STUDY OF ACUTE TOXICITY OF *CAESALPINIA DECAPETALA*

S.S. PINGALE[1], M.G. CHASKAR[2] AND U.B. SHELAR[3*]

[1]Department of Chemistry, ACS College Narayangaon, Junnar, Pune
[2]Department of Chemistry, B.G. College Sangavi, Pune
[3*]SSC College Junnar, Pune
Corresponding Author E-mail: uttamshelar@yahoo.com

ABSTRACT

Acute toxicity study of aqueous slurry of leaves powder of Caesalpinia decapetala was studied by present work. The leaves of Caesalpinia decapetala were collected from Junnar Tahsil, Pune, Maharashtra, India. The sample was preserved in air tight plastic container and was used for acute toxicity study as per the OECD guidelines. 1.00 gm/Kg to 9.00 gm/Kg body weight of sample powder was given orally in the form of aqueous slurry in distilled water to Swiss mice. The test animals were observed continuously for first 4 hours to see the behavioral changes. Then they are observed for 14 days. There was no any mortality record during study, no any changes observed in the behavior of test animals, food and water intake of the administered animals was absolutely normal. These observations support the non toxic nature of leaves powder of Caesalpinia decapetala. The leaves of Caesalpinia decapetala were found to be safe when administered orally without any adverse toxic signs during the study.

KEYWORDS: *Caesalpinia decapetala*, Acute Toxicity, Lethality.

INTRODUCTION

Medicinal plants from time immemorial have been used in virtually all culture as source of medicine[1]. They are considered to be the backbone of traditional medicine and are widely used to treat acute and chronic disease. World health organization (WHO) found that 80% of the inhabitants of the world depend upon the traditional medicines, therefore WHO allow to use naturally occurring herbal products. For national policies WHO reported that 119 plants derived drug. Out of these 74% of drugs are having active chemical compounds hence original plants can be used in traditional medicine[2]. The herbal plants were used for healing purpose and they are beneficial and do not have any side effects. Herbal medicines are used for traditional reasons to protect, restore and improve health. WHO has given guidelines to study toxicity of herbal medicines. Traditional and alternative medicines are used for prevention, diagnosis and treatment of various illnesses [3]. It is believed that naturalness is guarantee of harmlessness

Toxic Dose

Toxicity is the relative ability of substance to cause adverse effect in leaving organisms [4]. Toxicology is the study of the adverse effect of chemicals or physical agents on living organisms. The effect of toxicants on the cellular, biochemical and molecular mechanism of action as well functional effect such as neurobehavioral and immunological and assess the probability of their occurrence. Medicinal plants are assumes to be nontoxic and regarded safe due to their natural origin. They are used

to treat various forms of diseases. Plant extract can be used for various diseases like infections, cold, inflammation, GIT disorders, insomnia depression, heart disease, diabetics, cancer, acquired immunodeficiency syndrome and liver disease. However scientific study proves that some of the plants have cytotoxic, genotoxic and carcinogenic effects when used chronically [5]. Therefore safety dose level should be administrated to the animal under study. Toxicologist usually divides the dose (exposure) of animals into acute toxicity, sub-acute toxicity, sub-chronic toxicity and chronic toxicity [6]. Appropriate dose of drug should be determined by preliminary study of acute toxicity. Such study is essential to prevent any over dose.

There are three major ways for the absorption of toxic compounds the skin, lungs and gastrointestinal tract. The gastrointestinal tract is most important as most of toxic (foreign) compounds are injected orally. The lungs are important for airborne compounds while skin is only rarely a significant site for absorption.

LD_{50} The lethal dose (LD_{50}) is defined as dosage of a substance which kills 50% of the animals in particular group, usually determine in an acute, single exposure study.

Acute Toxicity It is the toxic effect produced by single exposure of drugs by any route for a short period of time.

Sub-acute Toxicity Repeated doses of drug are given in sub-lethal quantity for period of 14 to 21 days.

