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CHRONIC TOXICITY STUDIES OF *PLUMERIA ACUMINATA* AND *GALEGA PURPUREA* IN EXPERIMENTAL ANIMALS

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ABSTRACT

The use of medicinal plants and preparations derived from them as dietary supplements, nutraceuticals, functional foods and herbal medicinal products has become more widely accepted in developing countries. Therefore, it is important to evaluate the adverse effects of these plants and their preparations. The objective of this study was to evaluate the safety of the methanol extracts *Plumeria acuminata* (MEPA) and *Galega purpurea* (MEGP) by single the long-term oral administration in mice. The study of chronic toxicity was determined by oral feeding male mice daily with the MEPA and MEGP at the dose of 300, 600 and 1,200 mg/kg body weight continuously for 270 days. The results of chronic toxicity showed no abnormalities in the test groups as compared to the controls. Hematological and blood chemical values in treated groups were normal in comparison with the control group. Non-toxicity effect of MEPA and MEGP were present as no changes in body weight, internal organ weight, and general behaviors. Macroscopic or microscopic of internal organs or tissues in treated rats showed no changes. Therefore, the methanol extract of MEPA and MEGP given orally to male mice did not produce both acute and chronic toxicities.

INTRODUCTION: The widespread use of natural and synthetic drugs has necessitated the development of rapid and cost effective toxicity tests to protect humans and other biota [1, 2]. Generally, substances are absorbed from the blood stream and detoxified by the liver. After detoxification by liver they are generally excreted out of the body through kidney.

Therefore it is very much essential to determine the effect of a new agent on liver and kidney functions and metabolism. Toxicological evaluation was performed in blood and serum samples following the administration of dose regimens of these agents that were previously shown to be pharmacologically active in animal models.

Hepatorenal toxicity was evaluated by measuring enzyme activity or concentrations of: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, protein, total cholesterol, glucose, blood urea, nonprotein nitrogen and uric acid.

Plumeria acuminata belonging to the family Apocynaceae is widely distributed throughout the Southern parts of India. In traditional medicinal system different parts of the plant have been mentioned to be useful in a variety of diseases. The bark has been reported to be useful in hard tumors, diarrhoea and gonorrhea. The leaves are reported to have anti-inflammatory, rubefacient in rheumatism and have strong purgative effect.

Its branches are used like those of 'chitraka' to produce abortion [3]. Our previous findings revealed that the methanol extract of *P. acuminata* leaves showed significant anti-inflammatory activity [4], antipyretic and antinociceptive activity [5], antioxidant and free radical scavenging activity [6] and antimicrobial activity [7].

Galega purpurea (Papilionaceae) grows on hard stony ground too difficult to be rooted. The various parts of the plant are widely used in the folk medicine for the treatment of fever, pain, cough, asthma, bilious febrile attacks, arthritis and rheumatism. Decoction of the root useful in the management of enlargement and obstruction of the liver, spleen and kidney. Also the root is useful in the treatment of dyspepsia, chronic diarrhoea and ulcers [8] (Nadkarni, 1976b). Recently we reported the anti-inflammatory and antinociceptive activity [9], antimicrobial activity [7], *in vitro* antioxidant and free radical activity [10] and antitumor activity [11].

Hence, the present investigation was carried out to evaluate the toxic effect of the methanol extract of *Plumeria acuminata* (MEPA) and *Galega purpurea* (MEGP) and the parameters of the study are hematological profile and hepatorenal function.

MATERIALS AND METHODS:

Plant material: The leaves of *Plumeria acuminata* (Family: Apocynaceae) and the roots of *Galega purpurea* were collected from Erode district of Tamil Nadu, India. The plant materials were taxonomically identified by Botanical Survey of India, Kolkata. Voucher specimens (No. GMG 02/05 and GMG 03/05 respectively) has been preserved in our laboratory for future reference. The plant materials were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder materials were defatted with petroleum ether and the marc thus obtained was then extracted with methanol in a Soxhlet apparatus.

