

Toxicological evaluation of *Cynoglossum glochidiatum* Wall. ex. Benth (Tejaraj), a folklore aphrodisiac medicinal plant

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ABSTRACT : Roots of *Cynoglossum glochidiatum* Wall. ex. Benth of Family Boraginaceae identified as source plant of Tejaraj is being used by tribal people of Orissa (Kandha) for treating male sexual dysfunctions and infertility. A pharmacological study was carried out to evaluate toxic effect (if any) of the root of *C. glochidiatum* in two phases i.e. short term (15 days) in high dose (2500 mg./kg.) and long term (60 days) in human equivalent dose (500 mg./kg.) in albino rats. The parameters studied were ponderal, haematological, biochemical and histopathological changes. The test drug did not produce any mortality or significant toxic effect on different organs except for producing increase in blood urea level and moderate fatty degenerative changes in liver.

Key words : Toxicology, *Cynoglossum glochidiatum*, Tejaraj, folklore, aphrodisiac plant.

INTRODUCTION

The tribal people of Kandha (Bargarh district of Orissa) used to prescribe root of *Cynoglossum glochidiatum* Wall. ex. Benth (Tejaraj) of Family Boraginaceae for increasing sexual power, general growth etc.¹ The plant belongs to genera cynoglossum, of which many a plants are reported to possess toxic effects^{2,3}. As the drug is new, and not reported for its toxic effects a toxicity study was carried out in two phases i.e. long term effect in normal dose and short term effect on higher doses. Effect on the cytoarchitecture of different important organs like liver, heart, kidney, etc, were studied through histopathological studies and assessment of changes in body weight with estimation of certain biochemical parameters as markers of toxicity.

MATERIALS AND METHODS

Animals :

The study was carried out in Charles Foster strain albino rats of either sex (for higher dose) and only male rats (in long term effect), bred and maintained under ideal husbandry in the animal house attached to the pharmacology Laboratory of I.P.G.T.&R.A. They were fed Navchakan oil mills "AMRUT" brand rat pellet feed and tap water given *ad-libitum* and were maintained under prevailing ambient temperature, humidity and exposed to day and night cycles.

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Collection and preparation of Drug :

The whole plant of *C. glochidiatum* was collected from the Gandhamardan hills of Bargarh district of Orissa in the month of April with the help of the tribal people as source plant of Tejaraj and was authenticated by the botanist of CCRAS Unit of Jamnagar and Pharmacognosy laboratory of I.P.G.T. & R.A., Jamnagar⁴. The part of the plant used was roots. They were dried under shade for 10-15 days. After complete drying the material was powdered in the grinder & was passed through mesh 60 and preserved in polythene bags.

Dose and route of administration :

Dose of the drug was fixed by extrapolating the human dose (6g/day) to laboratory animals on body surface area ratio as per the Table of Paget and Barne's⁵. The animals in the drug treated group received root powder of *C. glochidiatum*, in a suspension form, in distilled water, orally with the help of a No. 3 gastric catheter sleeved on to a syringe.

The toxic effect of *C. glochidiatum* was assessed in two phases. Phase I study was a short term toxicity study, where a higher dose (2500 mg./kg.) for a short period (15 days) was administered. Phase II toxicity was evaluated, where the test drug was administered at doses equivalent to human therapeutic dose (500 mg./kg) for a long period (60 days).

The data generated during the study was subjected to student's 't' test for paired and unpaired data to assess the statistical significance.

Procedure :

I. Short term toxicity study

Total 10 rats of either sex were weighed and marked with a dilute solution of picric acid and divided

into two groups having five rats each group. One group was administered Tejarâj in the prescribed dose as mentioned earlier and to another group animals tap water was administered, for a period of 15 days. The rats were weighed again on the 15th day and sacrificed by cervical dislocation. Blood was collected immediately by severing the neck blood vessels into two different ampoules one containing anticoagulants for pathological investigations like TLC count, Hb%, neutrophil and lymphocyte count and other to plain bulb for biochemical investigations⁶. Further the rats were dissected and organs like heart, liver, kidney, spleen, testis, uterus were separated and kept in normal saline (0.9%) carefully. All the organs

were weighed with a monopan balance and transferred to a glass bottle containing 10% formalin. These samples were sent to the laboratory to carryout histopathological studies by following the standard procedure⁷. The slides were viewed under microscope at various magnifications to note down their histopathological features. The photomicrograph of the sections were taken with the help of Carl Zeiss binocular microscope with photomicrographic attachment.