Chronic Toxicity In chronic toxicity study, drug is given in different doses for a period of 90 days to over a year to determine carcinogenic and mutagenic potential of drug.

Test Limit

High doses of all chemicals show toxicity. When a high dose is given to any animal then it may leads to the gastrointestinal blockage which produces gastrointestinal tract dysfunction. This toxicity does not related with the intrinsic characteristics of the test substance but it is due to the physical blockage caused by the biological inert substance. After giving acute dose, the substance should be nontoxic and nonlethal. It is called the test limit. Test limit for oral toxicity is generally considered to be 5.0g/kg body weight. If no mortality is observed at this dose level, a higher dose level generally is not necessary.

MATERIALS AND METHODS

Acute toxicity study of Caesalpinia decapetala

An acute toxicity study was carried out for *Caesalpinia decapetala* decoction of leaves by using mice as the experimental model. The study was carried out to assess the acute toxicity of the decoction of leaves of *Caesalpinia decapetala* on oral administration (Table 1).

Table 1: Study Protocol

Name of the study	Acute toxicity study
Test material	Plant powder (Slurry)
Animal model	Albino Swiss Mice
Animals procured from	Raj Biotech (INDIA) Ltd., Pune
Sex	Male and Female
Weight range of animals	Between 35 to 55 g
No. of dose groups	Three groups
Animals per group	3 males and 3 females

Route of administration	Intragastric administration with the help of gavage No. 16
Dose volume	2.0 ml per animal
Vehicle for administration	Distilled water
No. of administrations	Single
Concentration of dose	1.0, 3.0, 5.0, 7.0 and 9.00 gm/Kg body weight
Study duration	Acclimatization for 14 days, one day drug administration and 14 days observation period including holidays
Parameters observed	Cage side observations, daily food and water intake, daily body weight and daily mortality record etc

Animal Maintenance

The animals were housed in polyurethane cages. The cages were provided with rice husk bedding and were cleaned daily. The animals were provided with drinking water and were fed on commercially available Mice feed supplied by AMRUT FEED (Table 2).

Table 2: Composition of feed

Name	**Percentage**
Crude Protein	20 - 21 % minimum
Ether Extractive	04 - 05 % minimum
Crude Fiber	04 % maximum
Ash	08 % maximum
Calcium	1.2%
Phosphorus	0.6 % minimum
NFE	54 %
ME Kcal/Kg	3600
Pallet Size	12 mm

The feed was enriched with stabilized vitamins such as Vit. A and D_3, Vit. B_{12}, Thiamine, Riboflavin, Folic acid and supplemented with all minerals and microelements. Measured quantities of water and feed were supplied daily in each cage. The consumption of water and food was estimated from the amount of water remaining in feeding bottles and from the amount of feed remaining in the feed hopper.

RESULTS AND DISCUSSION

Cage Side Observations

Assessment of the behavior of animals was carried out by general observations of each animal on a daily basis from the stage of dosing to the end of the study. Cage-side observations included daily recording of condition of the fur, damaged areas of skin, subcutaneous swellings or lumps (the size, shape and consistency), areas of tenderness, abdominal distension, eyes - for dullness, discharges, opacities, pupil diameter, ptosis (drooping of upper eyelid), the colour and consistency of the faeces, wetness or soiling of the perimenum, condition of teeth, breathing abnormalities, gait, etc. Any changes or

abnormalities recorded could be an indication of toxicity. The test animals at all dose levels showed no significant changes in behavior before and after the administration of an oral dose of *Caesalpinia decapetala* decoction in the form slurry. Following Table 3 shows the dosage regime. Table 4 shows the general observations for the parameters studied and for all plants. Table 5 shows the mortality record.

