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ANTI-MICROBIAL AND ANTI-DIABETIC ACTIVITY OF *PROSOPIS CHILENSIS* EXTRACT AGAINST ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

India is one of the world's twelveth leading biodiversity centers with the presence of over 45000 different plant species. Herbs and its active constituents are being used to treat the infectious organisms, which no longer responsive to conventional medicine. The herbal drugs been used throughout the world have received greater attention in recent times, because of its diversity of curing diseases and well tolerated remedies compared to the conventional medicines. The aim of this study was to evaluate the anti-diabetic activity, anti-microbial activity and pharmacological activity of leaves extracts of *Prosopis chilensis*. All the extract was subjected to phytochemical analysis. It shows the presence of Saponins, Carbohydrates, Cardiac glycosides, The percentage yield of solvent extracts method and yield from leaves of *Prosopis chilensis* of petroleum ether (6.5%), chloroform (6.9%), ethanol (14.6%) and water extracted (27.14%) and Solvent Extraction method and yield from leaves of *Prosopis chilensis* of petroleum ether (6.1%), chloroform (5.9%), ethanol (12.6%) and water extracted (21.11%). The present study concluded that the anti-microbial activity and anti-diabetic activity of various extracts of leaves of *Prosopis chilensis* extracts showed significant therapeutic activity, but in specific ethanolic extract exhibit maximum activity may be due to presence of steroids, triterpenes.

Key Words:-*Prosopis chilensis*, Anti-diabetic activity and Anti-microbial Activity.

INTRODUCTION

India is one of the world's twelveth leading biodiversity centers with the presence of over 45000 different plant species. India is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world (Akgun *et al*., 2006). Herbs and its active constituents are being used to treat the infectious organisms, which no longer responsive to conventional medicine. The herbal drugs been used throughout the world have received greater attention in recent times, because of its diversity of curing diseases and well tolerated remedies compared to the conventional medicines. World health organization (WHO) has defined herbal medicines as finished labeled medicinal product that it contains active ingredients, aerial of underground parts of the plant or other plant material or combinations (Evans CW *et al*., 1996). In poly herbal ayurvedic preparations it will be very difficult if we want to estimate each and every ingredient in term of their chemical constituents. But if few major constituents having particular therapeutic action indicated in the labeled can be pin pointed then theses constituents should be estimated quantitatively along with the other parameter through which presence of all ingredients can be confirmed. Plant

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are oldest source of pharmacologically active compounds, and have provided human kind with medically useful compounds other such as cynogenic glycoside glucosinolates, occur as inactive precursor and are activated in response to issue damage or pathogenic attack. This activation often involves plant enzyme, which are released as a breakdown in cell integrity (Adarn NKB *et al*., 1992).

MATERIAL AND METHODOLOGY

The leaves of *Prosopis chilensis* were collected from wild source of surrounding A.M Reddy Memorial college of Pharmacy located at Petlurivaripalem, Narasaraopet, Guntur. Collected leaves of *Prosopis chilensis* were dried under shade and pulverized to make coarse powder.

The leaves of *Prosopis chilensis* were dried in shade, under environmental conditions and then subjected to size reduction to get a coarse powder. Such powdered was carried out successively with the solvents of Petroleum ether, Chloroform, Ethanol and aqueous, aqueous extraction was carried out by 7day in room temperature. Each time before extraction with next solvent, the powdered material was air dried below 50°C and then extract was concentrated by distilling of the solvent to obtain the crude extract. The drug was extracted with each solvent till complete extraction was effected (above 30 cycles) (Kirithikar KR *et al*., 1995).

Extraction procedure

Collected plant material was dried under shade and powdered coarsely, taken 120gms of each crude drug were successfully extracted with Petroleum ether, Chloroform, Ethanol by soxhelation and Aqueous extracted by macerated with distilled water. All extracts were individually filtered, evaporated to dryness (40°C). After completion of extraction stored in all the extracts viz. petroleum ether, chloroform, ethanol and aqueous were stored in desiccators over phosphorous pentaoxide and self-indicating silica gel G.

