

COMPARISON OF THE EFFECT OF *PTEROCARPUS MARSUPIUM* WITH PIOGLITAZONE IN DEXAMETHASONE-INDUCED INSULIN RESISTANCEKOYAGURA NARENDAR^{1*}, I M NAGENDRA NAYAK², M G JAMADAR¹, ASHOK M PATIL³, SANJIT ANAND²¹Department of Pharmacology, Al Ameen Medical College, Vijayapur, Karnataka, India. ²Department of Pharmacology, K.S. Hegde Medical Academy, NITTE University, Mangalore - 575 018, Karnataka, India. ³Department of Pathology, Al Ameen Medical College, Vijayapur, Karnataka, India. Email: narendar.kumar23@gmail.com

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ABSTRACT

Objective: This study was undertaken to evaluate the preventive effect of heartwood of *P. marsupium* in dexamethasone-induced hyperinsulinemia and hyperglycemia and compare it with that of pioglitazone.

Methods: Male albino wistar rats were divided into five groups (n=6). Plain control group received gum acacia (2%) orally from d 1 to d 12. Dexa control group received gum acacia (2%) orally for d 1 to d 12 and Dexa (8 mg/kg) intraperitoneal (i.p.) from d 7 to d 12, during the study period. Two test groups received ethanolic extract of *Pterocarpus marsupium* heartwood (PME) (1 and 2 g/kg/) per oral (PO), and standard control group received pioglitazone (60 mg/kg/PO) from d 1 to d 12. During the 12-d study period, the two test groups and standard control group received Dexa (8 mg/kg/i.p.) from d 7 to d 12. On last day of the study, the blood samples were collected by retro-orbital sinus puncture and used for estimation of serum insulin and glucose levels. Homeostatic Model Assessment (HOMA) method was employed to calculate the degree of insulin resistance (IR). Results were analyzed by using one-way analysis of variance followed by Scheffe's multiple comparison test (p<0.05).

Results: Treatment with ethanolic extract of *P. marsupium* and pioglitazone significantly (p<0.05) reduced the elevated insulin and glucose levels as well as HOMA-IR and HOMA-IS values in dexa treated animals.

Conclusion: Ethanolic extract of *P. marsupium* heartwood effectively countered dexamethasone-induced hyperinsulinemia and hyperglycemia. Insulin-sensitizing activity of *P. marsupium* heartwood was found to be more effective than pioglitazone.

Keywords: *Pterocarpus marsupium*, Insulin resistance, Hyperinsulinemia, Hyperglycemia.

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INTRODUCTION

Diabetes mellitus is a global disease estimated to affect around 438 million people by 2030 [1]. Insulin resistance (IR) plays a major role in the pathogenesis of diabetes, obesity, and cardiovascular diseases. It develops many of the consequences associated with metabolic syndrome [2]. Psychological stress and sedentariness occurred due to changes in modern life style which led to a pandemic of metabolic syndrome. The psychological stress and sedentariness occurred due to changes in modern life style which led to a pandemic of metabolic syndrome. Glucocorticoids (GCs) may play a major role in conditions of stress [3]. Excess GCs may trigger the development of IR and elevation of blood glucose levels [4]. Adaptive response from the β -cells of the pancreas leading to hyperinsulinemia prevents hyperglycemia initially. However, further increase in IR causes relative insulin deficiency and hyperglycemia. GCs promote lipolysis in peripheral tissues which, in turn, increases free fatty acids and glycerol. The excess of fatty acids and glycerol accumulates in the liver and peripheral tissues as fat, involved in pathological abnormalities [5,6].

The homeostatic model assessment (HOMA), developed by Matthews *et al.*, has been commonly employed to quantify the IR in research. This method is most convenient, and easy technique compared to the euglycemic clamp method [7].

Many medicinal herbs have been proved to be efficacious with fewer side effects in the diabetes treatment. Although synthetic drugs are effective, adverse effects are minimizing the use of these drugs in the treatment of diabetes mellitus [8].