II. Long term toxicity study :

The procedure was same as above except that the drug was administered to male rats in the dose of 500 mg./kg. for 62 days.

RESULTS

PHASE-I : SHORT TERM TOXICITY STUDY :

TABLE NO. 1 : EFFECT OF *C. GLOCHIDIATUM* ON BODY WEIGHT OF ALBINO RATS :

Group (n)	Dose (mg./kg.)	Changes in Body weight at different stages of drug administration (g)						
		Initial	Actual weight	Days 7		Days 15		
				Weight change in gm.	Weight change in %	Actual Weight	Weight change in gm.	Weight change in %
Control (5)		139.0 ±7.49	158.2 ^{axx} ± 9.00	19.2 ±3.45	13.79 ±2.38	165.2 ^{axx} ±8.87	26.2 ±5.02	19.03 ±3.63
Tejarâj (5)	2.5	205.8 ±14.35	220.2 ^{axx} ±13.60	14.4 ±3.00	7.29 ±1.77	231.2 ^{axx} ±14.22	25.4 ±4.67	12.68 ±2.64

Data : Mean ± SEM, a** =p<0.01 when compared with initial value.

TABLE NO. 2 : EFFECT OF *C. GLOCHIDIATUM* ON WEIGHT OF DIFFERENT ORGANS OF ALBINO RATS :

Organ : Group (n)	Dose (g./kg.)	Weight of the Organs(gm)		
		Absolute weight (g) Mean ± SEM	Relative weight (g/100g body) Mean ± SEM	
Liver :	Control (5)	---	6.05 ± 0.25	3.01 ± 0.13
	Drug (5)	2.5	6.89 ± 0.66	2.96 ± 0.13
Heart :	Control (5)	---	6.56 ± 4.4	3.24 ± 1.7
	Drug (5)	2.5	7.02 ± 3.5	3.05 ± 1.0
Spleen :	Control (5)	---	4.07 ± 2.2	2.03 ± 1.4
	Drug(5)	2.5	5.00 ± 6.1	2.17 ± 2.4
Kidney :	Control (5)	---	1.46 ± 0.10	0.72 ± 0.02
	Drug (5)	2.5	1.47 ± 0.08	0.64 ± 0.03

TABLE NO. 3 : EFFECT OF *C. GLOCHIDIATUM* WALL. EX. BENTH ON BLOOD UREA LEVEL OF ALBINO RATS :

Group (n)	Dose (g/ kg.)	Blood Urea (Mg/100ml) Mean ± SEM	Percentage Change (%)
Control (5)	---	44.48 ± 2.537	---
Drug (5)	2.5	35.96 ± 7.840	19.15↓

↓= Decrease

TABLE NO. 4 : EFFECT OF *C. GLOCHIDIATUM* WALL. EX. BENTH ON TOTAL LEUCOCYTE COUNT AND HAEMOGLOBIN CONTENT & DIFFERENTIAL LEUKOCYTE COUNT OF ALBINO RATS :

Group n = 5	Dose (g/kg.)	Mean ± SEM			
		TLC Count	Hb (gm %)	Neutrophil (%)	Lymphocytes (%)
Control	---	2630 ± 330	14.36 ± 0.29	35.2 ± 4.47	63.6 ± 4.57
Drug	2.5	2360 ± 440	13.32 ± 0.39	39.2 ± 3.22	60.00 ± 2.92

PHASE-II : LONG TERM TOXICITY STUDY :

TABLE NO. 5 : EFFECT OF *C. GLOCHIDIATUM* ON BODY WEIGHT GAIN IN ALBINO RATS :