Preliminary phytochemical screening of extract of leaves of Prosopis chilensis

The plant may be considered as a biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, flavinoids and sugars etc., that exert physiological effects. These compounds are responsible for therapeutic effects, usually the secondary metabolites. All the extracts of the plant material were subjected to preliminary phytochemical screening for the detection of various plant constituents. The result was follow table No.1 (Singh SB *et al*., 1984).

Antimicrobial activity

By far the most widely used assay for identifying anti-microbial activity by diffusion method, which exploit diffusion of anti-microbial compounds through agar media and fungi variations on the underlying principles of agar diffusion has been in existence since the latter infect, this principle was used by Alexander Fleming in interpreting his observations for, the penicillin consequently lead to the discovery of first anti biotic, penicillin. The use of agar diffusion continues into modern times in the microbial assays recommended by the US food and drug Administration and the National Committee for clinical laboratory standards. The basic idea of diffusion assays is as follows a suspected anti-microbial compound or treatment is presented with in a reservoir created on an inoculated plate of agar media, zone of inhibition forms where concentrations of the diffused molecules are sufficient to inhibit microbial growth. On the surface, the theory is quite simple. Diffusion of antimicrobial compounds forms a reservoir over time produce an outward gradient of decreasing concentration of the compound. Where concentration of the compound is sufficient to inhibit the growth of microorganisms, the growth is blocked resulting in observed zone, which extend outward from the reservoir at which the minimum concentration required for inhibition exists (Aflolayom AJ *et al*., 1997: Almagboul AZ *et al*., 1985).

Tested extracts

1. Petroleum ether extract
2. Chloroform extract
3. Ethanolic extract
4. Aqueous extract

Preparation of solution extract

Before testing of these extracts for antimicrobial activity, they were completely dried at normal conditions. It was dissolved in DMSO and diluted with sterilized water. Prepare the stock solution of each extract (Bhatia IS *et al*., 1975).

1. Gram positive control
   Gentamycin 10 µg/ml
2. Gram negative control
   Vancomycin 10 µg/ml

Test cultures

For evolution of antimicrobial five cultures were used. Escherichia coli, Streptomyces gresieus, Bacillus

subtilis, Pseudomonas aureginosa and Strptococus epidermis.

Preparation of inoculums

The bacterial cultures were grown on nutrient agar for 24 hours at 27°C in incubator. They were stored at 4°C and sub culturing was done after one work.

For evolution antimicrobial activity, 24 hours fresh culture of bacteria was suspended in sterile distilled water to obtain uniform suspension of microorganism.

Preparation of culture media

Evolution of antimicrobial activity following culture media was used.

Nutrient agar medium

The media was used for growth of bacteria. The formula for the preparation of the medium is given below.

Formula

Peptone - 5.0g, Sodium chloride - 5.0g, Beef extract - 1.5g, Yeast extracts - 1.5g, Agar - 15.0g, Water up to 1000ml, PH (at 25°C) - 7.5±0.2.

Procedure for disk diffusion method

A previously liquefied medium inoculated with 0.2ml of microbial suspension of test organisms having uniform turbidity at temperature of 40°C and 20ml of inoculated medium was poured immediately into the sterile Petri dish having internal diameter of (100×70mm). Care was taken for uniform thickness of the layer of the medium in different plates. After complete solidification of liquefied inoculated medium, the cups were made aseptic. In each of these plate paper disc wetted with the sample solution.

The selected disc was held with forceps and it was placed on the inoculated plate. The discs were placed on the agar plates as follows. With the help of the fire pointed pair of forceps, this was in alcohol, flamed and cooled. After placing the paper disc the Petri plates were kept in room temperature for 2 hours, as a period of pre-diffusion. Then the plates were kept in incubator for 18 hours at a temperature of 27°C for bacterial growth. After incubation period, the diameter resultant growth inhibition zones were measured. Each extract and antibiotic was tested against each organism and zone of inhibition was calculated.

Testing Procedure for Antidiabetic Activity (Gupta NP *et al*., 1984)

Animal models

Wister rats (200 to 225gm) of either six were employed in this study. The rats were maintained under standard in laboratory conditions at 25°C, relative humidity 50±15% and normal photo period (12h dark/ 12h light) were used for the exp. Commercial pallet diet (chakan oil mils, sangli, India) and water. Were provided the regulatory body of Government has approved by the Institutional animal ethics committee and ND libitum the experimental protocol.

