Pharmacological Study

Chronic toxicity study of *Butea monosperma* (Linn.) Kuntze seeds in albino rats

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Abstract

In the present study, toxic effects of powder of seeds of *Butea monosperma* (Linn.) Kuntze were evaluated for a period of 3 months in albino rats. Control group received distilled water. The powder suspension was orally given to the treated group at a dose of 800 mg/kg/day for 90 days. Parameters like body weight, weight of important organs, biochemical, hematological parameters, bone marrow cytology and histopathology of vital organs were studied. Test drug administration did not affect the body weight, organ weight and bone marrow cytology to a significant extent. Among the 18 hematological parameters studied, significant changes were observed in three parameters, namely, significant decrease in hemoglobin content, red blood cell count and hematocrit. Of 16 biochemical parameters studied, significant changes were observed in 5 parameters, namely, decrease in total protein, albumin, bilirubin and significant increase in very low density lipoprotein and triglyceride. The histopathology of 18 organs revealed changes such as fatty changes, glomerular congestion and tubular hemorrhage in the kidneys, decrease in the cellularity of the spleen, epithelial disruption in jejunum, decrease in spermatogenesis in the testis, epithelial proliferation in ventral prostate and decrease in epithelial proliferation in the uterus. Thus, toxicity profile obtained from the present study shows that *B. monosperma* seeds are likely to produce toxic effect when administered in a powder form.

Key words: Albino rats, *Butea monosperma* (Linn.) Kuntze, chronic toxicity, seeds

Introduction

One of the most important requirements for any drug used in therapeutics is unequivocal inference with regards to its safety and efficacy. Though many medicinal preparations are used in Ayurvedic therapeutics, comprehensive data about their safety is not available for majority of them. Further, the safety profile sometimes changes drastically with the nature of the formulation used for final therapeutic application.

*Butea monosperma* (Linn.) Kuntze is a medicinal plant, commonly known in Ayurveda as “Palasha”. It is distributed over a large area in Asia, for example, in Sri Lanka, Burma and India. Seed of this plant is a popular folk medicine which has been used as a contraceptive⁴ and anthelmintic.⁵ However, it is reported under poisonous legumes,⁴ and several studies have reported that the seed powder can occasionally cause nephrotoxicity and anemia, the seed extract can cause liver, lungs and spleen congestion,⁵ and the seed suspension can cause teratogenic effect in rats.⁶ Further, the seed extract exhibited antifertility effect in experimental animals⁷-¹⁰ and physostigmine like action in experimental studies.¹¹ In vitro the seed oil showed a significant bactericidal and fungicidal effect.¹² It is also reported that Butin flavone of seed is being used as an antifertility agent,¹³ and the crude saline extract (0.9%) of seeds agglutinates the erythrocytes of several animal species.¹⁴ The seed extract exhibited low mortality in mice on acute toxicity test.¹⁵ However, there has been no report of long-term toxicity study...
of this drug at conventional Ayurvedic therapeutic dose level. Keeping these points on focus, the chronic toxicity study of B. monosperma seed powder was performed in rats.

Materials and Methods

Preparation of test drug

B. monosperma (Linn.) Kunthze (Family Bataceae) seeds were obtained from the pharmacy of Gujarat Ayurved University, Jamnagar. The botanical identifications were made by referring descriptions of Sharma et al.[16] Adarsha Kumar Agnihotri and Shashi Bala,[17] and Shastri[18] in the Department of Pharmacognosy attached to IPGT and RA, Gujarat Ayurved University, Jamnagar. The fine powder (80 mesh) was prepared and stored in an air-tight container for experimental purposes.

Animals

Charles Foster strain albino rats of either sex, weighing between 180 and 220 g, were selected for the study from the animal house attached to the institute. They were housed at 22 ± 2°C with constant humidity 50-60%, on a 12-h natural day and night cycle. They were fed with diet Amrut brand rat pellet feed supplied Pranav Agro Industries and with tap water ad libitum. The experiments were carried out in accordance with the directions of the Institutional Animal Ethics Committee (IAEC), after obtaining its permission.

Dose selection and schedule

The dose of the test drug was calculated by extrapolating the human dose to animals (800 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1969).[19] The test drug was suspended in distilled water (80 mg/ml) and administered orally at a volume of 0.5 ml/100 g body weight with the help of gastric catheter of suitable size sleeved on to a syringe nozzle. The animals of control group received equal volume of distilled water.

Statistical analysis

Student’s t test for unpaired data was used for analyzing the data generated during the study. A "P" value less than 0.05 was considered as statistically significant and the level of significance was noted and the results were interpreted accordingly.

