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INVESTIGATION OF ANTIDIABETIC ACTIVITY AND ISOLATION OF COMPONENT FROM ETHANOL EXTRACT OF *PROSOPIS STAPHANIANA*

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ABSTRACT

Background: The different parts of the *Prosopis* were being used in Ayurveda for curing various diseases. e.g. leaf for skin-bites and scorpion stings and bark for leprosy, dysentery and rheumatism. Very less research has been done on the plant *Prosopis staphaniana* and no publication has been found on antidiabetic activity. **Objectives:** This work is therefore contemplated to investigate *Prosopis staphaniana* for antidiabetic activity by alloxan induced diabetic model by using albino rats (Wistar strain) and insulin as standard. **Methodology:** The bark of the plant were washed and air-dried to get a constant weight, cut into small pieces and pulverized to powder using pestle and mortar to get a powder The ethanol extract was subjected column chromatography obtained fractions were treated for antidiabetic activity. **Results:** Significant (Sig. P <.005, HS. n = 6) dose-dependent effects were observed columned fractions of bark of *Prosopis staphaniana*. The results revealed that ethanol extract is having considerable diabetic activity. Ethanol extract was subjected to purification and isolated the new fattyacid. **Conclusions:** The results of this study revealed that the ethanol bark extract of of *Prosopis staphaniana* possesses antidiabetic activity through its phytochemical components.

KEYWORDS: *Prosopis staphaniana,* Antidiabetic activity, Insulin.

1.0 INTRODUCTION

Diabetes mellitus is a chronic disease described by high blood glucose levels due to unquestionable or relative circulating insulin levels. Though different types of oral hypoglycemic agents are accessible along with insulin for the treatment of diabetes mellitus, there is a enermous interest in herbal remedies, due to side effects associated with synthetic therapeutic agents.[1-8] The Indian traditional systems of medicine, especially Ayurveda and Siddha, have put forward a number of therapeutic claims for plant drugs. However, it is important to provide experimental proof and show the various medicinal uses proclaim in traditional systems. The leaves of different species of prosopis were reported to contain variable quantities of free amino acids and flavonoids[9] with alkaloids and diketones isolated as active ingredients. The concentration of alkaloids varies between species. Concentrations were significantly higher in younger rather than in older leaves.[10] Of these alkaloids, two piperidine alkaloids have been studied.[11] Prosopine is a weak excitant of the nervous system while prosopinine has a relieve anxiety but also has local anaesthetic effects three times vigorous than cocaine. However, being strong irritants precludes their use in modern medicine. Aqueous and alcoholic extracts show some antibacterial activity.[12] A flavone glycoside, tutrim, has been isolated from the flowers.[13] Other studies have shown significant activity of plant extracts against lung carcinoma[14] Recently Alejandro Tapia *et al.,*[15] investigated the biological activity of extracts from the aerial parts of five Argentinian *Prosopis* species and the exudate of *P.flexuosa* were assessed for DNA binding, ß-glucosidase inhibition and free radical scavenging effect using the DPPH decoloration assay. DNA binding effect was found mainly in the basic fraction. The alkaloids tryptamine, piperidine and phenethylamine derivatives were isolated from the basic extracts. Encouraged by these facts it was contemplated to investigate *Prosopis staphaniana* for antidiabetic activity in more detail.

2.0 MATERIALS AND METHODS

2.1 Plant material

The bark of the plant *Prosopis staphaniana* were collected from Vidyanagar (Shimoga, Karnataka, India). The plant was authenticated by Professor Kamalakar, Department of Botany, Sahyadri Science College (Shimoga, Karnataka, India). A voucher specimen has been deposited in the Department of Botany, Sahyadri Science College.

2.2 Preparation of extract

The powdered plant material (350 g) was repeatedly extracted in a 2000 mL round bottomed flask with 1500 mL solvents of increasing polarity begin with petroleum

ether, chloroform, ethanol and double distilled water. The reflux time for each solvent was 40 cycles. The extracts were cooled at room temperature, filtered and dried under reduced pressure in a rotatory evaporator (Buchi Rotavapor).

2.3Preliminary phytochemical screening

The phytochemical screening of bark of *Prosopis staphaniana* contains alkaloids, flavonoids, glycosides, saponins, steroids, phenolics and tannins.[16-17]

2.4 Isolation and identification of the compound

The ethanolic extract of the plant *Prosopis staphaniana* was chromatographed over silica gel (60-120 mesh) on column of 15 cm length and 3 cm diameter. Elution was carried out with solvents and mixture of solvents with increasing polarities. Fractions were collected in 10 ml portions and monitored on TLC (silica gel as absorbent, solvent system Chloroform: Methanol: 70:30 V/V and the fractions showing similar spots were combined. Removal of solvent under reduced pressure and controlled temperature. The chromatographic details are presented in Table 1