Experimental design

Diabetes was induced by using alloxan mono hydrate (100mg/kg) only alloxanised hyper glycemic animals were used for further studies. Animals were fasted for 18 hours before experiment. And divided into 5 groups (6 animals in each group) the first group (control) received normal saline and to second group received alloxan monohydrate alone. The three test group received 200mg/kg of different extracts before the dose of alloxan. All the animals were regularly observed for their general behavior (Graham SA *et al*., 2005).

RESULTS

Effects on blood glucose levels

Dried aqueous, alcoholic and petroleum ether (60 to 80°C) extracts of leaves of *Prosopis chilensis* and *Prosopis chilensis* (100mg/kg of each) were suspected in 1% bentonite and subjected for hyperglycemic activity in Wister rats (200-225gm). Diabetes was induced by I.V. administration of alloxan 100mg/kg) after anesthesia with ethyl ether. 48h later the blood (1ml) was collected from orbital sinus into tubes and immediately used for determination of glucose. Only animals that prevented with glycemic levels equal to or above 200mg/DL were submitted to treatment, which consists of daily administration of extracts of crude drugs for seven days. The oral treatment of all groups carried out at the same time (in the morning) and under the same conditions. One hour after the last administration the blood was collected again blood glucose measurements using Glucometer.

The percentage yield of petroleum ether, chloroform, ethanol and water extracted were calculated and presented (Table No.2.) Solvent extracts method and yield from leaves of *Prosopis chilensis*:

Antimicrobial activity

The petroleum ether, chloroform, ethanol and aqueous extracts were screened for antimicrobial activity. Filter paper discharger diffusion method was used. Gemtamycin (10g/ml) and Vancomycin (10g/ml) were used as standard for bacterial culture.

Preliminary phytochemical screening

The present work deals with successive extraction of the leaves of increasing polarity, Viz petroleum ether, Chloroform, Ethanol and Aqueous extractive values were found to 6.1%, 5.9%, 12.6%, and 21.11%. Preliminary phytochemical screening of *Prosopis chilensis* of different extracts was performed by using qualitative chemical test. The results indicate the presence of Saponins, Carbohydrates, Cardiac glycosides, Steroids, Triterpenes and Alkaloids, Tannins, Phenolic compounds, Flavonoids, Proteins and Amino acids respectively.

Table 1. Preliminary phytochemical screening of extract of leaves of *Prosopis chilensis*

S.No.	Plant constituents	Test/Reagent	PTE	CHE	ELE	AQE
1	Steroids	Salkovaski	--	--	--	--
2	Alkaloids	Dragendroff's Test	++	++	++	++
		Hager's Test	++	++	++	++
		Mayer's Test	++	++	++	++
		Wagner's Test	++	++	++	++
3	Saponins	Foam Test	++	++	++	++
		Haemolysis Test	++	++	++	++
4	Fats & Oils	Filter paper Test	--	--	--	--
5	Tannins & Phenols	Ferric chloride Test	++	++	++	++
		Lead acetate Test	++	++	++	++
		Potassium dichromate Test	++	++	++	++
		Bromine water Test	++	++	++	++
6	Flavinoids	Shinoda Test	++	++	++	++
		Lead acetate Test	++	++	++	++
7	Carbohydrates	Molish Test	--	--	--	--
		Fehling's Test	--	--	--	--
		Barfoeds Test	--	--	--	--
8	Proteins	Millon's Test	++	++	++	++
		Biuret Test	++	++	++	++
9	Amino acids	Ninhydrine Test	++	++	++	++

Table 2. Solvent extracts method and yield from leaves of Prosopis chilensis

S.No.	Solvents	Polarity	Extraction	%Yield
1	Petroleum Ether	0.0	Soxhlation	6.1
2	Chloroform	4.1	Soxhlation	5.9
3	Ethanol	5.2	Soxhlation	12.6
4	Aqueous	9.0	Maceration	21.11

