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Phytochemical and Pharmacological Screening of the Plant Crateva Magna Against Alloxan Induced Diabetes in Rats.

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Abstract:
The plant Crateva magna belonging to family Capparaceae is used in anti spasmodic, hypotensive, anti inflammatory, hypoglycemic, anti protozoal, analgesic purposes. The present study was carried out to evaluate the effect of Crateva magna whole plant (ethanolic and aqueous extract p.o.) on alloxan induced diabetes in appropriate animal model. The study was carried out on alloxan induced diabetic model. The diabetes was induced by using Alloxan and Glibencamide (5 mg/kg) was used as standard drug. The aqueous extract of leaves of Crateva magna results maximum yield value than that of petroleum ether extract, chloroform extract and alcohol extract through successive maceration process. The aqueous extract of leaves of Crateva magna showed maximum control of blood sugar in hyperglycemic Wistar rats than other experimental extracts. The test extract also reduces the blood sugar level to a maximum extent in case of normal animals. So we finally came to the conclusion that the plant Crateva magna increases healing of diabetes and prevents the development of experimentally induced diabetes in Wistar rats.

Key Words; Diabetes, alloxan, Glibencamide, hyperglycemic.

Introduction [1-8]

The plant Crateva magna belonging to family Capparaceae is a well known plant in herbal world for its wide range of use in medicinal purposes. It is used as an anti spasmodic, hypotensive, anti inflammatory, hypoglycemic, anti protozoal, Anthelmintic, analgesic purposes. The major constituent is the Triterpines, which has been shown to have these various activities. Other constituents are the Alkaloids, minor flavonoides, sterols, Triterpines and the isothiocyanate glucosides.

Diabetes Mellitus is one of the oldest & most prevalent chronic disease, which is a serious, costly and heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism [9]. This is affecting nearby 25% of the population [10]. This is also characterized by a state of chronic hyperglycemia (peripheral insulin resistance), glucosuria, polydipsia, polyurea, ketoacidosis etc [11,12]. The name Diabetes Mellitus corresponds to the groups of disorders characterized by absent or deficient insulin secretion [13]. Diabetes mellitus is an independent risk factor for the development of coronary artery disease, myocardial infraction, hypertension and dyslipidemia.

Many medicinal plants used in ethno medical practices in India are unknown or little known to scientific world. The pharmacological activities of most of the plants remain to be studied. One such plant is "Crateva magna", belonging to the family Capparaceae.

This prompted us to investigate the pharmacological activities that possess by this plant. The purpose of this investigation was to explore the potential effect of plant Crateva magna. Its pharmacological effect was not studied previously, and no pharmacological literature is available.

The present study was undertaken to evaluate the phytochemical constituents and pharmacological evaluation of the effect of the plant Crateva magna on the healing of experimentally induced diabetes in rats.

Materials and Methods:

Plant material

The whole part of plant Crateva magna plant was collected from young matured plant from the rural belt of Balasore, Orissa during the month of Nov-Dec and identified by the botanist of Department of Botany, Utkal University, Bhubaneswar by comparing with the voucher specimen present in the herbarium. After authentification fresh plant materials were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The coarse powder was used for further studies.

Experimental animals

Male albino Wistar rats weighing between 180 to 250gm were used. The experimental protocol is approved by the institutional Animal Ethics committee I.A.E.C/U.D.P.S/990/2005-Vanivihar,Bhubaneswar.Following C.P.C.S.E.A guideline.

Drugs and Chemicals

Chemicals used in the study were of analytical grade and were procured from Merck specialties private limited, Mumbai, India. Alloxan monohydrate procured from S.D.Fine chemicals, Boisar, Maharastra. The standard drug Glibenclamide is procured from Aventis Pharma Ltd., Goa-22.

Extraction of plant material and preparation of test dose

About 200 gm of coarse dried powder of plant of the Crateva magna was taken in the soxhlet apparatus and extracted successively using different solvents according to their increasing order of polarity, for the present investigation. (i.e. Pet. ether→Chloroform→ Ethanol→ Aqueous). The extraction for each solvent was carried out for 18 to 24 hours. The extract was collected by evaporating the solvents by slow heat treatment. Total 2kg of pulverized whole plant was subjected under solvent extraction to produce the required amount of test extract.