Table 1: Chromatographic details of ethanolic extract of *Prosopis staphaniana.*

Fractions	Eluent	Colour and Nature	Yield
Fraction 1.1	Chloroform: 100 V/V	Dark brown paste	0.30 gm
Fraction 1.2	Chloroform: Ethyl acetate 80:20 V/V	Brown powder	0.50 gm
Fraction 1.3	Chloroform: Methanol 70:30 V/V	Brown powder	0.50 gm
Fraction 1.4	Chloroform: Methanol 60:40 V/V	Brown powder	3.51 gm
Fraction 1.5	Chloroform: Methanol 40:60 V/V	Brown powder	1.35 gm
Fraction 1.6	Chloroform: Methanol 20:80 V/V	Brown powder	0.60 gm
Fraction 1.7	Methanol: 100 V/V	Brown powder	3.00 gm

2.5 Animals

Male wistar albino rats (160 – 200 g) were used in the experiment. Animals maintained under environmental conditions, were feeding with a principal diet (Hindustan Lever, India) and water *adlibitum*. The animals were fasted for 18hr before experimentation but allowed free access to water.

2.6 Fixation of dose

Acute toxicity study was performed using albino mice and doses were fixed as per OECD guideline No 420 and adopted CPCSEA protocol for the screening of pharmacological activity.

3.0 METHODS TO SCREEN ANTIDIABETIC ACTIVITY

3.1 Alloxan Induced Hyperglycemia

Hyperglycemia and glycosuria occur after administration of alloxan in several species.[18-19] Investigators found that alloxan has a selective destructive effect on the β-cells of islet of Langerhans of the pancreas. However, this effect varied with species and the dose.

3.2 Experimental Induction of Diabetes in Rats

Diabetes was induced by intraperitonial injection of alloxan monohydrate in physiological saline at a dose of 60 mg/kg body weight in chilled citrate buffer pH 4.5. Alloxan monohydrate was purchased from the IOBA Chemie Bombay. After 48 hr rats showing blood glucose levels of 250-350 mg/dl were considered as diabetic and were employed in the study.

3.3 Experimental Design

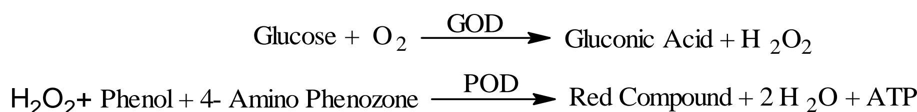
The rats were housed in polyethylene cages and divided into 5 groups of six animals each. Group-I served as solvent control, group II served as diabetic control, group III received insulin 0.6 u/mg, S.C., group-4 and group-5 received ethanolic extract of *Prosopis staphaniana* at 100 mg/kg and 50kg/mg., i.p. for 2 weeks respectively. On the 0 week, 1 week and 2 week the animals were fasted for 18 hr and blood samples were drawn by orbital sinus puncture under mild ether anesthesia. The blood samples were collected in Eppendroff's tubes containing 50 µl of anti-coagulant (EDTA). Plasma was separated by centrifugation at 5000rpm for 10mts and analysed for glucose content in autoanalyser Microlab by enzymatic method (GOD/POD method-Beacon-Diagnostic PVT.LTD). The study was approved by the Institutional Animals Ethics Committee (CPCSEA).

3.4 Statistical Analysis

In all the above experiments the results have been expressed as mean ± S.E.M., ten animals in each group. Statistical significance test were performed by Newman-keul's Test and P-values were calculated by comparing with controls. $P<0.05$ implies significance.

3.5 Principle of Estimation of Glucose

Glucose is oxidized by glucose oxidase (GOD) into gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of Peroxidase (POD) oxidized the chromogen 4-aminophenazone and phenol to a red colored compound. The intensity of this red color produced is proportional to the glucose concentration and is measured at 505nm Microlab by enzymatic method (GOD/POD method-Beacon-Diagnostic PVT.LTD).



4.0 RESULTS

4.1 Antidiabetic activity of ethanol extract of *Prosopis staphaniana*, Linn in rats

Alloxan has been shown to destruct β-cells of pancreas producing hyperglycemia. In our experiments the diabetes was characterized by hyperglycemia. The results are shown in below Table 2.

Table 2: Antidiabetic activity of crude ethanol extract of *Prosopis staphaniana*, Linn in rats.

Treatment Groups	O Week	First Week			Second week		
		Mean ±SEM	Diff	% Change	Mean ±SEM	Diff	% Change
I	84.3±0.9	83.8±1.2	0.5±0.9	-0.6	81.8±1.2	2.5±1.3	-2.9
II	266.7±4.0	293.3±4.2	26.7±7.0	+10.2	328.3±6.9	61.7±7.9	+23.3
III	245.0±3.4	162.0±1.0	83.0±3.4	-33.8	128.2±1.9	116.8±4.0	-47.6
IV	269.2±4.0	187.0±2.4	82.2±3.4	-30.5	157.5±2.5	111.7±3.6	-41.5
V	276.7±3.1	235.8±6.4	40.8±5.2	-14.8	228.3±6.5	48.3±5.9	-17.5
ANOVA	----	F=72.9 P <.001, HS			F=186.5 P <.001, HS		