Group (n)	Dose (mg./kg)	Body weight gains at different stages of drug administration						
		15 days				30 days		
		Initial	Actual weight	Weight gain in gm.	Weight gain in %	Actual Weight	Weight gain in g.	Weight gain in %
Control (6)		63.83 ± 3.20	113.84 ^{axxx} ± 4.6	47.0 ± 3.20	71.08 ± 5.75	135.0 ^{axxx} ± 3.87	68.16 ± 3.36	100.89 ± 6.78
Tejarâj (6)	500	69.00 ± 3.55	83.83 ^{ax} ± 6.99	14.83 ^{**} ± 5.21	21.23 ^{**} ± 7.08	132.2 ^{axx} ± 12.37	60.8 ± 12.85	89.89 ± 19.64

TABLE NO. 5-A : EFFECT OF *C. GLOCHIDIATUM* ON BODY WEIGHT OF ALBINO RATS :

Group (n)	Dose (mg./kg)	Body weight changes at different stages of drug administration						
		45 days				60 days		
		Initial	Actual weight	Weight change in gm.	Weight change in %	Actual Weight	Weight change in gm.	Weight change in %
Control (6)		63.83 ± 3.20	176.33 ^{axxx} ± 4.42	109.5 ± 3.17	165.62 ± 8.90	199.5 ^{axxx} ± 5.44	127.67 ± 6.90	194.71 ± 17.84
Tejarâj (6)	500	69.00 ± 3.55	183.6 ^{axx} ± 16.41	112.2 ^{**} ± 16.87	154.84 ± 24.38	208.8 ^{axxx} ± 18.26	137.4 ± 17.98	193.89 ± 26.10

Data : Mean ± SEM, a^{xxx} = P<0.001, a^{xx} = P<0.01 when compared with their respective initial values.

** = p<0.01 when compared with control

The data pertaining to the test drugs effect on body weight are summarized in Table -1. A significant increase in body weight in test drug and control group was observed, after 7th and 15th day of drug administration in comparison to initial weight. Non significant decreased in weight gain was observed when compared to control group.

The data, on the effect of *C. glochidiatum* on different organ weights can be found in Table-2. The weight of liver, when presented as absolute value, showed an apparent but statistically non significant increase. However, an apparent but statistically non significant decrease was observed when the data were presented as relative values.

The weight of heart, when presented in absolute form, showed an apparent increase in the weight in comparison to control group. In contrast, a decrease in heart weight was observed when the data were presented in relative form. However, the observed changes were not statistically significant.

The weight of spleen, when presented in absolute form, show an apparent increase in weight in drug (22.85%) administered group in comparison to control. When the data were presented in relative form an apparent increase in drug administered group was observed. However, the changes observed in both the form of data presentation were found to be statistically non significant.

The test drug did not produce any significant effect on the weight of the kidney irrespective of presentation in the form of either relative or absolute weight.

In drug administered group blood urea level was decreased by 19.15% in comparison to control group. But due to variation in data this decrease did not reach a statistically significant level (Table 3). Test drug did not affect the T.L.C count and showed an apparent decrease in Hemoglobin percent i.e. 7.24% in comparison to control. However, this decrease was not statistically significant (Table 4). An apparent increase (11.36%) in neutrophil count in drug administered group was observed in comparison to control group. However due to variation in data the change did not reach statistically significant level.

A marginal decrease i.e. (5.66%) in Lymphocytes count was observed in drug group treated group was observed in comparison to control. However, the change was statistically non significant.

Observations on Histopathological examinations :**Phase I (2500 mg./kg.) :**

Liver : Section of rats receiving test drug from this group shows moderate fatty degenerative changes. (Fig. -1)

Spleen : Test drug could not affect the cytoarchitecture of spleen to significant extent.

Kidney : No significant disturbance in the cytoarchitecture of kidney could be observed in test drug administered group.

Heart : The test drug did not produce any disturbance in the cytoarchitecture of heart.

