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ORIGINAL ARTICLE

Evaluation of phytochemicals and histopathological evidence of antidiabetic potential of some medicinal plants in Wistar albino rat

Rashmi Aashay Morey
Department of Zoology, Prof. Ramkrishna More College, Akurdi, Pune
Affiliated to Pune University, Pune (Maharashtra), INDIA
Email: moreyrashmi@gmail.com

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ABSTRACT

The increase in the number of patients suffering diabetes is alarming and needs to be taken care of in the most appropriate way. The complications in this disorder are often fetal. People suffering from diabetes are marked with the tendency to have a high level of blood sugar and the metabolic involvement of this sugar causing further complications. Though several medicines are in market today, still the problem remains persistent. Alternative strategies are suggested and worked upon primarily to lower the sugar level. Several traditional practices are also used for the same. In this study an attempt has been made to evaluate the recovery of the vital organs in rat through the administration of the extractsof of Cymbopogan nardus and Psidium guajavawhich are known to have medical properties and are often used inhouseholds. The results reveal the presence of alkaloids, flavonoids, saponins, tannins, steroidal compounds, phenolic compounds and terpenoids. The body weight of the treated rats showed increase indicating recovery of the degenerative changes in the vital organs which was also evident in the histopathological observations.

Key words: *phytochemicals, histopathology, body weight,of Cymbopogan nardus,Psidium guajava*

INTRODUCTION

The condition of Diabetes is related to high blood sugarlevel, detection of sugar in urine andaltered nitrogen equilibrium and it is mainly due to decrease of insulin secreted by the beta cells of pancreas and desensitization of insulin receptors for insulin. Occurance of diabetes is quite common these days (Vats 2000). It causes number of complications like retinopathy, neuropathy, and peripheral vascular insufficiencies (Chehade 2000) and is characterized by the elevated blood glucose level and disturbed carbohydrate, fat and protein metabolism.Type 1 diabetes, which is insulin dependent (IDDM) usually begins in the childhood, caused by autoimmune destruction of β cells which produce insulin. Destructions of the β cells results in a complete loss of insulin production. Type 2 diabetes, known as non- insulin dependent diabetes mellitus (NIDDM) usually occurs after 40 years of age. This type is frequently associated with insulin resistance and normal or elevated level of insulin. Some times during pregnancy the gastro intestinal diabetes also occurs and usually disappear after the child is delivered. However even though diabetes during pregnancy may be short- lived it can compromise the health of both mother and fetus.

Diabetes is associated with a number of significant medical problems. Severe hyperglycemia may result in coma, disturbances of liver and kidney or even death. Mild hyperglycemia if present for many years may increase the risk of cardiovascular disease, which can manifest as heart attack, heart failure stroke and gangrene. Visual loss due to retinopathy, cataract is also common. About 6% of all diabetic patients develop glaucoma, which is the main reason for blindness. In addition to neuropathy, poor wound healing and impotency are the common complications of diabetes in man.

It is now accepted that maintaining blood glucose level near the normal range may reduce the complications associated with diabetes. Conventional therapy to maintain the normal level of glucose includes combination of diet, exercise and sugar lowering medicines. This approach is helpful to some extent, but it has limitations because first the conventional dietary approaches fail to emphasis high fiber carbohydrate food or specific food which may increase the blood glucose level. Secondly insulin therapy may not achieve glycemic control in insulin resistance person. When these drugs are effective, they must be used with proper dose because fatal hypoglycemia limits its

usage (Grover et, al 2002). Furthermore, insulin cannot prevent the development of disease so safer methods of lowering blood glucose level are needed.

Plants are known to have a variety of important active molecules which have various properties and structures. They have a remarkable role in the traditional medicine in different countries. The protective effect of plant products is due to the presence of several components which have distinct mechanisms of actions; some of them are enzymes and proteins and other are low molecular weight compounds such as vitamins, carotenoids, flavenoids (zhang and Wan 2002), Anthocynins and other phenolic compounds (SanchezMoreno etal ;1998)

Compounds of plant origin are generally regarded as safe. Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important.. Hence, treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels. Mode of action in treatment is obscure, most of the plants have been found to contain glycosides, alkaloids, terpenoids, flavonoids etc. (Zhang and Wang, 2002) that are suggested to have antidiabetic potential.

