Acute Toxicity Study Of An Aqueous Extract Of Dried Leaves Of 'Gymnosporia Spinosa' On Albino Mice

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Abstract: Background: Leaves of G. Spinosa have been used by people for treatment of jaundice. No information is available regarding toxicity studies which prompted us to carry out this work.Method: Acute toxicity study was carried out using aqueous extract of G.Spinosa leaves. for that 28 inbred Swiss albino mice of either sex were randomly divided in to 4 equal group. First group received distilled water (control). Second, third and fourth groups received single dose of drug orally as 40,120 and 240mg/100 g. of Bd. Wt. respectively. Animal were observed for various sign and symptoms. After 72 hrs blood was collected for blood counts and biochemical parameters. Liver, lungs and kidney were subjected to histo-pathological studies. Result: Throughout study there was no mortality in study except degenerative changes in the liver, other organ showed no changes. Analysis of biochemical data showed elevation of S.Alkaline phosphatase and Random blood sugar level.Conclusion: The data showed that hepato-toxicity at higher dose level which is about 100x human therapeutic dose. [Kubavat R Natl J Integr Res Med, 2019; 10(6):23-26]

Key Words: Acute toxicity, Aqueous extract, Gymnosporia Spinosa

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Introduction: Traditional herbal medicines are naturally occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. Traditional herbal medicines are getting significant attention in global health debates[1,2].

One of such herb 'Gymnosporia spinosa has been used for various disorders like it cures biliousness, purifies the blood, cures ulcers, piles, remove "kapha", inflammation, burning, thirst, corneal opacity and as vermifuge[3,4]. Leaves of Gymnosporia spinosa have been used by people living in Gujarat for treatment of liver disorders.

Hepatoprotective activity of 'Gymnosporiaa spinosa' leaves has been studied[5]. Antispasmodic effects were also reported with crude extract of this plants[6]. No information available regarding toxicity study on Gymno sporiya Spinosa with our search from limited available information prompted us to carry out of this work For this reason we confined our work on Acute toxicity study of aqueous extract of dried leaves of 'Gymnosporia spinosa'.

Materials and Methods: Drug: The plant material (leaves of Gymnosporia spinosa) were collected during August-September 2001, from village area near Jamnagar and identified with the help of a pharmacognosist of Gujarat Ayurvedic University, Jamnagar.

The shade dried leaves were ground and passed through sieve. Then finally the aqueous extract of the leaves powder was prepared. Thus for 800mg dried leaves powder, 20ml of distilled water was used to prepare aqueous extract.

Animals: The albino Swiss mice of either sex were inbreed in our departmental animal house and they were fed with standard pelleted laboratory diet and ordinary tap water. The study was designed to evaluate acute toxicity of the drug.

Acute Toxicity Study: For evaluating acute toxicity, albino mice of either sex were housed; four animals per cage for one week to acclimatized and were provided food and water ad - libitum. Then the mice were housed one per cage for 5 days for food training i.e. (They were provided food only for 6 hrs. / Day). Then the mice were divided into 4 equal groups: (7 mice in each group) Allocation was done in randomized fashion. Average food intake for three days was measured in all animals, before starting the experiment.

First group received plain water only, and was used as control. Group 2, 3 and 4 were treated orally with 40 mg, 120mg and 240 mg per 100 g of the Body-weight single dose of aqueous extract of Gymnosporia spinosa respectively. This amounts exposed the animals to 30, 90 and 180 times the therapeutic dose of the drug. The monitoring of the parameters commenced immediately after administrating the drug. Animals were observed at 0 hr., 1 hr., 2 hrs., 4 hrs., 6 hrs., 24 hrs. and 72 hrs.

Parameters For Observations: Mortality of animals, Motor activity, Tremors, Convulsions, Posture, Spasticity, Opisthotonicity, Ataxia, Righting reflex, Sensations, Pilo-erection, Ptosis, Lacrymation, Exopthalmos, Salivation, Diarrhoea, Writhing, Skin color and Respiratory rate. blood was drawn from each of the animal by cardiac puncture to determine: Hemoglobin, R.B.C. count, W.B.C. count, Blood Urea, Blood Glucose, Serum Creatinin, Serum Cholesterol and S.G.P.T. (Blood chemistry was carried out in the Pathology Laboratory using semi auto Analyzer).