Pterocarpus marsupium is a deciduous tree, commonly known as Bijasal or Indian Kino tree, belongs to the family *Fabaceae*. It possesses

different properties such as antioxidant, analgesic, hepatoprotective, antifungal activity, and anti-inflammatory activity [9]. The heartwood of this tree is a medicinally important part having various flavonoids which may be responsible for the anti diabetic effect of this plant [10]. However, insulin-sensitizing activity of *P. marsupium* is not yet studied in steroid-induced IR *in vivo*. This study will be useful to identify the more potent drug to counteract the Dexa induced diabetic complications in clinical therapies. Therefore, the present study was aimed to explore the insulin-sensitizing effect of *P. marsupium* and compare with that of pioglitazone in dexamethasone-induced IR in Wistar rats.

METHODS

Animals

The study was done using male albino Wistar rats weighing around 250-300 g. Permission was obtained from the Institutional Animal Ethics Committee (Reference: KHEMA/IAEC/02/2013) before the study. As per the regulations of Committee for the Purpose of Control and Supervision on experimental animals, animals were accommodated under standard conditions, at temperature (23±2)°C, humidity 50 ± 5%, 12:12 hrs light-dark cycles. They were kept in polypropylene cages, rat pellets (Hindustan Lever Limited, Mumbai) and water were given *ad-libitum*.

Drugs and chemicals

A purified form of pioglitazone was obtained from Mahalakshmi Chemicals, Hyderabad. Dexamethasone injection was procured from Zydus Pharmaceuticals, Mumbai. Ketamine injection was collected from Neon Laboratories Limited, Mumbai, India. The serum glucose levels were estimated by commercially available kits. Measurement of serum insulin levels was done using ultrasensitive rat insulin enzyme-linked immunosorbent assay (ELISA) kit obtained from Gen X Bio Health Sciences Private Limited, New Delhi.

Collection and identification of plant material

Heartwood part of *P. marsupium* was obtained from S.D.M Ayurveda College, Udupi, Karnataka. The material has been identified and authenticated by Dr. Sunil Kumar KN, Senior Research Officer, Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi.

Preparation of the ethanolic extract of *P. marsupium* heartwood

The heartwood material was weighed accurately (1.8 kg) in a round bottom flask. About 16 L of absolute ethanol was added to it and allowed to stand for 24 hrs. The contents were filtered, and the extract was concentrated by distillation and solvent was removed by evaporation on a water bath. It was completely dried under vacuum. Finally, around 94 g of dried extract was obtained.

Phytochemical analysis

Ethanolic extract of *P. marsupium* was subjected to preliminary phytochemical analysis for identification of chemical constituents such as alkaloids, steroids, carbohydrates, tannins, flavonoids, resins, saponins, triterpenoids, and phenols [11].

Experimental design

Male albino wistar rats were divided into 5 groups of 6 animals in each group. Study was conducted for a period of 12 d. In this study, Group 1 served as plain control which received gum acacia (2% per oral[PO]/12d) and Group 2 as Dexamethasone control which received vehicle for 12 d and Dexamethasone (8 mg/kg/intra-peritoneal[i.p.]) from 7th d to 12th d during the study period. Rats in group 3 and 4 were treated with graded doses of ethanolic extract of *P. marsupium* heartwood (PME) 1 and 2 g/kg/PO for 12d respectively. Rats in group 5 were treated with a standard drug, pioglitazone 60mg/kg/p.o for 12d (PIO60mg/kg). All the groups except the control group received dexamethasone (8mg/kg/i.p.) from 7th d to 12th d (Table 1). On the last day of the study period, fasting blood was collected by retro-orbital sinus puncture. Blood samples were centrifuged at 2000RPM for 20 minutes, and serum was separated and utilized for the estimation of glucose and insulin levels.

Estimation of biochemical parameters

Serum glucose levels (mg/dl) were measured by glucose oxidase and peroxidase method [12]. Serum insulin levels (μ U/ml) were estimated by ELISA method [13].

HOMA

The HOMA-IR and HOMA-insulin sensitivity (IS) were used to determine the degree of hepatic IR and IS, respectively [14].

$$\text{HOMA-IR} = (\text{Fasting glucose [mg/dl]} \times \text{fasting insulin } [\mu\text{U/ml}]) / 405$$

$$\text{HOMA-IS} = 10,000 / (\text{fasting insulin } [\mu\text{U/ml}] \times \text{fasting glucose [mg/dl]})$$

Statistical analysis

The data were presented in mean \pm standard error of the mean. Results were analyzed by one-way analysis of variance followed by Scheffe's multiple comparison *post hoc* test using SPSS software. Level of significance was assumed at 5%, and $p < 0.05$ was considered as statistically significant.