Calculated amount of dried aqueous extract was suspended in 0.5% w/v of sodium-CMC in normal saline solution to get the test doses (200mg/kg per ml for both extract). The dose limits were selected on the basis of previously performed oral acute toxicity studies in mice, in accordance with the OECD guidelines [14].

Experimental Design:

- Phytochemical screening of aqueous extract of Crateva magna leaves using various reagents.
- Toxicity study of the potent extract of Crateva magna to determine LD_{50} and selection of dose.
- Determination of the most potent extract for reduction of the elevated blood glucose levels induced by alloxan in Wistar rats by single dose treatment model through measuring the fasting blood sugar levels.
- The selection of route of administration between oral and IP route by single dose treatment in both normoglycemic and hyperglycemic models.
- Diabetes is induced in rats by single dose Alloxan administration.
- Diabetes is confirmed by blood glucose estimation.

The study of antidiabetic activity of the most potent extract is to be done by the following models.

Effect of the test extracts on:-

(a) Single dose treated hyperglycemic rats.

(b) Multi dose treated hyperglycemic rats.

- Determination of percentage loss in body weights in alloxan induced diabetic rats treated with test extract at the end of 30 days.

Phytochemical screening: [14]

The aqueous extract was subjected to preliminary Phytochemical analysis in order to detect the presence of various groups of phytoconstituents by carrying out the following chemical analysis i.e. test for carbohydrate, protein, tannin, flavonoid, saponin, steroids, alkaloids, glycosides etc. are identified using various reagents.

The preliminary phytochemical screening revealed the presence of chemical constituents present in plant Crateva magna and results were shown in table no-1.

Acute toxicity studies:

In the acute toxicity test carried out in mice we take eleven doses and 10 mice in each dose of both aqueous and ethanolic extract i.e. 100, 500, 1000, 1500, 2000 mg/kg body weight. The treated mice were observed continuously for two hours and then occasionally for further four hours and finally overnight mortality recorded[5].

Table 1: Phytochemical screening of aqueous extract of Crateva magna

Carbohydrate	+ve
Proteins	+ve
Tannins	+ve
Flavonoids	+ve
Saponins	+ve
Steroids	+ve
Alkaloids	+ve
Acetic compounds	+ve
Glycosides	+ve

Table 2: The toxicity study of aqueous and alcoholic extract of Crateva magna

Group	Dose (mg/kg)	Route	Date/Total	Death %
I	100	Oral	00/10	0
II	500	Oral	00/10	0
III	1000	Oral	00/10	0
IV	1500	Oral	00/10	0
V	2000	Oral	00/10	0

Table 3: Effect of aqueous and alcoholic extract of Crateva magna single dose treatment in Normoglycemic rats in oral route

Group & Treatment	0 hrs	2 hrs	4 hrs	8 hrs	10 hrs	%age decrease at the end of 10hrs
Solvent (Normal Saline-2ml/kg)	95.33± 5.57	96.65± 6.17	91.48± 4.32	88.09± 3.94	90.31± 3.39	
Standard (Glibencamide-5 mg/kg)	96.47± 4.36	87.00± 3.76	81.11± 3.22	67.51± 2.37[b]	55.65± 1.08[c]	39.41
Aqueous Extract (200mg/kg)	105.87 ±6.11	87.91± 3.67	80.16± 2.07	77.45± 1.37 [b]	66.19± 2.00[c]	34.75
Alcoholic extract (200mg/kg)	94.63± 4.20	88.83± 3.34	83.23± 1.88	78.98± 0.01	74.94± 0.15[a]	15.92

The tabulated values are expressed in MEAN ± S.E.M of six animals. The superscripts a, b, c denotes statistical significance at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, in comparison to group-l.

All groups of test drug showed neither any toxic effect nor any lethal effect in the dose range of 100 to 2000 mg/kg body weight. During the course of study the behaviors of the mice were carefully observed and fall of time, reduction of spontaneous activity also determined using instruments like rotarod, actophotometer**.** [16]

After observing the animal behavior we had taken the same dose 200 mg/kg of body weight for both aqueous and ethanolic extract respectively for further screenings.

Table 4: Effect of aqueous and alcoholic extract of Crateva magna in single dose treatment in alloxan induced single dose hyperglycemic rats in oral route.