Table 3: Doses Regime

Sr. No.	Sex	Dose gm/Kg Body Wt.	No. of animals used	Total Vol. adm. in cm^3
1	Male	1.00	03	2.00
2	Female	1.00	03	2.00
3	Male	3.00	03	2.00
4	Female	3.00	03	2.00
5	Male	5.00	03	2.00
6	Female	5.00	03	2.00
7	Male	7.00	03	2.00
8	Female	7.00	03	2.00
9	Male	9.00	03	2.00
10	Female	9.00	03	2.00

Table 4: Cage Side Observations for All Animals

Sr. No.	Parameters	Cage Side Observations
1	Condition of the fur	Normal
2	Skin	Normal
3	Subcutaneous swellings	Nil
4	Abdominal distension	Nil
5	Eyes –dullness	Nil
6	Eyes – opacities	Nil
7	Pupil diameter	Normal
8	Ptosis	Nil
9	Colour & consistency of the faeces	Normal
10	Wetness or soiling of the perimenum	Nil
11	Condition of teeth	Normal
12	Breathing abnormalities	Nil
13	Gait	Normal

Table 5: Mortality Record

Group Gm/Kg	**1**	**1**	**3**	**3**	**5**	**5**	**7**	**7**	**9**	**9**
Sex	M	F	M	F	M	F	M	F	M	F
Hr. 1	-	-	-	-	-	-	-	-	-	-
Hr. 2	-	-	-	-	-	-	-	-	-	-
Hr. 3	-	-	-	-	-	-	-	-	-	-
Hr. 4	-	-	-	-	-	-	-	-	-	-

Day 1	-	-	-	-	-	-	-	-	-	-
Day 2	-	-	-	-	-	-	-	-	-	-
Day 3	-	-	-	-	-	-	-	-	-	-
Day 4	-	-	-	-	-	-	-	-	-	-
Day 5	-	-	-	-	-	-	-	-	-	-
Day 6	-	-	-	-	-	-	-	-	-	-
Day 7	-	-	-	-	-	-	-	-	-	-
Day 8	-	-	-	-	-	-	-	-	-	-
Day 9	-	-	-	-	-	-	-	-	-	-
Day 10	-	-	-	-	-	-	-	-	-	-
Day 11	-	-	-	-	-	-	-	-	-	-
Day 12	-	-	-	-	-	-	-	-	-	-
Day 13	-	-	-	-	-	-	-	-	-	-
Day 14	-	-	-	-	-	-	-	-	-	-
Mortality	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Body Weight Changes

Body weight is an important factor to monitor the health of an animal. Loss in body weight is frequently the first indicator of the onset of an adverse effect. A dose, which causes 10 % or more reduction in the body weight, is considered to be a toxic dose. It is considered to be the dose, which produces minimum toxic effect, irrespective of whether or not it is accompanied by any other changes. All the animals from treated groups did not show any significant decrease in body weights for all the 14 days as compared with the 0 day values. The variation in body weight changes of males and females and the data is given in Table 6.

Table 6: Daily Body Weight Record in Grams.

Group gm/Kg	**1**	**1**	**3**	**3**	**5**	**5**	**7**	**7**	**9**	**9**
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 0	42	37	51	34	43	34	54	47	38	40
Day 1	42	38	50	33	43	34	54	46	37	40
Day 2	43	38	52	33	42	35	55	45	37	41
Day 3	43	39	52	34	43	36	54	45	38	40
Day 4	44	40	51	35	44	36	55	46	38	41
Day 5	45	40	54	35	45	37	55	46	39	42
Day 6	45	41	54	34	45	36	56	46	40	42
Day 7	46	42	55	35	45	37	56	47	40	43
Day 8	47	42	55	36	46	37	56	47	41	43
Day 9	47	43	54	34	45	38	57	47	41	43
Day 10	48	44	54	36	46	38	56	46	42	44
Day 11	49	44	55	35	46	38	57	47	43	44
Day 12	49	45	55	36	46	37	57	47	43	45

Day 13	50	46	56	36	45	38	58	48	44	45
Day 14	51	46	55	36	46	38	58	48	44	45

(Values in the table are expressed as mean of three animals in each group)

Food and Water Consumption

There was no significant change in food and water intake of the test animals at all dose levels. The data for food and water consumption is given in Table 7 and Table 8, respectively.

Table 7: Daily Food Intake Record in Grams.