Uterus : In test drug administered group vacuolization of the sub mucosal layer was observed - A representative photomicrograph is given in figure.(Fig. -2)

Testis : In comparison to control rats features of slightly decreased spermatogenesis were observed in drug administered group. However there was no significant disturbance in the cytoarchitecture.(Fig.-3)

The data pertaining to the effect of *C. glochidiatum* on body weight gain of rats can be found in Table 5. The data shows a significant increase in actual body weight in all the groups of different degree after 15th and 30th day of drug administration in comparison to their initial values. The body weight gain, in both percentage and actual weight basis was found to be significantly less in drug administered group in comparison to control rat at 15th day of drug administration. The difference was more pronounced in drug group. An apparently less weight gain in drug administration group in comparison to control group was observed at 30th day also. However, the difference was not statistically significant. Similarly, highly significant increase in body weight was observed in both the groups on 45th and 60th day of drug administration. There was no statistically significant difference in the weight gain between drug administered groups and control groups though an apparent higher percentage gain was observed drug (at 60th day) group.

Histopathological study :

Phase II (500 mg./kg.) :

Liver : Sections obtained from rats of this group showed mild fatty generative changes in test drug group. (Fig.-4)

Kidney : Sections obtained from rat receiving test drug did not show any significant difference in the cytoarchitecture, However, in one of the rat, mild fatty degenerative changes were observed (Fig. 5).

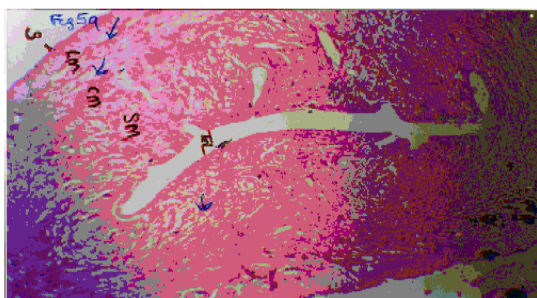


Fig.2 : Uterus section, vacuolization of submucosal layer (1x32 Magnification)

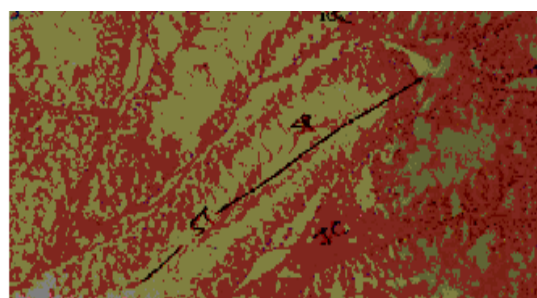


Fig.3 : Testis section, decrease spormetogenesis (1x100 Magnification)

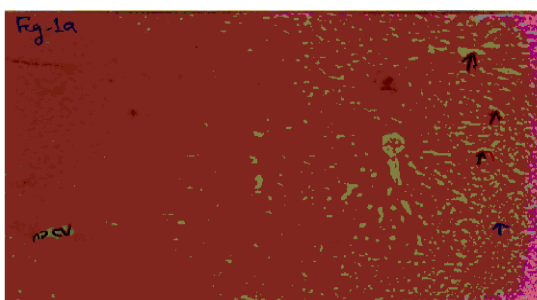


Fig.4 : Liver section, Mild fatty degenerative changes (1x100 Magnification)

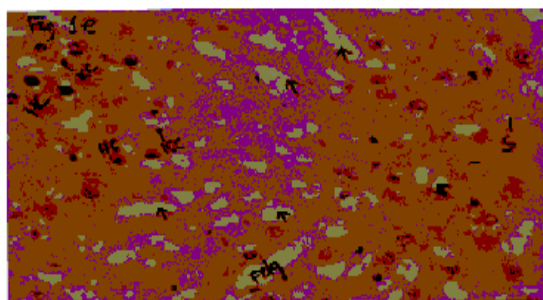


Fig.1 : Liver section, Moderate fatty degenerative changes (1x400 Magnification)

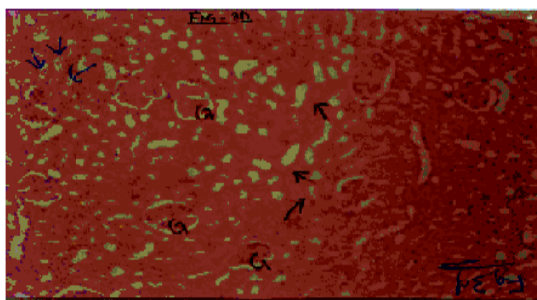


Fig.5 : Kideny section, Mild fatty degenerative changes (1x100 Magnification)

DISCUSSION

The toxic effect of *C. glochidiatum* was assessed in two phases. Phase I study was a short term chronic toxicity study, where a higher dose (2500 mg./kg.) for a short period (15 days) was administered. Phase II toxicity was evaluated during spermatogenesis study in which the test drug were administered at doses equivalent to human therapeutic dose (500 mg./kg.) for a long period (60 days).