Group & Treatment	**0 hrs**	**2 hrs**	**4 hrs**	**8 hrs**	**10 hrs**	**%age decrease at the end of 10hrs**
Solvent (Normal Saline-2ml/kg)	158.83 ±1.45	159.66± 1.6	160.33± 2.07	159.33± 2.108	161.50± 2.156	
Standard (Glibencamide-5 mg/kg)	156.5± 1.40[a]	151.66± 1.2 [c]	149.5±1 .176 [c]	147.33± 0.843[c]	148.66± 1.02[c]	5.86
Aqueous Extract (200mg/kg)	178.5± 1.64[c]	171.5±1 .38[c]	168.33± 0.91 [c]	164.66± 0.954[a]	167.33± 1.174 [a]	7.76
Alcoholic extract (200mg/kg)	172.33 ±1.90	162.66± 1.9[a]	159.83± 1.78[a]	155.66± 1.978[a]	158.5±2. 078 [b]	9.68

The tabulated values are expressed in MEAN ± S.E.M of six animals.The superscripts a, b, c denotes statistical significance at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, in comparison to group-l.

Table 5: Effect of aqueous and alcoholic extract of Crateva magna in multi dose treatment in normoglycemic rats in oral route.

Group & Treatment	**7th day**	**14th day**	**21st day**	**30th day**	**%age decrease at the end of 30th day**
Solvent (Normal Saline-2ml/kg)	81.45±1.05	77.42±1.01	73.42±0.99	70.39±0.95	
Standard (Glibencamide-5 mg/kg)	78.44±8.12	74.41±8.08	70.41±6.06	67.38±6.02	18.31
Aqueous Extract (200mg/kg)	79.97±4.57	75.94±4.53	71.94±2.51	68.91±2.47	17.00
Alcoholic extract (200mg/kg)	81.97±5.61	77.93±5.57	73.93±3.55	70.90±3.51	16.59

The tabulated values are expressed in MEAN ± S.E.M of six animals. The superscripts a, b, c denotes statistical significance at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, in comparison to group-l.

Chemically induced Diabetes: *[17-21]*

Alloxan induced Diabetes

For groups and six animals in each group were selected for screening. Control group received with alloxan (120mg/kg) in the Normal saline, standard received with Glibencamide 5mg/kg body weight (p.o.) once daily and the test groups were treated with 200mg/kg of both ethanolic and aqueous extract. Then the animals were kept on observation for seven days with their normal diet and the seventh day the blood glucose level of animals were tested by using gluco-check apparatus. Diabetes developed gradually was assessed after a week; an experiment was carried out to determine the blood sugar levels. Animals with blood sugar levels 200-500 mg/dl

Table 6: Effect of aqueous and alcoholic extract of Crateva magna in multi dose treatment in alloxan induced hypoglycemic rats in oral route.

Group & Treatment	**7th day**	**14th day**	**21st day**	**30th day**	**%age decrease at the end of 30th day**
Solvent (Normal Saline-2ml/kg)	156.33± 1.825	153.33± 2.086	142.33± 0.139	137.5± 1.032	13.43
Standard (Glibencamide-5 mg/kg)	131.10± 0.47[c]	122.5±0.763[c]	112.16± 0.476[c]	92.00± 0.577[c]	41.22
Aqueous Extract (200mg/kg)	138.66± 3.461[b]	128.33± 2.348[c]	115.83± 2.626[c]	102.5± 2.187[c]	47.06
Alcoholic extract (200mg/kg)	128.16± 1.492[c]	120.5±1.176[c]	113.66± 1.115[c]	105.83 ±1.572[c]	40.91

The tabulated values are expressed in MEAN ± S.E.M of six animals. The superscripts a, b, c denotes statistical significance at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, in comparison to group-l.

Table 7: % Loss of body wt. in aqueous and alcoholic extract of Crateva magna treated Alloxan induced diabetic rats.

Group & Treatment	**0th day**	**7th day**	**14th day**	**21st day**	**30th day**
		Body weight loss in percentage			
Solvent (Normal Saline-2ml/kg)	21.76	26.55	29.40	34.17	37.17
Standard (Glibencamide-5 mg/kg)	18.28	13.13	11.65	9.01	5.03
Aqueous Extract (200mg/kg)	42.84	15.21	12.87	9.15	7.18
Alcoholic extract (200mg/kg)	22.28	12.11	11.55	11.32	10.76

The tabulated values are expressed in MEAN ± S.E.M of six animals. The superscripts a, b, c denotes statistical significance at $p<0.05$, $p<0.01$ and $p<0.001$ respectively, in comparison to group-l.

were chosen on 7th day and considered as diabetic rats for antidiabetic screening.

