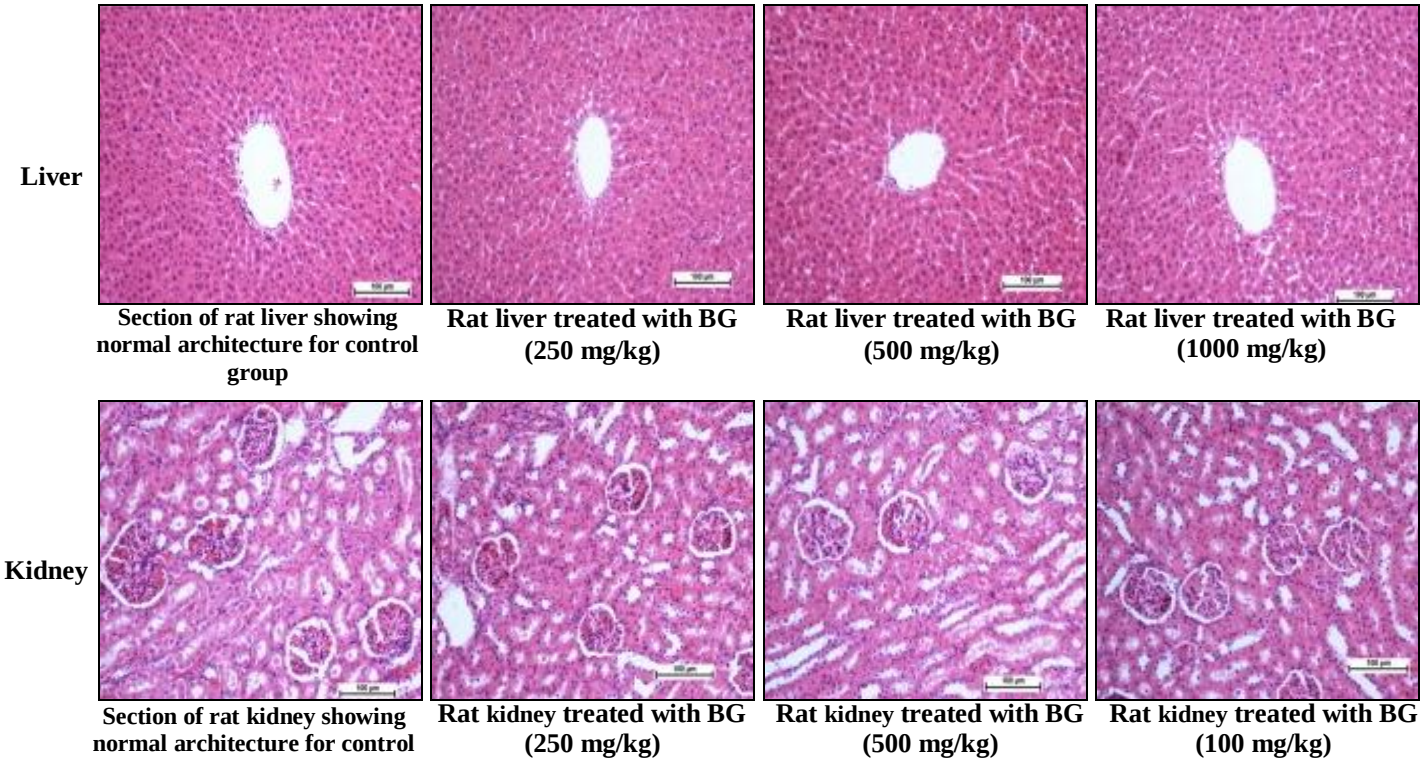


**FIG. 5: PHOTOMICROGRAPHS OF LIVER AND KIDNEY SECTIONS AFTER TREATMENT WITH METHANOL EXTRACTS OF *BOUGAINVILLEA GLABRA* FOR 90 DAYS**

**DISCUSSION:** The screening of the plant toxicity was critical in deciding the safety and efficiency of the plants. Acute and subchronic toxicity studies for both the methanol extracts of plants were carried out by investigating its effect on organ weight, hematological, and biochemical parameters. Decrease in weight of the body and also weight of inner organs were taken into consideration as sensitive parameters of toxicity after exposure to substances which are toxic [11].

Hence, the existing investigation was commenced to estimate the acute as well as subchronic toxicities of two plant extracts *Bougainvillea glabra* and *Mucuna pruriens* on an animal model [12]. Acute toxicity study was accomplished to recognize the supplementary range of doses in animal experiments and also to clarify the possible clinical signs prompted by the trial compounds under examination. One of the sensitive targets of toxic compounds is hematopoietic system and is a main index of pathological and physiological conditions in humans [13].

In this study, *Bougainvillea glabra* and *Mucuna pruriens* extracts at a dose of 2000 mg/kg did not have an adverse effect on the rats tested for observation of 2 weeks. Subchronic toxicity study provides a time period for eliciting any toxicity on long term feeding. Crucial data about persistent or cumulative toxic effects on target organ systems are generally acquired from these studies. Exposure to chemicals or any toxic drugs may result in a weight reduction of internal organs [14]. The slight changes in liver and spleen relative weights in the presence of *Bougainvillea glabra* and *Mucuna pruriens* extracts may specify that the extract did not show toxic effects on the liver and spleen.

Consequently, this study indicates that *Bougainvillea glabra* and *Mucuna pruriens* extracts did not confirm chronic toxicity effect at tested doses.