RESULTS

Phytochemical analysis

The phytochemical investigations of PME revealed the presence of alkaloids, flavonoids, resins, steroids, phenols, and triterpenoids (Table 2).

Fasting serum insulin and glucose levels

Dexamethasone administration to Wistar rats showed significant ($p < 0.05$) increase in serum insulin and glucose levels compared to control group. Treatment with PME (1 and 2 g/kg) and pioglitazone 60 mg/kg significantly ($p < 0.05$) reduced serum insulin and glucose levels compared to dexamethasone control group. There was a significant ($p < 0.05$)

Table 1: Grouping of animals

Groups	Treatments
Group 1-Plain control	Gum acacia (2%) oral (vehicle)
Group 2-Dexa control	Vehicle+dexamethasone 8 mg/kg/i.p.
Group 3-PME 1 g/kg	Ethanolic extract of <i>P. marsupium</i> 1 g/kg/oral+dexa 8 mg/kg/i.p.
Group 4-PME 2 g/kg	Ethanolic extract of <i>P. marsupium</i> 2 g/kg/oral+dexa 8 mg/kg/i.p.
Group 5-PIO 60 mg/kg	Pioglitazone 60 mg/kg/oral+dexa 8 mg/kg/i.p.

P. marsupium: *Pterocarpus marsupium*

Table 2: Phytochemical constituents in PME extract

Test	Present (+)/absent (-)
Alkaloids	+
Carbohydrates	-
Flavonoids	+
Resins	+
Phenols	+
Triterpenoids	+
Steroids	+
Coumarins	-
Carboxylic acids	-
Quinones	-
Saponins	-
Tannins	-
Amino acids	-

PME: Ethanolic extract of *Pterocarpus marsupium*

difference observed between PME1g/kg and PIO60mg/kg treated groups in terms of reduction in insulin and glucose levels. However, there was no significant ($p > 0.05$) difference between PIO60mg/kg and PIO 60 mg/kg groups (Table 3 and Fig. 1).

HOMA-IR and HOMA-IS

HOMA of IR and IS was used to estimate IR and IS, respectively. Dexamethasone control group showed a significant rise in HOMA-IR and decreased in HOMA-IS compared to plain control group ($p < 0.05$). Treatment with two doses of PME (1 and 2 g/kg) and PIO60 mg/kg had shown significant fall in IR and rise in IS compared over dexamethasone control group ($p < 0.05$). There was a significant difference between the mean values of HOMA-IR and IS ($p < 0.05$) in PME1g/kg and PIO60mg/kg treated groups. However, an insignificant difference was seen between PME1g/kg and PIO60mg/kg treated groups in HOMA-IR ($p > 0.05$). However, the difference in mean values of HOMA-IS was significant in between these two groups ($p < 0.05$) (Table 3).

DISCUSSION

Insulin is an anabolic hormone which plays a major role in the uptake of glucose in peripheral tissues [15]. But, GCs impair insulin signaling in peripheral tissues which lead to IR [16]. Dexamethasone is a potent synthetic steroid selected for the present study to induce IR [17]. Our study indicates that intraperitoneal administration of dexamethasone for six d significantly induced IR. It was proved by the rise in serum glucose and insulin levels as well as increase in HOMA-IR and decrease in HOMA-IS values of dexamethasone treated rats. Dexamethasone by impairing insulin signaling, it reduces insulin sensitivity and causes hyperinsulinemia and hyperglycemia [18,19]. The underlying mechanism of dexamethasone to induce IR might be due to inhibition of glucose uptake by antagonizing translocation of glucose transporters to the plasma membrane [20-22]. It enhances gluconeogenesis and also causes down regulation of insulin receptors [23].