The solvent was completely removed under reduced pressure and a semisolid mass was obtained. Phytochemical screening of the extract revealed the presence of flavonoids, tannins, alkaloids, glycosides and steroids. The dried methanolic extract of *Plumeria acuminata* (MEPA) and *Galega purpurea* (MEGP) were suspended in normal saline and used for the present study.

Animals Male Swiss albino mice weighing 25-28 g were used for the present investigation. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. They were housed in clean polypropylene cages and were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum.*

The animals were acclimatized to laboratory condition (temperature 25±2˚C) with dark/ light cycle (14 /10 hr) for one week before start of experiment. All procedures were approved by Jadavpur University Animal Ethical Committee, Kolkata, India (R.No. 367/01/C/CPCSEA).

Experimental Design: The method was performed according to WHO guideline[12] and the OECD guideline for testing of chemicals 452 [13]. Healthy male Swiss albino mice were divided into seven groups of 12 animals in each group. Group 1 received normal saline (0.9 %, w/v, NaCl, p.o) 5 ml/kg body weight daily (vehicle control). Groups 2, 3 and 4 received MEPA at the doses of 100, 250 and 500 mg/kg body weight (p.o) respectively and groups 5, 6 and 7 received MEGP at the doses of 100, 250 and 500 mg/kg body weight (p.o) respectively, daily for 270 days and kept for another 28 days post treatment.

Toxic manifestations such as signs of toxicity, mortality and the body weight changes were monitored daily. At the end of the study, all mice were fasted overnight and anesthetized for blood collection. Heparinized blood samples were taken for determining complete blood count, red blood cell count, platelet count and red cell indices. The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis.

All mice were sacrificed after the blood collection. The internal organs and some tissues were weighed to determine relative organs weights and observed for gross lesions. All tissues were preserved in 10% neutral buffered formalin solution for histopathological examination.

Hematological Profile: The effect of MEPA and MEGP on peripheral blood was investigated. Red blood cell count (RBC), haemoglobin content [14] and white blood cell count (WBC) [15] were measured by using the standard procedures.

Biochemical Parameters: The changes in the biochemical parameters due to the treatment of MEPA and MEGP were evaluated. Serum biochemical enzymes such as serum glutamic oxaloacetic (SGOT) and glutamic pyruvic transaminase (SGPT) activities[16] and alkaline phosphatase (SALP) [17] were determined. The total protein concentration and bilirubin were measured by the method of Lowry et al., 1951 [18] and Oser, 1965 [19].

Renal parameters: The changes in the renal parameters due to the treatment of MEPA and MEGP were evaluated by measuring serum urea [20], creatinine [21] and uric acid level [22].

Histopathology: The organs were then preserved in 10% buffered formalin solution. The organs were processed for histopathological studies[23]. Tissue slides were stained with hemotoxylin and eosin and were examined by a pathologist.

Statistical Analysis: The experimental results were expressed as the mean ± SEM. Data were assessed by the method of analysis by ANOVA followed by Student's *t*-test; P value of < 0.05 was considered as statistically significant.

RESULTS: The methanol extracts of *Plumeria acuminata* (MEPA) and *Galega purpurea* (MEGP) were evaluated for their short term toxicity in mice.

General behavior: All of the rats fed with the extract showed normal general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, and normal change in skin and fur.

Effect of MEPA and MEGP on body weight: The body weight of MEPA and MEGP treated animals at the doses of 100 and 250 mg/kg were more or less normal. But the treatment of MEPA and MEGP at the dose of 500 mg/kg was significantly lower than that of the normal animals. The body weights of the animals were reported in **Figure 1**.