Hematological fluctuations like anemia are often additions of toxicity of bone marrow and investigation of parameters of blood, with reference to studies of animals require high importance for humans [15]. Hematological parameters are important markers of the physiological and pathological state of the blood. Thus, variations in these parameters may indicate toxicity associated with the compound being assessed. Moreover, no imperative change in liver, kidneys, and heart was stated comparative to test animals [16]. In the chronic study, oral administration for 90 days did not lead to any significant differences in the hematological parameters of the treatment of methanol extracts of both the plants' groups when compared with controls. These results suggest that both the plants have no significant toxicological effects on the hemopoietic system. Assessment of serum biochemical parameters was carried out to find any probable variations in kidney and hepatic functions by plant extract.

The values of SGPT, ALP, SGOT and bilirubin are the conventional liver functions markers [17]. Drugs are metabolized in the liver, making it a vulnerable site for attack. Alteration in total protein, total bilirubin, and albumin indicate injury in hepatocytes secretory functions of liver [18]. Abnormal ranges of uric acid, serum creatinine, and urea are biomarkers of probable damage of kidneys [19]. Administration of methanol extract of *Bougainvillea glabra* and *Mucuna pruriens* to rats did not alter the biochemical parameters. The lack of changes in biochemical values indicates that the plant extracts does not affect the hepatic or renal metabolism of rats. It may be concluded that both plants did not bring any significant damage to the organs. The harvested organs did not indicate any treatment associated with histopathological modifications in treated and controlled rats. The cellular architecture was maintained in the tissues of treatment and controlled group animals of the subchronic toxicity study.

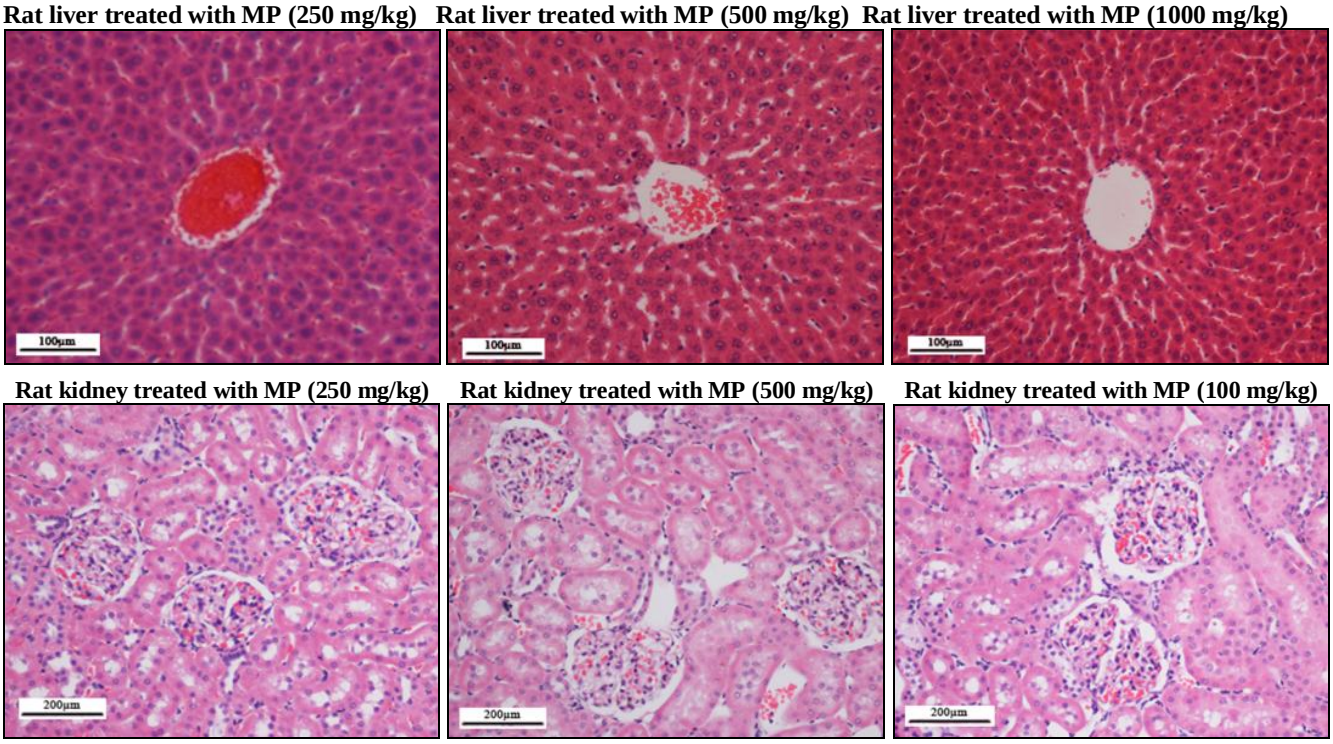


**FIG. 6: PHOTOMICROGRAPHS OF LIVER AND KIDNEY SECTIONS AFTER TREATMENT WITH METHANOL EXTRACTS OF MUCUNA PRURIENS FOR 90 DAYS**

**CONCLUSION:** Methanol extracts of *Bougainvillea glabra* and *Mucuna pruriens* administration with an oral dose of 2000 mg/kg did not exhibit toxicity signs, symptoms, or death in rats during the study. During 90 days of study, both the plants did not elicit any considerable variation in bodyweights, organ weights, biochemical parameters, and hematological parameters. The treatment doses given to rats also exhibited organ protective potential along with boosting the immune system. Overall findings indicate that the toxicity studies of methanol extract of

*Bougainvillea glabra* and *Mucuna pruriens* were practically nontoxic when administered orally.

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**CONFLICTS OF INTEREST:** None

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