Then the animals were sacrificed, their Liver & Kidney dissected out and their Gross and Histo pathological examinations were done by a pathologist who was not knowing the distribution of animal in study group and Control group * Data was analyzed by using student 't' test (unpaired/paired) 'p' value of < 0.05 was considered as statistical significant.

Results: Acute toxicity study of an aqueous extract of Gymnosporia spinosa Leaves (72 hours) are shown in table 1

Table1: Acute toxicity study of an aqueous extract of Gymnosporia spinosa Leaves

Dose of drug	Nil(control)	40mg/100mg	120mg/100mg	240mg/100mg
No. of animals	7	7	7	7
Animal weight(g)	28±0.97	27.14±0.93	26.33±0.85	30.16±1.48
Food intake(g) before drug	6.52±0.58	7.62±0.51	8±0.41	6.10±0.72
Food intake(g)After drug	6.42±0.24	5.66±0.37	4.94±0.5*	4.99±0.29*
Hb(g%)	7.16±0.51	8.32±0.60	7.27±0.42	7.87±0.15
W.B.C.(cells/cumm)	4542.9±642	4614.2±629.4	4914.3±597.1	4385.7±135.5
Blood Urea(mg/dl)	22.1±3.21	16.4±2.87	30.4±1.80*	28.5±1.77
Serum creatinine(mg/dl)	0.62±0.05	0.65±0.05	0.46±0.02*	0.77±0.05*
S.G.P.T. (units/l)	43.48±11.96	53.9±15.27	23.84±3.54	24.75±1.78
S.Alkaline $Po_{4.}$(units/l)	56.55±7.27	109.25±5.28***	40.9±3.07	118.91±11.77***
R.B.S.(mg/dl)	57.38±8.10	73.10±9.12	48.21±1.16	85.81±1.89**
Serum Cholesterol(mg/dl)	93.77±8.18	124.02±12.69	71.11±11.39	140.71±2.18**
S.Bilirubin (mg/dl)				
1)Total	0.91±0.04	0.88±0.04	0.75±0.03[8]	0.75±0.03[8]
2)Direct	0.30±0.02	0.28±0.02	0.27±0.02	0.29±0.02
3)Indirect	0.60±0.04	0.60±0.03	0.46±0.03*	0.45±0.03*
Wt. of liver(g)	5.00±0.19	4.20±0.22*	4.60±0.17	4.40±0.10
Wt. of kidney(g)	0.66±0.04	0.48±0.03***	0.70±0.03	0.42±0.03***
Wt. of lung(g)	0.33±0.06	0.27±0.02	0.50±0.05	0.27±0.03

***P≤0.05, **P≤0.01, ***P≤0.001**

There were no death of any animal in any of groups during the period of study hence it was not possible to establish LD_{50} in this series of experiments. In control animals treated with distilled water there was no change in food intake. But a definite decrease in food intake was noted in all three treated groups with statistical significance at 120mg/100g dose & 240 mg/100g. Body weight did not change significantly in any treated group.

No significant differences were noted in value of Haemoglobin and WBC count and treated mice.
Blood urea increased after drug statistically significant in 120mg/100g dose. S.Creatinine was significantly decreased in 120mg/100g but was increased in 240mg/100g, it was statistically significant.

S.Alkaline phosphatase is increase in animals treated with 40mg/100g & 240mg/100g and it is statistically significant.

Level of Random blood sugar increased in40mg/100g & 240mg/100g dose level statistically significance was noted at 240mg/100g dose. We found that Serum cholesterol level increase in 40mg/100g. Statistically significance was noted at 240mg/100g dose.

S.Bilirubin in treated mice it decreased in all doses. But it shows significant decrease in total and indirect S.Bilirubin at 120mg/100g & 240mg/100g. There was decreased in the weight of liver in all treated groups but statistically significant at 40mg/100g.

There was decreased in the weight of kidney at 40mg/100g, and 240mg/100g statistically significant.

On histopathological examination revealed normal kidney and lung structure in all animal studies Section of liver shows cloudy degenerative changes in all groups 6 animal out of 7 with a dose 40mg/100g and 240mg/100g in 4 out of 7 with 120mg/100g dose.

Discussion: When a drug is used, it is expected to benefit the recipient. At the same time, it is a fact that there is no drug, which is totally free from harmful effects. And therefore when any drug is used, it is necessary to consider not only the beneficial effect but also the harmful effects.

