RESEARCH ARTICLE e-ISSN: 2454-7867

Available online at www.ijtpls.com
International Journal of Trends in Pharmacy and Life Sciences
Vol. 3, Issue: 5, 2017: 67-70

ANTI-HYPERGLYCEMIC ACTIVITY OF BARK OF *THESPESIA POPULNEA* IN ALLOXAN INDUCED *WISTAR* RATS

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ABSTRACT
The bark of *Thespesia Populnea* was selected to evaluate its antidiabetic activity Subsequently a survey of literature suggested that no activity is reported on bark of *Thespesia Populnea* .It has been evaluated scientifically for its anti diabetic activity.. The treatment of hydro alcoholic extract of bark of Thespesia Populnea reduced the elevated blood glucose level, serum glucose and cholesterol levels indicating anti diabetic activity. The antidiabetic activity may be due to its antioxidant activity as antioxidants are supportive for antidiabetic activity. In conclusion, the total extract will be subjected to isolate the entity responsible for the antidiabetic activity.
Key Words: *Thespesia Populnea*, Diabetes mellitus, antidiabetic activity

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Received: 20/11/2017
Revised: 12/12/2017
Accepted: 25/12/2017

INTRODUCTION

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Barcelo and Rajpathak,2001). Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period of time, this can result in longterm damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels. Complications in some of these organs can lead to death (Pari and Saravanan, 2004). Survey reported by the National Commission on Macroeconomics and Health reveals that, India will be described as the 'diabetes capital of the World' with high incidence of diabetes (Raman B; Geetha B.2006). Currently, there are 41 million having diabetes and the number will be increased to around 70 million by the year 2030(Smitha R, Manu A, 2006). From the current level of about 26 to 27 million people known to be suffering from diabetes. The incidence of diabetes in urban population is 14% and 8 % (or) pre-diabetes over 7.20 lakhs die every year due to diabetes and its complications(Smitha R, Manu A, 2006) , and is one of the most common cause of nephropathy severe diabetes for 5 to 15 years will leads to kidney damage. After 20 years of diabetes 75% develop nephropathy. Two out of every 1,000 children suffer from acute diabetes both type-1 and type-2 (Smitha **R,** Manu A, 2006).

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. It has been estimated that about 80-85% of population both in developed and developing countries rely on traditional medicine for their primarily health care needs and it is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles (Ignacimuthu *et al.*, 2006; Elujoba et *al.*, 2005: Tomlinson and Akerele, 1998). The active principles of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents. Different species of medicinal plants are used in the treatment of diabetes mellitus. For diabetes treatment, before the discovery of insulin by Banting and Best in 1922, the only options were those based on traditional practices (Ribnicky *t al.*, 2006). Till today metformin is the only ethical drug approved for the treatment of non insulin dependent diabetes mellitus 'patients, which is derived from a medicinal plant *Galega officinalis* (Eshrat, 2002, Oubre *et al.*, 1997). Among those

plants used traditionally for the treatment of diabetic complications are, *Stevia rebaudiana* Bertoni (Gregersen *t al.*, 2004; Li et *al.*, 2004), *Ajuga remota* Benth (Abebe *et al 2003) and Thespesia Populnea.*. Hence, it is essential to study the antidiabetic effect *of Thespesia Populnea* and whether it retained its anti-hyperglycemic activity of *Thespesia Populnea.*

Collection And Authentication

Bark of Thespesia Populnea *was* collected from Nelogonda district .The herbarium of the plant was prepared for authentication. The plant has been authenticated by Mrs.Sujatha; Head of the Dept, Botany, New Bhavan's College, and Hyderabad. Andhra Pradesh. The authentication letter has been issued as a proof of authentication.

Preparation of extract

The bark was separated from the stem and was kept for shade drying. The bark was then grounded into coarse powder and extracted with 50% ethanol. The extraction was carried out for 10 hours. The extract was collected and was evaporated to get concentrated extract.

Phytochemical evaluation

The extracts were preliminary investigated for various phytochemical constituents such as alkaloids, Carbohydrates, Steroids, Proteins, Phenols, Tannins, Flavonoids, Glycosides, Gums, Saponins and terpenes.

Acute Oral Toxicity

Healthy Swiss albino mice of either sex weighing 25-30 g, starved overnight were divided in to 3 groups (n=6) and fed with increasing doses (30, 300 and 3000 mg/kg, p.o) of each extract and the toxicity was evaluated. The total drug extracts administered orally in doses of up to 3 gm/kg did not produce any evident toxicity and mortality in mice up to 14 days after administration.

Anti-diabetic studies using Alloxan induced diabetes in rats and estimation of biochemical parameters.

Healthy adult male *Wistar* rats (250-300g) 8-10 weeks old were obtained from animal house. The rats were housed in polypropylene cages and maintained under suitable nutritional and environmental (12-hr light: I 2-h dark cycle; 25+-3; 35-(>0' humidity) conditions throughout the experiment. The animals were fed with water and standard rat pellet.