MEAN ±SEM

Between groups one way ANOVA, followed by Newman-keul's Test

P<0.05,P <.001, Sig. P <.001, HS. n = 6

4.2 Antidiabetic activity of the coloumed fractions of *Prosopis staphaniana*

The ethanolic extract of the bark of *Prosopis staphaniana* has shown to possess a wide spectrum of activity. TLC of the crude extract was carried out using solvents and solvent combinations of varying polarities. It was observed that there was clear separation. Therefore the ethanol extract was subjected to column chromatography the results were shown in chapter-4. Out of all the following fractions only two major fractions were subjected to antidiabetic activity the results were shown in Table 3.

Table 3: Antidiabetic activity of crude ethanol extract of *Prosopis staphaniana*, Linn in rats.

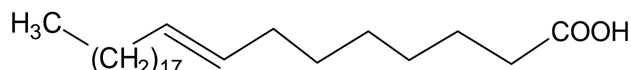
Treatment Groups	O Week	First Week			Second week		
		Mean ±SEM	Diff	% Change	Mean ±SEM	Diff	% Change
I	80.6±0.40	81.0±0.31	0.5±0.9	0.4	80.6±1.2	2.5±1.3	-2.9
II	260.8±0.58	301.80±0.86	26.7±7.0	-15.8	350.20±0.20	61.7±7.9	+23.3
III	260.60±0.24	123.60±0.40	83.0±3.4	52.6	110.40±0.40	116.8±4.0	57.6
IVEA:MeOH8:2	261.60±0.87	141.6±0.40	86.2±7.7	+45.9	123.6±0.40	124.2±5.3	52.8
V EA:MeOH6:4	260.60±0.24	123.60±0.40	83.0±3.4	52.6	110.40±0.40	116.8±4.0	57.6

Between groups one way ANOVA, followed by Dunnet's test Test

P<0.05,P <.001, Sig. P <.001, HS. n = 6

4.3 Identification of the compound

On isolation of the ethanol extract, a pale yellow paste semi solid was obtained. Structural determination of the compound was done using spectroscopy techniques and it was confirmed as fatty acid. The yield of fatty acid was 0.30 gm in *Prosopis staphaniana*. The compound was identified based on the following evidence: Molecular weight 409, IR-Spectra 3458 cm^{-1} (COOH), 1732 cm^{-1} (C=O), 1647 cm^{-1} (C=C), ^{1}H NMR (400 MHz): 0.83, 1.25, 1.6, 2.3, 5.1 and δ 5.3 ^{13}C NMR (200 MHz): δ 125, 130, 29.00, 14.07, 16.04, 18.89, 23.71, 26.45, 31.95, 32.27 and 39.76. The molecular formula of fatty acid is $C_{27}H_{52}O_2$.



4.4 Effect of experimental plant on Serum glucose levels

The effect of ethanolic extracts from medicinal plant on the blood glucose levels of experimental animals was determined at various time interval for 2 weeks after oral administration at 50, 100 mg dose kg-1 b.wt. (Table 2). There was a significant elevation in the blood glucose level by 2 times during experimental time period in alloxan-induced diabetic rats, when compared to normal rats. The administration of *P. staphaniana* extract caused the blood glucose levels of diabetic rats to -30.5, -14.8 (1 week), 41.5, 17.5 (2-week) at the dose of 50, 100 mg dose kg-1 b.wt. (p<0.05).

4.5 Effect of coloumed fractions of experimental plant on Serum glucose levels

The effect of coloumned fractions from two medicinal plants on the blood glucose levels of experimental animals was determined at various time interval for 2 weeks after oral administration at 50 mg dose kg-1 b.wt. (Table 3). The EA: MeOH (8:2) fraction from *P. Staphaniana* showed reduction in serum glucose level to +45.9(1 week), 52.8 (2 week) respectively.

5.0 DISCUSSION

Alloxan induces "diabetes" in a large variety of animal species by damaging the insulin releasing pancreatic ß-cell, resulting in a decrease in endogenous insulin release.[20-34] Numerous studies shows that a variety of plant extracts effectively lowered the glucose level in alloxan-induced diabetic. In the present study, the ethanolic extracts of *P. staphaniana* effectively decreased the blood glucose in alloxan- induced diabetic rats, which is nearly equal to that of insulin. The hypoglycaemic activity may be ascribed to the presence of saponins, alkaloids, flavonoids.

6.0 CONCLUSION

The results of this research work indicates that *Prosopis staphaniana* extract through its phytochemical constituents possess antidiabetic properties and also isolation one fatty acid component from the bark extract. Ayurveda for curing various diseases. e.g. leaf for skin-bites and scorpion stings and bark for leprosy, dysentery and rheumatism. Further investigation is hereby recommended to demonstrate cellular mechanisms and structural components of the active ingredients of this bark extract in order to standardize them.

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