The plant extracts of *Cymbopogan nardus* and *Psidium guajava* to be used would be screened for their histoprotective activity. This may aid in opening new avenue in the development of remedial strategies to lower diabetic complications.

SIGNIFICANCE OF THE STUDY

Medicinal plants have been used in almost all traditions as a source of medicine. It has been estimated that about 85% of population in developing and developed countries use traditional medicine for primary health care needs and it is assumed that a major part of traditional therapy involve the use of plant products or their active principles.(Ignacimuthu, et al;2006; Elujoba, et al; 2005;Tomlinson and Akerle,1998) .Different species of medicinal plants are used in the treatment of diabetes mellitus (Ribnicky, et al;2006).

It is imperative to work on some alternative strategy to keep the blood glucose level within the normal range and thereby avoid the further complications. In this regards plant products may prove to be potential candidates.

MATERIALS AND METHODS

Collection of the plants:

The leaves of *C.nardus* and *P. guajava* were collected from Pune region during August and September and were authenticated from the Department of Botany, Prof.Ramkrishna More College, Akurdi. Theese leaves were washed with water, air dried at room temperature for 10 days in the absence of sunlight and then powdered. 250 grams of the powder were then packed in soxlet apparatus and extracted in methanol for 12 hours. The extract was then subjected to rota-evaporator and the powders obtained were kept in brown glass bottles (tightly capped) at 4^{o}C until further use.

Phytochemical screening:

Test for alkaloids:

10 g of the air-dried powder of the test plants were extracted with about 50 ml dil. hydrochloric acid. The filtrate was then subjected with ammonium hydroxide to make it alkaline and extracted with about 20 ml chloroform in three successive portions The chloroform extracts were evaporated till dryness and the residues were dissolved in 2 ml of dilute hydrochloric acid and tested with Dragendorff's reagent (Fulton,1932).

Dragendorff's reagent:

When added to the residue solution an orange precipitate was formed, this indicates the presence of alkaloids. The semi-quantitative results of the method have been rated with number of '+' signs for less precipitate to heavy precipitate.

Test for flavonoids:

One ml of 10% ethanolic extract of the test plants were mixed with 0.5 ml of hydrochloric acid (10%) and magnesium metal. A reddish colour was developed indicating the presence of

flavonoids (Yisa 2009). The result of this method has been rated with number of '+' signs for less colour to intense colour.

Test for saponins:
One gram of the plants under investigation were boiled with 10 ml water for few minutes and filtrated. The filtrate was vigorously shaken. The persistent froth (1 cm height) was observed for 1 hr. indicates the presence of saponins (Wall, *et al.,* 1954).

Test for tannins:
About 2 g of the air-dried powder of the plants were extracted with ethanol (50 %) and tested for the presence of tannins using the following test (Trease and Evans, 1989).
5 ml of the alcoholic extract of the studied plant were mixed with 2 ml vanillin hydrochloric acid solution; formation of precipitate indicates the presence of gallic acid.

Test for unsaturated sterols and or/ triterpenes:
For testing the presence of unsaturated sterols and triterpenes, 1g of the air-dried powder of the test plants were extracted with few ml of ethanol then filtrated and the filtrate was evaporated till dryness. The residue was dissolved in 10 ml chloroform, filtered and the filtrate was divided into two equal portions for proceeding the following tests (Wall, *et al.,* 1954) .
(a) Libermann-Burchard test:
To the first portion of chloroform filtrate 1 ml of acetic acid anhydride was added, followed by 2 ml of sulphuric acid down the wall of the test tube. The appearance of a reddish violet colour at the junction of the two layers and a bluish green colour in the acetic acid layer indicates the presence of unsaturated sterols and or/triterpenes.
(b) Salkowiski's test:
Sulphuric acid was added in 1:1 proportion with the chloroform filtrate. The red colour indicates unsaturated sterol and /or triterpenes. The intensity of the colour formed for the above tests were rated by a '+' mark to roughly quantitate the compounds.

