Research Article

Experimental Study on *Holoptelia Integrifolia* Planch. in Relation to Diabetes Mellitus Type 2

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ABSTRACT

Holoptelea integrifolia (Ulmaceae) is a very important medicinal plant used in various indigenous systems of medicine for curing several diseases, diabetes mellitus is one of them. It is traditionally used in the treatment and prevention of several ailments like leprosy, inflammation, rickets, leucoderma, scabies, rheumatism, ringworm, eczema, malaria, intestinal cancer, and chronic wounds. In experimental study it was found that it has no toxic effect alongwith it has no hypoglycemic effect in normoglycemic rats. In Streptozotocin and hydrocortisone induced hyperglycemic rats the hypoglycemic activity of the drug was found highly significant. So this drug seems to be very rationale for the treatment of Diabetes mellitus. It has also antibacterial, antifungal, analgesic, antioxidant, anti-inflammatory, anthelmintic, antidiabetic, antidiarrhoeal, adaptogenic, anticancer, wound healing, hepatoprotective, larvicidal, antiemetic, CNS depressant, and hypolipidemic activities. This drug works on diabetes mellitus according to Ayurveda by its properties like it has Tikta, Kashaya Rasa, Laghu. Ruksha Guna, Ushna Virya and Katu Vipaka it subsides the Kaphapitta Dosha. So it is also helpful in following diseases like Shotha, Agni mandya, Chhardi, Udararoga, Shoola, Gulma, Arsha, Krimi, Raktavikara, Prameha, Kushtha, Charmaroga, Medoroga. So inspite of treating only Prameha it is used as a multipurpose drug also for curing several diseases.

Keywords: Antidiabetic, gulma, hypoglycemic, larvicidal, shotha

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INTRODUCTION

Therapeutic indications of diffèrent plant drug have been evolved through observations, trial and errors in clinical practice or experimental models. Before evaluating any new drug clinically, there should be a rigorous approach towards safe use of this plant drug preparations. In the recent time there is a reawakening of reaping the worth of ancient wisdom. In present study it has been planned to evaluate a drug on experimental models before its use in humans. One of the aims of this study is experimental evaluation of plant *Holoptelia integrifolia* (Chirabilva) for its hypoglycemic action in madhumeha (Diabetes mellitus). The plant Holoptelea integrifolia is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism [1]. Bark and leaves are used as itter, astringent, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism [2].

MATERIALS AND METHODS

The water soluble solid extract was prepared from the stem bark of *Holoptelia integrifolia* (Chirabilva) by classical method and studied. The experimental study was carried out in the laboratory of Centre of Experimental Medicine and Surgery (CEMS), I.M.S., B.H.U. It has been said that its leaf extract contains antidiabetic activity [3].

Drugs and Solutions

1. **Trial Drug –** 5% aqueous solution of water extract of *Chirabilva* has been prepared.
2. **Streptozotocin –** Citrate buffer of streptozotocin in citric acid and sodium citrate at pH of 4.5 has been prepared

freshly while injecting to the model animals. Dose was 70 mg per kg of body weight.

3. **Hydrocortisone sodium succinate** - Hydrocortisone succinate in a dose of 50 mg/kg body weight was injected intraperitonially .
4. Distilled water.

Figure 1 : Plant of *Holoptelia integrifolia*

Procurement and Maintenance of Animal Models

Healthy wistar rats of both sexes weighing 100 – 120 gm were arranged from the Department of Dravyaguna, I.M.S., B.H.U. These animals were kept in cages with rice husk bedding. Each cage contained 3 animals. The animals were allowed to acclimatize to the animal room conditions for 1 week and fed with rat pallet feed and tap water. Identical conditions were maintained for all the groups.

Evaluation of tolerance dose of the drug

Ten animals were maintained in the experimental conditions. The aqueous extract of the test drug was orally fed to the animals starting with 10 mg/kg body weight of the extract on the first day and increased by doubling the dose on each consecutive day until a dose of 15 gm/kg wt. was reached on the fourteenth day. The animals were observed over a span of over ten days for any symtpoms in intolerance like vomiting, refusal of food, at normal behaviour or bowels (Bryan Ballantyne, 1993).

Evaluation of the duration of tolerance drug

The test drug was administered in the form of aqueous extract (orally) to ten animals in a dose of 15 gm/kg weight daily for seven days to all those abnormal behavioral symptoms and mortality. No any otherwise effects were observed.[4]

Evaluation of toxic dose of the drug in experimental wistar albino rats

Ten animals were orally fed with aqueous extract of the test drug by starting with a dose of 10 mg/kg body weight of the extract and raised up till 15 gm/kg body weight and observed for any mortality or abnormal behaviour or symptoms for a period of fourteen days.