Study protocol

The selected animals were divided into two groups, with each group comprising three male and three female rats. The first group was kept as control, whereas the second group was administered with the test drug. The initial body weights of the animals of both the groups were recorded and the test drug was administered for 90 consecutive days. On the 91st day, the animals were weighed again and sacrificed by an overdose of ether anesthesia. Blood was collected immediately by jugular vein puncturing in two different types of tubes, one containing dilution fluid for cell counter and another for serum biochemical investigations. Further, the rats were dissected and organs were separated and weighed with a monopan balance and transferred immediately to a glass bottle containing 10% formalin for histopathological studies.

Hematological analysis was performed using an automatic hematological analyzer (Users’ manual, AC 970[20], AC 920[21], AC 910[22]-SWELAB). The parameters measured in the blood samples were: Red blood cell (RBC), hematocrit (HCT), hemoglobin (Hb), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC), granulocyte count, granulocyte percent, eosinophil count, eosinophil percentage, lymphocyte count, lymphocyte percentage, platelet count, platelet crit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW).

Biochemical analysis of serum samples was performed using an automatic chemistry analyzer (Erb Smart Lab). Biochemical parameters measured were blood glucose,[23] serum total protein,[24] albumin, globulin, A/G ratio, serum glutamate oxaloacete transaminase (SGOT),[25] serum glutamate pyruvate transaminase (SGPT),[26] blood urea,[27] alkaline phosphatase (ALP) activity,[28] serum creatinine,[29] total cholesterol,[30] high density lipoprotein (HDL) cholesterol,[31] serum triglyceride,[32] and total bilirubin.[33]

The histopathological slides of different organs like liver, kidney, heart, lung, trachea, jejunum, spleen, thymus, lymph node, testis, seminal vesicle, prostate, uterus, ovary, pituitary, forebrain, hind brain and adrenal gland were prepared by referring standard procedure of Raghuramulu et al.[34] The slides were viewed under binocular research Carl-Zeiss’s microscope, Germany (Carl Zeiss MicroImaging Standort Göttingen - Vertrieb DeutschlandKönigsallee 9-217081 Göttingen Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Results

In the seed powder (BMP) treated group, body weight gain was observed, but the magnitude was comparatively lesser in comparison to the control group. Regarding the organ weight, out of nine organs studied, none of the organ weights was significantly affected by administration of the test drug for 90 days [Table 1].

A total of 18 hematological parameters were studied; only the descriptions of Sharma

### Table 1: Effect of Butea monosperma seed powder on body weight and organ weight

| Organs                        | Control       | BMP (800 mg/kg) |
|-------------------------------|---------------|-----------------|
| Initial body weight (g)       | 184.67 ± 05.51| 201.00 ± 09.49  |
| Final body weight (g)         | 232.33 ± 12.17| 234.2 ± 14.95   |
| Weight of liver (g/100 g)     | 03.07 ± 00.15 | 03.71 ± 00.37   |
| Weight of heart (g/100 g)     | 00.32 ± 00.05 | 0.33 ± 00.03    |
| Weight of kidney (g/100 g)    | 00.70 ± 00.02 | 0.70 ± 00.04    |
| Weight of spleen (g/100 g)    | 00.21 ± 00.01 | 0.35 ± 00.07    |
| Weight of thymus (g/100 g)    | 00.24 ± 00.02 | 0.31 ± 00.05    |
| Weight of testis (g/100 g)    | 01.00 ± 00.02 | 0.11 ± 00.08    |
| Weight of prostate (g/100 g)  | 00.12 ± 00.02 | 0.13 ± 00.03    |
| Weight of seminal vesicle (g/100 g) | 00.36 ± 00.02 | 0.37 ± 00.05 |
| Weight of uterus (g/100 g)    | 00.26 ± 00.06 | 0.37 ± 00.09    |

BMP: Butea monosperma seed powder
3 parameters were affected to significant extent and all these were pertaining to RBC related parameters. There was significant decrease in Hb content (P < 0.001), RBC count (P < 0.001) and HCT (P < 0.001) in comparison to the control group [Table 2].

Among the 16 biochemical parameters studied, significant changes were observed in only 5 parameters. There was decrease in total protein (P < 0.001), albumin (P < 0.01), bilirubin (P < 0.01) and significant increase in the level of VLDL (P < 0.001) and triglyceride (P < 0.05) [Table 3].

Data related to the effect of test drug on bone marrow [Table 4] shows that test drug did not produced any significant effect on the cell components in bone marrow smear.

A total of 18 organs were examined as mentioned in Materials and Methods section and changes were observed in 6 organs. The observed changes were fatty changes, glomerular congestion and tubulur hemorrhage in the kidneys [Figure 1], slight decrease in the cellularity of the spleen [Figure 2], epithelial disruption in jejunum [Figure 3], moderate decrease in spermatogenesis in the testis [Figure 4], moderate epithelial proliferation in ventral prostate [Figure 5] and decreased epithelial proliferation in the uterus [Figure 6].