Ferric chloride test:
Fifty grams of extract is dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenol compounds.

Experimental Animals:
Adult male Wistar albino rats weighing about 150 to 200 g. were obtained from the animal house of Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune. These rats were used for further study. All animals were cared and the experiments were carried out according to the guidelines recommended by the Committee for the purpose of Control and supervision of Experiments on Animals (CPCSEA), Government of India. The clearance from the Institutional Animal Ethics Committee (IAEC) was also obtained.

Induction of Diabetes:
For induction of diabetes a single dose of Alloxan (150mg/kg body wt.) was administered intraperitoneally in 18 hrs. previously fasted rats. After one hour of administration animals were allowed food and water *ad libitum.* After 7 days the blood was collected from orbital sinus and used for further biochemical assays.

Experimental Design:
In this work total 36 rats were used. The rats were divided into 6 groups of 6 animals each as detailed below:

Group I--		Control	[6 animals]
Group II	--	Diabetic control	[6 animals]
Group III	--	Test Drug (*cymbopogan nardus*) Low dose*	[6 animals]

GroupIII -- Test Drug (*cymbopogan nardus*) High dose# [6 animals]
GroupIV -- Test Drug (*Psidium guajava L*) Low dose [6 animals]
GroupIV -- Test Drug (*Psidium guajava L*) High dose [6 animals]
(250 mg/kg body weight# 600 mg/kg body weight)*

The body weight was noted before starting the experiment and at the completion.

Drug Administration and Histopathological study:
The test drugs were administered orally as per the standard method, at the end of the study the animals from each group was sacrificed, liver, kidney and pancreas were excised washed with cold saline and fixed in 10% formalin for the histopathological examination using H and E staining. Liver tissue was also stored in 0.9%saline to be used for biochemical studies.
Histopathological study was carried out by routine standard method using 1010% neutral buffered formalin as fixative. Paraffin wax was used for embedding and sectioning (5µ thickness) was done. The sections were stained with Haematoxlin–Eosin as a double staining method. The slides were observed under Research microscope (Olympus CH20i-Tr) with microphotography attachment. The magnification was 400X.

STATISTICAL ANALYSIS

The data were expressed as mean SD (±). The results obtained were analyse using Student's t-test. Values were considered statistically significant at $P<0.05$ and $P<0.01$.

RESULTS AND DISCUSSION

The results obtained are tabulate below:

Table 1: C hanges in body weight of control and experimental animals

	Initial Weight (Gm)	Final Weight (Gm)
Control Group I	188.40 ± 1.26	194.50 ± 2.11**
Diabetic control Group II	176.20 ± 2.25	164.30 ± 1.29*
C.nardus low dose Group III	179.40 ± 3.12	182.80 ± 3.43*
C.nardus high dose Group IV	181.50 ± 1.96	187.30 ± 2.78**
P. guajava low dose group V	180.60 ± 3.02	186.40 ± 1.56*
P. guajava high dose group VI	184.20 ± 3.61	192.50 ± 4.59 **

* $P<0.01$, **$P<0.05$ compared with initial weights.

Table 2: Phytochemical analysis of selected plants

	Alkaloids	Flavanoids	Saponins	Tanins	Steroidal Compounds	Phenolic Compounds	Terpenoids
C.nardus	++	++	++	+	+	++	+
P. guajava	+	++	++	++	++	++	++

Diabetes mellitus is a common and major endocrine disorder that affects more than 200 million people worldwide. The number is expected to grow to 400 million by 2030, corresponding to 7.8% of the adult population. In addition to the primary effects of diabetes, this disease is accompanied by increased risk factors such as hyperglycemia, hypertension, dyslipidemia, decreased fibrinolytic activity, severe atherosclerosis and increased platelet aggregation (Williams and Pickup, 2004; Rajalakshmi, *et al.,* 2009).
Remarkable development has been made in the treatment of diabetes mellitus by synthetic drugs. Though effective it certainly has some side effects such as hypoglycemia at higher dose administration, low oral bioavailability due to degradation in stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, and poor permeability across the intestinal epithelium, make it necessary to find other alternatives (Mohini, *et al.,* 2012; Mukherjee, *et al.,* 2006). Therefore, it is imperative to discover natural treatments without side effects that can reduce these risk factors in diabetic patients.