No toxicity has been observed even at dose of 15 gm/kg body weight, so drug was found at all non-toxic, proved to be safe without any toxicity.

Calculation of the dose of test drug in experimental animals

1.The animal dose for experimental study was calculated as following -

The dose of water soluble solid extract was calculated on the basis of churna. The dose of churna according to Sharangdhar is one karsha (i.e. 12 gms.) per day.

While preparing the test drug we found that by 25 kg of churna 5 kg of Ghansatva has been obtained. It means that 1 kg (1000 gm) churan yields 1/5 kg (200 gm) Ghansatva. It means that

One Karsha of churna = 12 x 1/5 = 2.4 gm of Ghansatva.

Thus, the human dose of Chirabilva Ghansatva calculated for an average of 70 kg man is 2.4 gm per day.

Therefore, human dose = 2.4 / 70 = 0.03 gm/kg (approx.)

= 30 mg/kg (approx.)

Animal dose =10 x human dose of same weight

=10 x 30 = 300 mg/kg or 30 mg/100 gm

Schedule of the Experiment

Thirty six animals were randomly divided in six groups having 6 animals in each group with following details -

Group I - Normal group

12 animals were maintained in the experimental condition with no drug treatment and no hyperglycemia was produced. First of all fasting blood samples of all rats of this group were collected from the inner canthus, then these animals were divided into two groups of six each.

Group A (Treated group): 5% aq. solution

of Chirabilva in a dose of 30 mg/100 gm body weight per day was given for 21 days.

Group B (Control group): These rats were kept as control group. Drug was not given to this group. Distilled water 1 ml/100 gm body weight was given to them daily for 21 days. Blood samples from all the rats were collected through inner canthus on 7th, 14th and 21st day after previous overnight fasting.

Group II - Streptozotocin induced hyperglycemic rats

***Step I*:** Twelve rats of either sex weighing 100 – 120 gms were selected in this group. Then they were kept on overnight fasting, after collecting blood samples from the inner canthus of eye, normal diet has been given to the rats.

***Step II*:** 120 mg of Streptozotocin was dissolved in 6 ml of citrate buffer (pH 4.5). After dissolving in citrate buffer Streptozotocin was injected peritoneally in a dose of 70 mg/kg body weight to each rat. Then rats were kept on normal feed and water. A steady state has been reached after 96 hrs.

***Step III*:** Fasting blood samples were collected from inner canthus of all the rats. After confirming the induction of hyperglycemia the rats were divided into two groups.

Group A. (Treated group): 5% aq. solution of Chirabilva in a dose of 30 mg/100 gm were given to this group for 20 days. Fasting blood sample were collected at 7th, 14th and 21st day.

Group B. (Control group): Distilled water 1 ml/100 gm body weight was given to them daily for 20 days. Blood sample were collected through inner canthus at 7th, 14th and 21st day.

Group III - Hydrocortisone induced hyperglycaemic rats

Twelve rats of either sex weighing 100 – 120 gms were selected for the experiment. Initially fasting blood samples were collected.

***Step I*:** Hydrocortisone succinate solution in distilled water in a dose of 50 mg/kg body weight was given intraperitoneally to every rat for seven days. On seventh day fasting blood samples were collected from the all rats.

***Step II*:** All the hyperglycemic rats were divided into two groups consisting of the six rats in each group.

Group A. (Treated group): 5% aq. solution was given by intragastric tube each rats in dose of 30 mg/100 gm body weight for 20 days. Blood samples after overnight fasting were collected on 7th, 14th and 21st day.

Group B. (Control group): Rats of this group were kept as control group and distilled water in a dose of 1 ml/100 gm body weight was given orally for 20 days. Their blood samples were collected on 7th, 14th and 21st day.