A careful analysis of the data on haematological, ponderal and histopathological changes observed after administration of test drug shows that the drug does not seem to have high toxic potential. If it was highly toxic then there would have been instances of mortality during the study. However there were many perceptible qualitative and quantitative differences with respect to its toxicity profile. Biochemical data presented in table 3 shows a non-significant decrease (19.15%) of blood urea level in test drug administered group. A decrease in blood urea level may be suggestive of positive nitrogen balance. This type of activity is normally observed with drugs possessing adaptogenic property. The more important aspect that should be noted clearly is that, there is no significant tissue destruction due to this drug administration. The exact nature of mechanism of this decrease would be interesting to study.

Haematological data presented in the table 4 shows an apparent but non-significant decrease in the percentage of hemoglobin i.e. 7.24 in drug group. Decrease in haemoglobin in blood is a normal consequence of sequestration of RBC in non-vascular compartments or their destruction by haemolysis. Since the decrease is only marginal in case of test drug, it can be suggested that it may be due to RBC sequestration in storage sites. The data on the leucocyte count shows that no significant change was observed in test drug administered group. This clearly shows that the test drug does not produce any significant adverse effect on blood forming tissues.

When the changes in body weight in short-term group are taken into consideration, a non-significant decrease was noticed in test drug group in comparison to control group. Decrease in body weight may be due to direct drug toxicity, if it enhances catabolic activity. Further the decrease in body weight may be suggestive of tissue degeneration or destruction as it is a well-known fact that body weight decrease is a very good indicator of tissue destruction. Tissue destruction is often accompanied by elevation in blood urea level. However decrease in blood urea was

observed in test drug administered group. This rules out tissue destruction.

In long term toxicity study the test drug showed significant decrease in body weight after 15 days. However no significant change in body weight was noticed after 30, 45 and 60 days. This shows that on chronic administration the toxic potential to large extent is attenuated by body's defense mechanism the nature of which remains to be investigated. As regards ponderal changes in both the phases of study *C. glochidiatum* did not affect the weight of the liver, spleen, heart, kidney, testis, and seminal vesicle significantly.

Histopathological examination of nine important organs i.e. liver, spleen, heart, kidney, testis, seminal vesicle, ventral prostate, uterus and ovary obtained from both the groups were carried out. The examination did not reveal any significant histopathological changes in 3 organs (spleen, heart and ventral prostate) from the test drug administered groups.

Phase I (2500 mg./kg.) study liver showed moderate fatty degenerative changes in *C. glochidiatum* group in contrast, in second phase (500 mg./kg.) it showed mild fatty degenerative changes in the liver.

Fatty degenerative changes occur as a result of increased syntheses and mobilization of triglycerides and decreased formation of lipoproteins. These changes in the lipid metabolism may cause decreased tissue perfusion leading to degenerative changes. The pattern of changes observed in histopathological changes support the suggestion made earlier that on long duration body's defense mechanism gets adjusted to the drugs toxic potential. Liver on short term administration causes moderate liver toxicity. This proclivity is very much reduced on chronic administration indicating perhaps the great regenerative potential of liver.

In case of testis, features of slightly decreased spermatogenesis was observed in test drug group. However in both the phase no disturbance in cytoarchitecture was noticed. In long term phase (500 mg./kg.) in contrast to short term increase in spermatogenesis was observed in test drug group. In long term phase (500 mg./kg.) hypertrophy of the capsule and epithelial layer of seminal vesicle was observed in the treated group in comparison to control in addition, extensive branching of epithelium was also noticed. This is indicative of presence of androgenic activity in the test drug.

No disturbance in cytoarchitecture of kidney was observed in both the phase of study. But surprisingly in one rat of long term (500 mg./kg.) group receiving test drug mild fatty degenerative changes were observed.