A drug can be used only after a careful weighing the benefit: risk ration. When this ratio is favorable to the patient in a given situation, we can use the drug. This is true for any drug, whether it is synthetic chemical compound or a plant product. All the drugs have to pass through stringent toxicity testing, before approval can be granted for their use in general population[1,2].

In recent years there is increasing trend for using alternative system of medicine. It is argued, that such drugs are not only effective but also very safe as compared to allopathic drugs for the similar indications. The claim that natural plant product are safe should be accepted only after the plant product passes through toxicity testing using modern scientific methods[1,2].

Acute toxicity studies in animals are of value in predicting potential toxic effects of a chemical in human beings exposed to near fatal doses. From these studies nature of acute response in man may be anticipated. It also gives rough idea about the organ system involvement.

Gymnosporia spinosa is habitat in the tropics and subtropics of world. Its roots, bark, fruits, milky juice, leaves have been used for medicinal purpose[4].

In this study leaves of G.spinosa showed that food intake in mice was decreased, anorexiant effect might be due to feeding of 1.5 to 2.5 ml of liquid extract during treatment. This study is not a complete study some precise study is required to assess the anorexiant effect of G.spinosa.

Blood urea and Serum creatinine level were significantly increased at higher doses

De'S.et al 1994, evaluated Hepatoprotective activity of methanol extract of defatted leaves of G.montana by noting its effects on administration led to a significant reversal of majority of the altered biochemical parameter. Beside, a significant antagonism of the ccl_4 induced changes in the liver cytoarchitecture was observed (Figure1 & 2).

Figure 1: Normal liver section

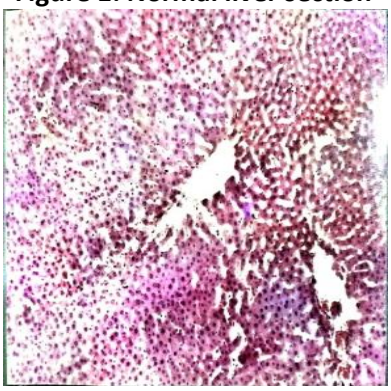
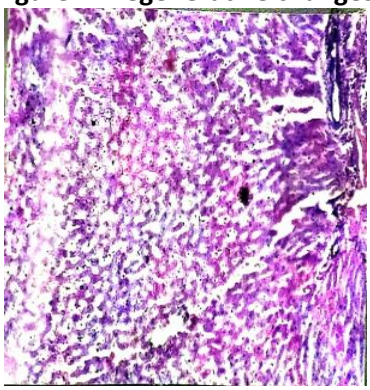


Figure 2: Degenerative changes



Our study shows that there were decrease in SGPT and S.Bilirubin level. S.Alkaline phosphatase level was significantly increased at higher dose.

Liver weight was decreased after drug and histopathological examination of liver shows cloudy degeneration with fatty changes of liver. This is surprising finding in view of protective actions reported by De et al in ccl_4 induced liver damage.

Random blood sugar was increased in mice it shows that G.Spinosa has hyperglycemic effect; it might be due to inhibition of insulin secretion or damage β cells of pancreas. Other possible mechanism is glycogenolysis or glucogenesis in the liver. The use of G.spinosa may worsen the

manifestation of diabetes. Serum cholesterol levels were increased in mice at higher dose.

There was no apparent change in spontaneous motor activity, no apparent changes in muscle coordination at higher dose. This is the first study regarding the acute toxicity of this plant as far as our knowledge goes so we were not able to compare our data with other studies. Our study has some limitations. The possibility of such toxicities cannot be ruled out and further studies are required.

Conclusion: 1) Aqueous extract of Gymnosporia spinosa did not have Any Lethal effect, animals ware exposed to 30, 90 and 180 times of human therapeutic dose. 2) In acute toxicity study SGPT and S. Bilirubin levels were decreased, but significantly increased in S.Alkalin phosphatase, Blood urea and S.Creatinine. Histopathological examination of liver shows cloudy degeneration. 3) Liver toxicity was demonstrated in acute toxicity study. 4) Random blood sugar level was increased in study it may worsen the manifestation of diabetes. 5) Though this study is carried out on the limited number of the animals, the possibility of such toxicities cannot be ruled out and further studies are required.

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