Table 3. Effect of Ethanolic extract of *Prosopis chilensis* on alloxan-induced diabetic rats

Groups	Treatment	Blood glucose level (mg/dl)		
		Basal value	1 Hour	3 Hours
I	Control (Distilled water)	388.54 ± 14.12	379.3 ± 13.34	377.57 ± 12.78
II	Ethanolic extract (100mg/kg)	378.46 ± 13.68	350.78 ± 12.88*	320.36 ± 16.78*
III	Ethanolic extract (200mg/kg)	382.48 ± 16.52	342.92 ± 14.38*	310.64 ± 12.66*
IV	Standard drug Metformin (500 µg/kg)	378.58 ± 12.42	338.22 ± 16.92*	308.18 ± 15.46*

Values are mean ± S.E.M; (n=6), determined at different time (hours) after treatment a statistically significant difference of the value when compared with the zero time (*P<0.001)

Results of in vitro antimicrobial activity against ***Escherichia coli*** on leaf extract of **Prosopis chilensis**

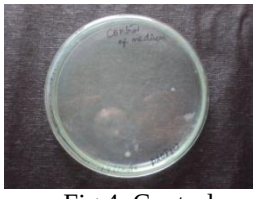

Fig 4: Control

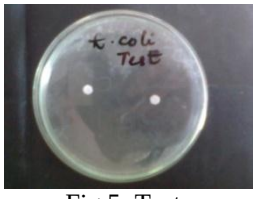

Fig 5: Test

Fig 6: Standard

Results of in vitro antimicrobial activity against ***Streptomyces gresieus*** on leaf extract of **Prosopis chilensis**

Fig 7: Test

Fig 8: Standard

Results of in vitro antimicrobial activity against ***Bacillus subtilis*** on leaf extract of **Prosopis chilensis**

Fig 9: Test

Fig 10: Standard

Results of in vitro antimicrobial activity against ***Pseudomonas aureginosa*** on leaf extract of **Prosopis chilensis**

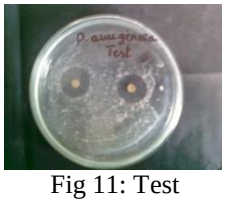

Fig 11: Test

Fig 12: Standard

Results of in vitro antimicrobial activity against ***Strptococus epidermis*** on leaf extract of **Prosopis chilensis**

Fig 13: Test

Fig 14: Standard

DISCUSSION AND CONCLUSION

In the present study, the hypoglycemic activity of crude extract from *Prosopis chilensis* was evaluated in normal hyperglycemic and alloxan induced diabetic rats. The Antidiabetic activity of *Prosopis chilensis* on glucose tolerance and normal rats summarized in table 3, the treatment of normal rats with ethanolic extract and Metformin, a known hyperglycemic drug, resulted from a significant decrease ($P<0.001$) in blood glucose levels 90min after oral drug administration when compared with initial level and control. The maximum glucose tolerance was observed at 30^{th} mins for ethanolic extract.

The blood glucose levels of diabetic rats treated with ethanolic extract at doses of 100 and 200 mg/kg showed significant difference at 1 end 3^{rd} hour from initial levels ($P<0.001$) The doses of the crude extract produced the max glucose lowering (19 and 16 percent respectively) in diabetic rat serum and, with the higher dose a significant time dependent hypoglycemic effect was shown throughout the period studied.

Based on the results of the study we conclude that the ethanolic extract of Prosopis chilensis, given orally at a dose of 100mg/km b.w., posses significant hypoglycemic in both normal and glucose loaded rat. Also in diabetic induced rat significantly. It is generally consider that alloxan treatment causes permanents destruction of β-cells. The free radial scavenging potential could also help in reducing the known complication of diabetic mellitus.

Thus the observed Antidiabetic effects may be least partly due to its anti-oxidant activity. Literature review shows that some flavonoids, glycosides and saponins isolated from medicinal plants significantly reduce the blood glucose level. Flavonoid, glycosides stimulates the secretion of insulin in β-cells of pancreas. In glucose loaded animals, it is possible that the extract may act by above evidence it is possible that the presence of glycosides and tannins are responsible for their activity.

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