Group Gm/Kg	**1**	**2**	**3**	**3**	**5**	**5**	**7**	**7**	**9**	**9**
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 0	16	15	14	11	15	14	20	14	14	16
Day 1	15	16	14	12	15	14	20	15	14	17
Day 2	16	16	15	11	14	15	19	14	15	16
Day 3	17	17	13	11	15	15	20	15	16	17
Day 4	17	17	15	10	15	14	20	15	15	17
Day 5	17	18	15	11	16	14	21	14	16	17
Day 6	18	18	16	12	16	14	21	14	17	18
Day 7	18	19	15	12	16	14	21	14	17	17
Day 8	19	19	16	11	16	14	22	14	18	19
Day 9	19	20	17	11	16	14	21	15	18	18
Day 10	20	20	17	12	17	15	22	14	17	17
Day 11	20	21	17	12	17	14	22	14	19	18
Day 12	21	19	18	12	18	14	22	15	19	18
Day 13	21	20	18	11	18	14	22	15	18	17
Day 14	22	22	19	11	18	14	23	13	19	19

(Values in the table are expressed as mean of three animals in each group)

Table 8: Daily Water Intake Record in ml.

Group gm/Kg	**1**	**1**	**3**	**3**	**5**	**5**	**7**	**7**	**9**	**9**
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 0	22	19	15	11	15	14	20	13	22	20
Day 1	23	20	15	11	15	14	20	13	21	21
Day 2	21	22	14	12	14	13	19	14	22	20
Day 3	23	21	14	12	14	13	21	14	23	22
Day 4	22	22	15	12	14	15	21	14	21	21
Day 5	23	22	16	13	13	15	21	14	23	22
Day 6	22	23	16	12	15	14	20	13	22	23
Day 7	23	21	16	12	15	14	22	11	23	21
Day 8	24	23	17	12	16	14	22	12	22	23
Day 9	24	22	17	11	15	14	22	12	22	22

Day 10	25	23	17	12	16	13	23	12	21	23
Day 11	25	24	18	11	16	13	21	12	23	22
Day 12	26	23	18	12	17	14	23	13	22	22
Day 13	25	23	19	11	17	13	22	13	22	21
Day 14	26	24	19	11	18	14	23	12	23	23

(Values in the table are expressed as mean of three animals in each group)

Mortality

Mortality is the main criteria in assessing the acute toxicity (LD_{50}) of any drug. There was no mortality recorded even at the highest dose level i.e. 9.0 gm / Kg. body weight.

CONCLUSION

From the results of this study, it was observed that there was no any abnormal change in the body weight, food and water consumption by the animals from all dose groups (1.00 gm/Kg body weight to 9.0 gm/Kg body weight), There was no mortality recorded even at the highest dose level i.e. 9.0 gm / Kg body weight, which proves that the plant powder have no any significant toxic effect.

REFERENCES

[1] M. G. Cragg, D. J. Newman, "Natural product drug discovery in the next millennium," Pharma. Biol., vol. 39, pp. 8-17, 2001.

[2] N. R. Faransworth, "The role of medicinal plants in drug development," in Natural Products and Drug Development, P. Krogsgaard-Larson, C. S. Brogger and H. Munksgaard, Eds., Alfred Benzon Symposium, Copenhagen, Denmark, Munksgaard, pp. 17-30, 1984.

[3] L. J. Subramanion, Z. Zuraini, C. Yeng, L. L. Yee, Y. L. Lachimanan and S. Sreenivasan, "Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice," Mole., vol.16, pp. 5268-5282, 2011.

[4] Toxicology and exposure guidelines, University of Nebraska Lincoln Environmental Health and Safety · (402) 472-4925.

[5] "Cytotoxicity evaluation of the crude extracts against Vero African green monkey kidney cell lines," Book of University Van Pretoria.

[6] S. S. Pingale and N. R. Kakade, "Evaluation of toxicity of *Caeselpinia bonducella* decoction," WJPR, vol. 3(2), pp. 2321-2329.