Then the animals were treated with test and standard drug orally once daily for 30 days and kept observed in 2, 4, 8, 10 hrs in single dose treatment and 7th, 14th, 21st, 30th day in multi dose treatment for estimation of diabetes.

Models for antidiabetic screening

1. Antidiabetic activity of both aqueous and alcoholic extracts of Crateva magna in single dose treatment to diabetic animals.
   - In normoglycemic animals.
   - In Diabetic animals
2. Antidiabetic activity of both aqueous and alcoholic extracts of Crateva magna in multi dose treatment to diabetic animals.
   - In normoglycemic animals.
   - In diabetic animals.

Determination blood glucose levels:

A small amount of blood collected without sacrificing the animal by orbital sinus puncture or by snipping off the tip of the tail. The rats were made semi-conscious

with ether using the sterile blunt needle; puncture the orbital sinus at the inner canthus of the eye, by rotating the needle with sufficient but not excessive pressure, two or three times as described in sub-acute toxicity study.

As bleeding starts, the animal was held close to the haemogluco test strip and allows the drop of blood to fall on the strip. The bleeding was stopped by applying pressure on the inner canthus for a short while.

The Johnson & Johnson Ltd. (One touch - Life Scan) glucometer was switched on. When the instrument gives a beep sound after 1 min, the test strip was inserted. Then as bleeding starts, the animal was held close to the haemogluco test strip and allows the drop of blood to fall on the strip. Then the instrument was allowed to react for one minute, Then the blood glucose level was displayed on the screen was recorded. Blood glucose levels were measured in all four groups at different time intervals like 2, 4,8,10 hrs in single dose treatment in both normoglycemic and diabetic animals. Blood glucose levels were measured on 7^{th}, 14^{th}, $21st$, 30^{th} day in multi dose treatment in both normoglycemic and diabetic animals.

The percentage change in drug induced glycemia was calculated at a time function using the following formula.

$$\text{Percentage Glycemic Change} = \frac{(\text{Initial Conc.} - \text{Final Conc.})}{\text{Initial Conc.}} \times 100$$

The body weight also measured on 0 th 7^{th}, 14^{th}, 21^{st}, 30^{th} day.

Body weight Analysis:

Initial body weight of rats was recorded. The final body weights of rats were recorded on 0 th 7^{th}, 14^{th}, 21^{st}, 30^{th}. The percentage loss in body weight was calculated after 30 days and the weight variation was noted from treated groups and compared with normal, Alloxan control group.

Stastical Analysis:

The statistical significance was estimated using unpaired, tailed students "t" test. The values are represented as Mean $\pm$ S.E.M. The data obtained from various studies were subjected to one – way analysis of variance (ANOVA). [22]

Results:

Both the extracts of the plant Crateva magna produced a significant reduction in the blood sugar level as well as body weight in both single and multi dose treatment, when compared to control. The standard drug Glibencamide showed best and potent action than any other dose. The aqueous extract of the plant showed better result than the ethanolic extract.

Discussion:

The aqueous extract of leaves of Crateva magna results maximum yield value than that of petroleum ether extract, chloroform extract and alcohol extract through successive maceration process.

Toxicological study revealed that the Crateva magna leaves was showed toxic effect at 2000mg/kg b. wt. and does not alter normal physiological and behavioural activities.

The aqueous extract of leaves of Crateva magna showed maximum control of blood sugar in hyperglycemic Wistar rats than other experimental extracts. The test extract also reduces the blood sugar level to a maximum extent in case of normal animals.

Among the study of effects of Crateva magna whole plant in both normoglycemic and hyperglycemic model through oral route. The oral route represents maximum therapeutic benefits. Hence, the oral route is most appropriate route for the test extracts.

Administration of Crateva magna significantly reduces the elevated glucose level in alloxan induced diabetic rats confirms its anti-diabetic activity. This also reduces normal glucose level, which reveals the hypoglycemic property.The results of the present investigation indicates that the Crateva magna whole plant may have a place in the therapy of

DM as anti-diabetic and/or hypoglycemic agent. The hypoglycemic and/or anti-diabetic effect of the test extract may be due to the influence on glycogenesis or glycogenolysis metabolic activity property of one or more of its constituents.

Conclusion:

Thus it is concluded that the aqueous extract of leaves of Crateva magna is beneficial in lowering the blood sugar concentration and in management of other diabetic complications without any doubt.

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