**Discussion**

In the seed powder (BMP) treated group, gain in body weight gain was observed like that of the control group, but the magnitude of body weight gain was comparatively lesser. This indicates that there might have been non-drug influence on the body weight changes. Since none of the observed changes reached statistically significant level, it can be suggested that the seeds at the dose level studied have no significant influence on the body weight, even on long duration of administration.

The test drug produced significant decrease in Hb content (P < 0.001), RBC count (P < 0.001) and hematocrit (P < 0.001) in comparison to the control group. One of the causes may be disturbed iron metabolism. The drug or one of its components may be interfering with the absorption of iron to cause anemic condition. The role of diet can be ruled out since the animals were always provided with plenty of food and this type of disturbance is not observed in other groups, which are co-equal. Increased destruction of RBC would lead to increase in the level of bilirubin in the serum. This can be ruled out since this group significant decrease in serum bilirubin (P < 0.02) was observed. Bone marrow failure also does not seem to be the main cause since other cellular elements were not affected to a significant extent. Chronic inflammatory changes may have some contribution since inflammatory changes in jejunum and hemorrhage in kidney tubule were observed in histopathological study. Neoplastic changes were not observed in any of the tissues; hence, it can be ruled out. In iron deficiency anemia, there is a fall in MCV, MCH and increase in RDW. In powder administered group, these changes were not observed; thus, iron deficiency may not be the major contributing factor. Hemoglobin decrease is due to a decrease in the number of RBC. The decrease may happen due to sequestration of RBC in non-vascular compartments also. Further, hormones like prolactin and growth hormone also have hematopoiesis enhancing effect. This hormonal effect is partly direct and partly through secretion of another set of growth factors like insulin like growth factor-1. It is possible that the test drug may be interfering with the secretion of these hormones or cell response to them. The exact mechanism of

| Parameters                  | Control         | BMP (800 mg/kg) |
|-----------------------------|-----------------|----------------|
| Hemoglobin (g/dl)           | 17.50 ± 0.54    | 13.92 ± 0.2*** |
| RBC (x 10^6 cells/mm³)      | 08.61 ± 0.12    | 6.74 ± 0.28*** |
| Hematocrit (%)              | 47.26 ± 1.42    | 37.62 ± 0.69*** |
| MCH (pg)                    | 20.27 ± 0.52    | 20.76 ± 0.72   |
| MCHC (g/dl)                 | 37.08 ± 0.91    | 37.08 ± 0.59   |
| MCV (fl)                    | 54.85 ± 1.30    | 55.94 ± 1.35   |
| RDW %                       | 7.01 ± 0.21     | 6.84 ± 0.16    |
| WBC (x 10^9 cells/mm³)      | 06.23 ± 1.10    | 5.26 ± 0.66    |
| Granulocyte (10^3 /µl)      | 0.73 ± 0.11     | 0.76 ± 0.07    |
| Granulocyte (%)             | 12.83 ± 2.30    | 15.36 ± 1.59   |
| Eosinophil count (10^3 /µl) | 0.38 ± 0.07     | 0.36 ± 0.05    |
| Eosinophil percentage       | 05.67 ± 0.86    | 6.56 ± 0.48    |
| Lymphocyte count (10^3 /µl) | 5.12 ± 0.099    | 04.14 ± 0.056  |
| Lymphocyte percentage       | 81.50 ± 03.11   | 78.08 ± 01.83  |
| Platelet count (10^3 /µl)   | 628.50 ± 115.0  | 511.60 ± 128.10|
| Platelet crit percentage    | 0.44 ± 0.08     | 0.34 ± 0.08    |
| MPV (µm³)                   | 6.17 ± 0.09     | 5.94 ± 0.25    |
| PDW (µm³)                   | 10.48 ± 0.27    | 10.08 ± 0.59   |

Table 2: Effect of **Butea monosperma** seed powder on hematological parameters

Table 3: Effect of **Butea monosperma** seed powder on serum biochemical parameters