Effect of MEPA and MEGP on organs weight: The organs were weighed after being sacrificed. There were no significant changes in the actual organ weights were seen in all animals treated with MEPA and MEGP at the doses of 100 mg/kg and 250 mg/kg body weight. But the animals treated with MEPA and MEGP at the dose of 500 mg/kg showed significant increase in the organ weights when compared with the normal animals. The results were shown in **Figure 2**.

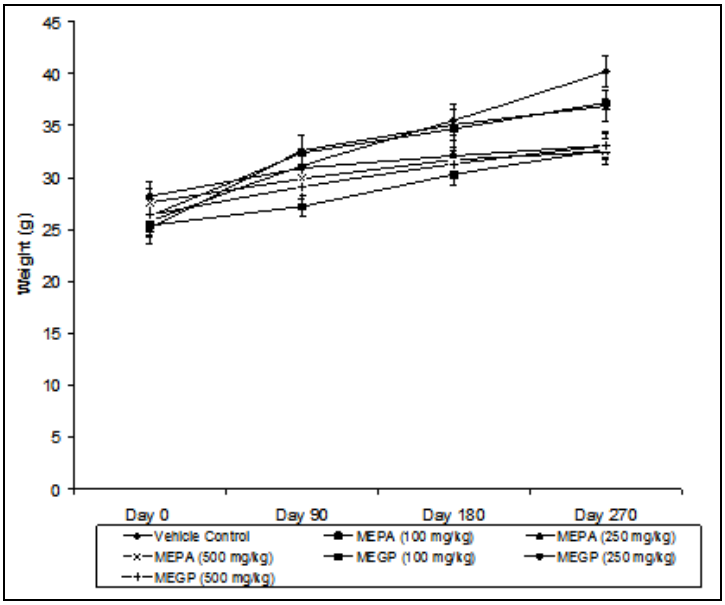

FIG. 1: EFFECT OF MEPA AND MEGP ON BODY WEIGHT

Values are mean ± SEM; P<0.05, Experimental groups compared with control groups

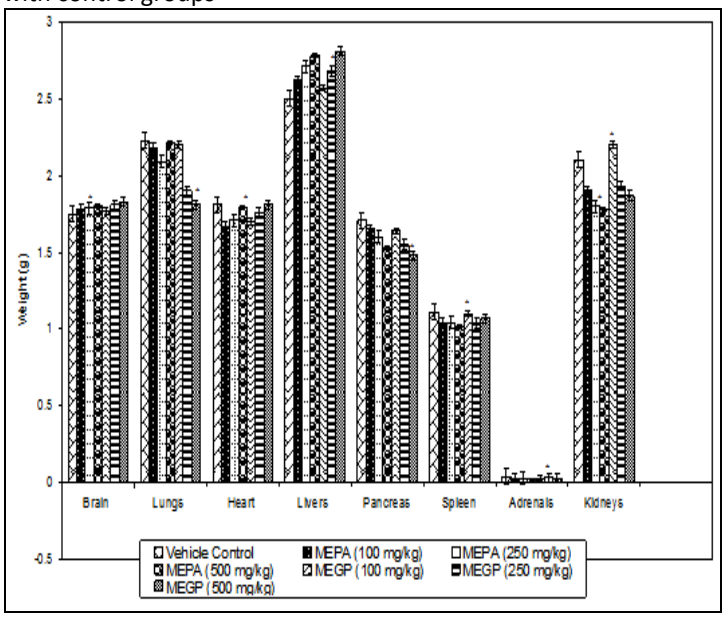

FIG. 2: EFFECT OF MEPA AND MEGP ON ORGANS WEIGHT

Values are mean ± SEM; *P<0.05, Experimental groups compared with control groups

Effect of MEPA and MEGP on hematological parameters: The treatment of MEPA and MEGP at the doses of 100 mg/kg and 250 mg/kg body weight did not alter the hematological parameter of animals. But the treatment of MEPA and MEGP at the dose of 500 mg/kg body weight altered the RBC count, total WBC count and hemoglobin content. The results were given in **Table 1**.