Induction of Diabetes

The experimental animal in this model is the male, adult Wistar weighing 150 to 200g. After a 24-hour fast, the rats were weighed and numbered accordingly. A solution of alloxan at 10% w/v diluted with saline at 0.9% was administered to the animals in a single dose corresponding to 100mg alloxan per kg of animal weight injected intraperitonially. Food and water were presented to the animals only 30 minutes after the drug administration. The animals showed the following signs of the condition: polydipsia (abnormal thirst), polyuria (increased urine volume), asthenia (weakness due to the inability to use glucose as a source of energy), and dehydration (due to the animal body's attempt to get rid of the excess blood glucose as the normal process of storing glucose in the body cells is impaired). In order to assess the effect of alloxan and to chemically establish the diabetic condition, an incision was done in any of the four veins in the tail of the rat using a 15 scalpel blade 72 hours after induction. A sample of the rat's venous blood was collected on a reagent strip 72 hours after the diabetes induction procedure for blood glucose level determination using a portable glucose analyzer. The level of serum glucose considered to be normal in *mints novergicus* ranges from 50 to 135mg/100m1.In this study, rats with glucose levels above 200mg/dl were considered as having severe diabetes.

Mechanism of action of Alloxan

Alloxan and streptozo^{c}in are widely used to induce experimental diabetes in animals. The mechanism of their action in β-cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of both these diabetogenic agents is mediated by reactive oxygen species; however, the source of their generation is different in the case of alloxan and streptozotocin. Alloxan and the product of its reduction dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells.

Procedure for induction of Diabetes mellitus

Lying on its back, the animal is given an injection of Alloxan intra peritonially

Fig. 1: Intra Peritonial Administration of Alloxan

Preparation of drug solution

Hydro Alcoholic extract of Anogiessus latifolia was dissolved in distilled water for oral administration. The concentration of extract selected was 300 mg/ kg body weight. Glibenclamide was suspended in tween 80 and administered at a dose of 5mg/kg body weight.

Experimental Design

Four groups of rats, six in each received the following treatment schedule.

Group I : Normal Control
Group II : Toxicant (Alloxan only)
Group Ill : Standard (Glibenclamide 100mg/kg p.o)
Group IV : Hydro-alcoholic extract of Thespesia Populnea (300mg/*kg*, p.o)

Biochemical parameters evaluated

Serum glucose: Blood samples (2 ml) were collected from the rats and mice by retro-orbital puncture under mild ether anesthesia on the 1 st, $_4$th, $_7$th, $_{10}^{th}$ 15th, and 21st day of the study. The Serum was immediately separated by centrifugation and the glucose level was measured by GOD/POD method using glucose reagent kit and by auto-analyzer.

Serum Triglycerides: Blood samples (2 ml) were collected from the rats and mice by retro-orbital puncture under mild ether anesthesia on the 1^{st,} $_4$th, $_7$th, $_{10}^{th}$ 15th and 21st day of the study. The Serum was immediately separated by centrifugation and the glucose level was measured by Serum Triglyceride Determination Kit and Triglyceride Reagent obtained from SPAN DIAGNOSTICS Pvt.limited, Surat.

Serum Cholesterol: Blood samples (2 ml) were collected from the rats and mice by retro-orbital puncture under mild ether anesthesia on the 1st, $_4$th, $_7$th, $_{10}^{th}$ 15th and 21st day of the study. The Serum was immediately separated by centrifugation and the glucose level was measured by Cholesterol determination kit obtained from SPAN DIAGNOSTICS Pvt.limited, Surat.

Statistical analysis

All the values of serum glucose, cholesterol, and biochemical estimations were expressed as mean-standard error of mean (S.E.M.).Data was analyzed by student's t-test. Differences between groups were considered significant at $P < 0.01$ levels.

RESULTS

The estimation of the biochemical parameters yielded following results:

Group	Serum glucose (mg/dl) Days					Cholesterol (mg/dl)
Triglycerides (mg/dl)	1	4	7	10	15	
Normal 80.80±5.4	88.0±2.8	89.3±4	85.3±5.5	88±5.1	86±3.9	142±6.1
Diabetic Control 361±11.3	298±6.3	370±8.9	410±12	360±16	265.31±14.9	199.81 ±10

Diabetic+TP 118±4** (300mg/kg, p.o)	285±8.3	214±10**	163±8.5**	132±7.1**	158.49± 6.4**	112.5 112.5
Diabetic's 114±4.1** (Glibenclamide, 5mg/kg, p, o)	286±5.8	206±9.8**	166±8.4**	128±8.7**	141.81±5.0**	105 ± 6.0**

* Values are mean's; n=6 in each group, *P<0.01 (compared to diabetic control or normal control). **P<0.001 (compared to diabetic control or normal control); ***P<0.01 (compared to normal control) and P<0.001 (compared to diabetic control); P<0.01 (compared to diabetic control). NS- not significant.
** Values are given as mean±SEM for groups of six animals

SUMMARY AND CONCLUSION

The bark of *Thespesia Populnea* was selected to evaluate its antidiabetic activity Subsequently a survey of literature suggested that no activity is reported on bark of *Thespesia Populnea* .It has been evaluated scientifically for its anti diabetic activity.. The treatment of hydro alcoholic extract of bark of Thespesia Populnea reduced the elevated blood glucose level, serum glucose and cholesterol levels indicating anti diabetic activity. The antidiabetic activity may be due to its antioxidant activity as antioxidants are supportive for antidiabetic activity. In conclusion, the total extract will be subjected to isolate the entity responsible for the antidiabetic activity.

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