| Parameters                  | Control         | BMP (800 mg/kg) |
|-----------------------------|-----------------|----------------|
| Total protein (g/dl)        | 0.86 ± 0.028    | 06.94 ± 00.15***|
| Albumin (g/dl)              | 04.41 ± 0.22    | 03.52 ± 00.08** |
| Globulin (g/dl)             | 04.18 ± 00.11   | 03.44 ± 00.93  |
| A/G ratio                   | 01.02 ± 00.48   | 01.00 ± 00.00  |
| SGOT (IU/l)                 | 220.00 ± 17.54  | 215.62 ± 44.20 |
| SGPT (IU/l)                 | 110.52 ± 12.33  | 124.76 ± 26.84 |
| ALP (IU/l)                  | 141.25 ± 37.07  | 201.80 ± 32.90 |
| Bilirubin (mg/dl)           | 00.73 ± 0.07    | 00.43 ± 00.60**|
| BUN (mg/dl)                 | 50.90 ± 09.81   | 44.68 ± 02.93  |
| Creatinine (mg/dl)          | 01.20 ± 00.13   | 01.26 ± 00.05  |
| Glucose (mg/dl)             | 105.88 ± 03.22  | 109.80 ± 03.23 |
| Total cholesterol (mg/dl)   | 43.95 ± 08.81   | 64.18 ± 03.02  |
| HDL cholesterol (mg/dl)     | 11.40 ± 01.73   | 11.44 ± 02.16  |
| VLDL cholesterol (mg/dl)    | 15.63 ± 03.15   | 29.52 ± 03.24***|
| LDL cholesterol (mg/dl)     | 16.92 ± 05.22   | 23.23 ± 05.08  |
| Triglyceride (mg/dl)        | 078.15 ± 15.73  | 147.58 ± 16.19*|

*P < 0.05, **P < 0.01, ***P < 0.001, BMP: Butea monosperma seed powder; SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase, BUN: Blood urea nitrogen, ALP: Alkaline phosphatase activity, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein.
Figure 1: Photomicrograph of representative section of kidney (1x100). G-Glomerulus, CT-Convoluted tubule, Cp-Capsule, FC-Fatty changes

Figure 2: Photomicrograph of representative section of spleen (1x100). WP-White pulp, Cp-Capsule, RP-Red pulp

Figure 3: Photomicrograph of representative section of jejunum (1x100). S-Serosa, ML-Muscular layer, EL-Epithelial layer

Figure 4: Photomicrograph of representative section of testis (1x100). ST-Seminiferous tubules, IC-Interstitial cells

Figure 5: Photomicrograph of representative section of prostate (1x100). EL-Epithelial layer, AV-Alveoli, CP-Capsule

Figure 6: Photomicrograph of representative section of uterus (1x100). EL-Epithelial layer, SM-Submucosal layer, CM-Circular muscle, LM-Longitudinal muscle, S-Serosa

this decrease observed in the above-mentioned factors remains to be elucidated.

Significant changes in serum biochemical parameters produced by the test drug are decrease in total protein (P < 0.001), albumin (P < 0.01), and bilirubin (P < 0.01), and significant increase in the level of VLDL (P < 0.001) and triglyceride (P < 0.05). One of the reasons for the observed hypoalbuminemia could be dietary deficiency. Here, this is ruled
Histopathological changes observed after drug administration are fatty changes, glomerular congestion and tubular hemorrhage in the kidneys [Figure 1], slight decrease in the cellularity of the spleen [Figure 2], epithelial disruption in jejunum [Figure 3], moderate decrease in spermatogenesis in the testis [Figure 4], moderate epithelial proliferation in ventral prostate [Figure 5] and decreased epithelial proliferation in the uterus [Figure 6]. The exact reason for renal changes is not known. The cell decrease observed in the spleen may be due to either cytotoxic effect of the drug directly on the spleen or through enhanced secretion of corticosteroid hormones from the adrenal cortex. Jejunal epithelial disruption may be due to direct effect of the drug on the jejunal epithelium. The other important change observed is the decrease in the sperm count of the testis. This indicates that the test preparation interferes with sperm formation or has a cytotoxic effect on the developing sperms. Increase in the proliferation of epithelium in the prostate may reflect the feedback mechanism in it as a reaction to the drug-induced decrease in spermatogenesis. This may not be considered as the pathological change. Uterus from the majority of rats showed features of quiescent; this may be indicative of the presence of anti-estrogenic effect of possible modulation of the secretion of follicle stimulating hormone from the anterior pituitary. Thus, taking all the factors into consideration, it can be suggested that the powder, though did not produce marked degenerative changes in any of the organs studied, has the proclivity to produce moderate pathological changes in at least five organs. It is to be pointed out here that the powder group did not produce any disturbance in the cytoarchitecture of the testis. Only the sperm present indicated moderate decrease. The germinal layer remained intact in this group and differentiation of spermatids was normal. In cases of severe oligospermia, the interstitial cells of Leydig show hyperplasia and hypertrophy. No such changes were observed in this group. Thus, the observed decrease may be due to the cytotoxic effect on the formed sperms.

## Conclusion

The toxicity profile obtained shows that *B. monosperma* seeds are likely to produce toxic effect when administered in a powdered form.

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