Changes in body weight of the control and experimental animals are given in table 1 which reveals that the body weight increased normally in the control group and the treated groups also showed significant increase in weight during the experiment which may be due to the administration of the test plant extracts. The untreated group showed decrease in the body weight.

Table 2 dipicts the preliminary phytochemical analysis of the selected plants. The results confirm the presence of Alkaloids, Flavanoids, Saponins, Tanins, Steroidal Compounds, Phenolic Compounds and Terpenoids. These compounds have shown to have some antidiabetic action.

Many investigators reported that *C.nardus* and *P. guajava* possesses a variety of medicinal properties such as hypoglycaemic and hypolipidaemic activities, but there are a very few records on the antioxidant activity of *P. guajava* and antidiabetc activity of *C.nardus.*

Histopathological study of group II ratsshow degeneration of islet cells ofpancreatic, which was due to alloxan used during the experiment. The untreated diabetic rats, showed severe necrotic changes of the pancreatic islets, particularly the cells of the islets. Degenerative and classical nuclear changes occurring during necrosis viz. pyknosis, karorrhexis, karyolysis, residues of the destroyed cells were visible under microscope. Relative reduction in the size and number of the islets together with severe reduction in the β- cells were demonstrated in these animals. The cellular integrity and architecture of pancreases were intact in the control group. However, signs of regeneration of β-cells have been reported following consumption of some plant extracts (Shanmugasundaram, *et al.,* 1990; Ayber, *et al.,* 2001; Suba, *et al.,* 2004; Yadev *et al.,* 2008). Pancreas of the diabetic treated rats with *C.nardus* and *P. guajava* showed a positive difference with respect to the general architecture of the islets of Langerhans and section of pancreas in general. This finding reveals the hypoglycemic effects of *C.nardus* and *P. guajava* whichmight be due to the action of substances like flavonoids or due to an increase in the insulin response (Eddouks, *et al.,* 2004; Jelodar, *et al.,* 2005).

The kidneys of the normal control rats showed normal architecture and the proximal and distal convoluted tubules, renal corpuscles, glomerulus and glomerular capsule. Microscopic examination of the kidneys of the treated and untreated diabetic rats showed mild tubular necrosis with moderate degenerative and necrotic changes in the glomerular epithelium and diffused interstitial and glomerular hemorrhages in the untreated rats while significant recovery of the general structure was seen in the rats treated with the extracts of *C.nardus* and *P. guajava*.

The histopathological sections of the liver of the untreated diabetic rats showed degenerative changes in the hepatocytes represented by disorganization of the hepatic cords, congestion of the central veins with mild hepatocellular necrosis and the sinusoids were infiltrated by mild nonspecific inflammatory cells. The hepatocytes of the untreated rats showed morphological change such as pyknosis, karyorrehexis, chromatolysis and cytoplasmic vacuolization. However, the liver of the treated diabetic rats with *C.nardus* and *P. guajava* extracts revealed slight improvement in the structure of the hepatic tissue compared to those of the untreated diabetic ones, except for a few mildly degenerated hepatocytes around the central vein of the *P. guajava* treated rats which still had some cytoplasmic vacuoles, other hepatocytes and portal and sinusoidal areas were almost normal. There was little evidence of regeneration in the liver of diabetic rats that received the *C. nardus* extract. The liver of the normal control rats had normal structure.

CONCLUSION

In conclusion, the present study demonstrated that repeated oral administration of *C.nardus* and *P. guajava*extracts for 28 days evokes a beneficial effect on the histoprotection in the diabetic rats. However, the *P. guajava* extract was found to be more effective than *C.nardus* extract, the pancreas of the treated rats also showed an improvement in their histological architecture. These results support the traditional usage of the *C.nardus* and *P. guajava*extracts in treatment of diabetes mellitus.

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