Effect of MEPA and MEGP on biochemical parameters: No significant changes were observed in the groups of animals treated with MEPA and MEGP at the doses of 100 and 250 mg/kg body weight. But the levels of SGOT, SGPT, ALT, ALP were slightly altered by the treatment with MEPA and MEGP at the dose of 500 mg/kg body weight (**Table 2**).

Effect of MEPA and MEGP on renal parameters: The results of renal parameters of MEPA and MEGP treated groups are summarized in **Table 3**. In the groups of mice treated with MEPA and MEGP 100 and 250 mg/kg body weight, the concentration of urea, uric acid and creatinine levels were significantly lowered than that of the control group. Treatment of animals with MEPA and MEGP at the dose of 500 mg/kg body weight significantly increased the levels of renal parameters.

TABLE 1: EFFECT OF MEPA AND MEGP ON HEMATOLOGICAL PARAMETERS OF MICE

Treatment	Haemoglobin (g%)	RBC (10^6/mm^3)	Total WBC (10^6/mm^3)
Vehicle Control	11.60±0.22	6.40±0.41	5.20±0.51
MEPA (100 mg/kg)	11.00±3.40	6.10±0.50*	5.70±0.58
MEPA (250 mg/kg)	10.10±0.19	6.30±0.12	6.60±0.29
MEPA (500 mg/kg)	10.80±0.13	6.60±0.40	7.40±0.48
MEGP (100 mg/kg)	10.20±0.03	6.50±0.21*	6.20±0.05
MEGP (250 mg/kg)	10.30±0.01	6.10±0.10	6.90±0.20
MEGP (500 mg/kg)	10.80±0.03	6.70±0.04	7.10±0.14

Data are expressed as the mean of results in 8 mice ± S.E.M. *P<0.05, Experimental groups compared with the normal group

TABLE 2: EFFECT OF MEPA AND MEGP ON BIOCHEMICAL PARAMETERS OF MICE

Parameters	Vehicle Control	MEPA (100 mg/kg)	MEPA (250 mg/kg)	MEPA (500 mg/kg)	MEGP (100 mg/kg)	MEGP (250 mg/kg)	MEGP (500 mg/kg)
SGPT (U/L)	65.01±0.25	68.10±0.53**	72.80±0.20	75.10±0.51**	68.30±0.30**	73.80±0.02	75.10±0.15**
SGOT (U/L)	39.50±0.03	42.60±0.52	44.10±0.18	45.40±0.42	43.60±0.02	45.30±0.08	48.40±0.23
Serum urea (mg/dl)	22.60±2.80	20.20±0.24	21.30±0.19**	23.40±3.70	20.90±2.04	21.40±0.09**	23.00±0.06
Serum calcium (mg/dl)	10.10±5.90	10.30±4.80	10.40±0.12	10.70±1.20	10.10±0.04	10.40±0.12	10.60±0.04
Serum phosphate (mg/ml)	4.20±4.70	4.60±1.70	4.80±0.49	5.20±0.15**	4.60±0.01	4.80±0.03	5.30±0.15**
LPO (nmol MDA/mg protein)	0.94±0.41	0.89±0.07	0.96±0.04	0.90±0.19	0.89±0.70	0.92±0.04	0.90±0.11
GSH (mg/g wet tissue)	2.33±0.68	2.40±0.29	2.44±0.22***	2.52±0.37	2.48±0.29	2.52±0.26***	2.57±0.24
SOD (U/mg protein)	4.47±0.21	4.51±0.42	4.60±5.90	4.72±0.17	4.55±0.40	4.60±0.50	4.71±0.17
CAT (U/mg protein)	26.20±0.44	26.98±0.42**	27.70±2.30	28.10±0.05**	27.68±0.20**	28.70±0.23	29.10±0.50**