OBSERVATIONS

Table 1 : Effect of Chirabilva on fasting blood sugar level (mg%) in normoglycemic albino rats

Group	BT	After treatment (Mean ± S.D.)			$BT - AT_3$	t value & p value
		AT_1	AT_2	AT_3		
Group A (Treated)	63.23 ± 9.90	57.30 ± 7.99	61.47 ± 9.13	60.64 ± 7.74	2.59 ± 8.95	t=0.71 p >0.05 NS
Group B (Controlled)	61.52 ± 13.13	63.33 ± 11.03	61.62 ± 10.44	63.02 ± 11.59	-1.49 ± 6.31	t=0.58 p >0.05 NS

Table 2 : Effect of extract (water soluble) of Chirabilva on fasting blood sugar level (mg%) in Streptozotocin induced hyperglycemic albino rats

Group	BT_1	BT_2	After treatment (Mean ± S.D.)			BT – AT_3	t value & p value
			AT_1	AT_2	AT_3		
Group A (Treated)	55.7 ± 9.40	290.11 ± 23.67	280.00 ± 24.56	213.35 ± 23.13	103.90 ± 9.89	186.10 ± 35.17	t=14.3 p<0.001 HS
Group B (Controlled)	38.5 ± 4.36	220.80 ± 21.38	210.0 ± 19.23	174.8 ± 23.42	60.30 ± 8.76	160.50 ± 28.10	t=10.4 p<0.001 HS

Table 3: Effect of extract (water soluble) of Chirabilva on fasting blood sugar level (mg%) in hydrocortisone induced hyperglycemic albino rats

Group	BT_1	BT_2	After treatment (Mean ± S.D.)			$BT - AT_3$	t value & p value
			AT_1	AT_2	AT_3		
Group A (Treated)	55.7 ± 16.20	87.63 ± 18.3	70.5 ± 16.89	62.5 ± 13.31	47.00 ± 8.27	40.63 ± 8.9	t=12.7 p<0.001 HS
Group B (Controlled)	53.8 ± 9.67	72.32 ± 10.53	85.40 ± 11.70	61.00 ± 8.92	97.4 ± 7.946	-43.60 ± 94.80	t=1.2 p<0.05 NS

RESULT

Intergroup Statistical Comparsion of Table 1

	t value	p value
A vs B	1.67	p > 0.05 NS

As the above mentioned data showed that the initial mean ± S.D. of Group 'A' (treated) was 63.23 ± 9.90 was at 3rd follow up reduced to 60.64 ± 7.74. So the result was statistically insignificant.

In group B (Control group) initial mean ± S.D. was 61.52 ± 13.13 which became 63.02 ± 11.59 on 3rd follow up so the result was not significant.

Inter group Statistical Comparsion of Table 2

	t value	p value
A vs B	1.78	p < 0.05 NS

Regarding the blood sugar level in streptozotocin induced hyperglycemia in wistar albino rats, above data shows that initial (after induction of hyperglycemia) mean ± S.D. of Group 'A' (treated) was 290.11 ± 9.40 which after 21st day of treatment reduced to 103.90 ± 9.89 result was highly significant. In group B (Control group) initial mean ± S.D. was 220.80 ± 21.38 which reduced after 21 day was 60.30 ± 8.76 which shows highly significant. Intergroup comparison was insignificant.

Inter group Statistical Comparsion of Table 3

	t value	p value
A vs B	3.89	p<0.01 HS

Above mentioned data show the level of blood sugar in hydrocortisone induced hyperglycemia and reduction in that level after 21 days treatment with trial drug. After induction of hyperglycemia in group A initial mean ± S.D. was 87.63 ± 18.3 which after 21 days of treatment reduced to 47.00 ± 8.27 which was highly significant. In control group (i.e. initial mean ± S.D. was 72.32 ± 10.3 after 21 day was non significant where intergroup comparison between treated and control was highly significant.

CONCLUSION

- Preliminary toxicity study was done.
- Hyperglycemia was induced by both Streptozotocin and hydrocortisone in separate groups of animals.
- Mortality and other symptoms was not observed in rats after Streptozotocin.
- Study was performed for 21 days.
- No hypoglycemic effect was produced after giving the trial drug in Normoglycemic rats.
- In Streptozotocin and hydrocortisone induced hyperglycemic rats the hypoglycemic activity of the drug was found highly significant.

REFERENCES

1. Warrier, P.K., V.P.K. Nambiar and C. Ramakutty, 1995. Indian Medicinal Plants a compendium of 500 species, Orient longman private Limited, 3: 162.
2. Prajapati, N.D., S.S. Purohit and A.K. Sharma, 2003. A Handbook of Medicinal Plants a Complete Source Book. Agrobias India Jodhpur, 273.

3. Sharma S,Khatri ,Pandey A, Jakh etiaV, Chaturvedi L, Dwivedi N.Anti-diabetic screening of leaves extract of Holoptelea integrifolia Roxb. International Journal of Pharma. Research & Developmet 2010;2(10):66-71.
4. Ghosh MN (1984). Fundamentals of Experimental Pharmacology. 2nd rev. ed. Scientific Book Agency, Calcutta, India. 144-158.