Data are expressed as the mean of results in 8 mice ± S.E.M. *P<0.05; **P<0.01 and ***P<0.001 Experimental groups compared with the normal group

TABLE 3: EFFECT OF MEPA AND MEGP ON RENAL PARAMETERS OF MICE

Treatment	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Vehicle Control	22.60±2.80	2.5±0.41	0.40±0.01
MEPA (100 mg/kg)	20.20±0.24	2.45±0.50*	0.38±0.03
MEPA (250 mg/kg)	21.30±0.19**	2.51±0.1	0.42±0.02
MEPA (500 mg/kg)	23.40±3.70	2.7±0.04	0.45±0.04**
MEGP (100 mg/kg)	20.90±2.04	2.4±0.21*	0.39±0.02
MEGP (250 mg/kg)	21.40±0.09**	2.55±0.06	0.44±0.04
MEGP (500 mg/kg)	23.00±0.06	2.79±0.03	0.47±0.01

Data are expressed as the mean of results in 8 mice ± S.E.M. *P<0.05, **P<0.01, Experimental groups compared with the normal group.

Histopathology: The internal organs of all rats such as kidneys, livers, adrenals, sex organs, thymus, stomach, intestine, muscle, pancreas, brain and spinal cord were performed on gross and microscopic. Because these blood chemical parameters are the index of liver and kidney function, it suggests that the extract dose not induce toxicity to the liver and kidney. The results also showed no significant histopathological change in the internal organs.

DISCUSSION: Liver function can be assessed by serum transaminase activity [24]. In normal subjects the levels of serum transaminases is usually low but after any tissue injury these enzymes are liberated into serum. It has been found that, patients with acute hepatic diseases have increased SGOT, SGPT levels and thus increased transaminase level may serve as an indicator of liver damage [25]. Moreover, according to the reports of Friend et al SGOT and SGPT activity is also found in mice liver cell injury caused by the viral hepatitis [26]. The results indicate that the MEPA and MEGP at the tested doses do not have any significant alteration in SGOT, SGPT and SALP levels. Bilirubin is formed from degeneration of hemoglobin in the whole of the reticulo endothelial system. The level of bilirubin was not altered by the 270 days treatment of MEPA and MEGP at the tested doses.

Kidney primarily serves to eliminate the waste products of metabolism from the body. It has been found that in case of renal failure, insufficiency of the kidneys to excret urea often leads to an increase in blood urea level [27, 28]. Similarly, when accumulation of other waste products like nitrogenous substances takes place, the NPN titre of blood increases. The urea concentration over a period is proportional to the amount of protein in the diet [28] and urea nitrogen forms the greater part of plasma nonprotein nitrogen. Variations in nonprotein nitrogen mainly reflect alterations in blood urea level and as urea rises its nitrogen forms increase the percentage of nonprotein nitrogen. Blood urea and consequently nonprotein nitrogen level is found raised in the terminal stages of chronic nephritis, in some cases of acute nephritis and in congenital cystic kidneys [23].

Creatinine is the least variable nitrogenous constituents of blood. The value is increased in early nephritis and in chronic hemorrhage nephritis with uremia. Similarly increased blood content of creatinine has been reported in renal injury subsequent to trauma or anuria, in traumatic injuries to the muscles and in muscular dystrophy. Treatment with MEPA and MEGP at the tested doses does not have any significant alteration in blood creatinine level.

CONCLUSION: From the above investigation it can be concluded that MEPA and MEGP at the doses of 100 and 250 mg/kg did not alter the hematological parameters, liver and kidney functions significantly. However at the dose of 500 mg/kg the animals showed significant alteration in the hematological and hepatorenal functions and metabolism. From the above discussion it can be concluded that MEPA and MEGP up to the dose of 250 mg/kg body weight are free from toxication. The chronic toxicity at 500 mg/kg body weight may be due to the presence of various chemical constituents in the extracts. Further work is needed to find out the exact mechanism of toxicity.

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