The synthetic groups of drugs such as biguanides, thiazolidinediones, and glucagon-like peptide-1 (GLP 1) mimetics have proved their benefit in GC-induced IR syndrome [24-26]. However, certain adverse effects

Table 3: Antidiabetic activity of PME extract

Parameter	Plain control	Dexa 8 mg/kg	PME 1 g/kg	PME 2 g/kg	PIO 60 mg/kg
Fasting glucose (mg/dl)	101.18±0.97	268.23±2.13 ^{*a,c,d,e}	158.23±5.41 ^{*b,e}	125.19±1.95 ^{*b}	135.21±1.91 ^{*b,c}
Fasting insulin (µU/ml)	67.48±2.28	438.4±8.74 ^{*a,c,d,e}	214.16±9.53 ^{*b,e}	129.92±7.81 ^{*b}	150.24±7.88 ^{*b,c}
HOMA-IR	16.88±0.72	290.38±6.53 ^{*a,c,d,e}	83.73±4.81 ^{*b,e}	40.06±2.18 ^{*b}	50.10±2.47 ^{*b,c}
HOMA-IS	1.47±0.06	0.09±0.001 ^{*a,c,d,e}	0.30±0.01 ^{*b,e}	0.63±0.36 ^{*b,e}	0.49±0.03 ^{*b,c,d}

*Significant at 5% level (p<0.05), ^aPlain control, ^bDexa control, ^cPME1g/kg, ^dPME2g/kg, ^ePIO60mg/kg: Values are expressed as mean±SEM for six rats in each group (n=6). SEM: Standard error of mean, HOMA: Homeostatic model assessment, IR: Insulin resistance, IS: Insulin sensitivity, PME: Ethanolic extract of *Pterocarpus marsupium*, PIO: Pioglitazone

limit their use in the treatment of IR. Metformin though effective causes gastrointestinal adverse effects and lactic acidosis [27]. Glitazones as a group are hepatotoxic, cardiotoxic, and also there is an increased incidence of bladder cancer [28,29]. GLP-1 mimetics can cause thyroid carcinoma [30]. These factors necessitate the need for new compounds with better safety profile.

P. marsupium has been used in Ayurvedic medicine for the treatment of diabetes mellitus. It has a high amount of polyphenolic compounds [31]. In the present study, phytochemical analysis of heartwood of PME revealed the presence of phenols, flavonoids, terpenoids, alkaloids, resins, and steroids (Table 2). Several studies reported that flavonoids, terpenoids, and phenols are considered as effective antidiabetic principles and produce synergistic activity in diabetes [32,33].

Early detection and prevention of IR may reduce the risk of the development of diabetes and other disturbances of metabolic syndrome [34]. Our study confirmed that the treatment with PME extract led to effective prevention of IR induced by dexamethasone. It was reflected by a significant reduction in serum insulin and glucose levels in rats treated with PME extract compared to PIO60mg/kg treated group (Table 3). The possible insulin-sensitizing activity of heartwood of PME extract might be due to the stimulation of glucose uptake into the skeletal muscles as described by Mishra *et al.* [35]. Phenolic compounds of *P. marsupium* heartwood like marsupin and pterostilbene may play a major role in improving glycemic control, and in turn, IR induced by dexamethasone [36]. It was also reported that the flavonoid constituents of *P. marsupium* heartwood could upregulate the peroxisome proliferator's activator receptor-γ gene expression, and in turn, the glucose metabolism by decreasing cytokine tumor necrosis factor-α levels [37].

The HOMA index was used to measure the IR and IS in all study groups. This calculation is one of the useful tools to measure the degree of insulin resistance [38]. This calculation is one of the useful tools to measure the degree of insulin resistance [38]. In the present study, dexamethasone-treated animals revealed a state of IR and decreased IS. This was indicated by the increase in HOMA-IR and decrease in HOMA-IS values compared to control group (Table 3). The elevated values of HOMA-IR with dexamethasone were effectively prevented by both doses of PME extract (1, 2 g/kg) and PIO60 mg/kg treatment. However, treatment with PME 2 g/kg exhibited superiority over PIO60 mg/kg in the improvement of HOMA-IS, which suggests that PME2 g/kg is most effective in the treatment of IR compared to PIO60mg/kg (Table 3). These effects were in correlation with the insulin and glucose levels. Overall, the insulin-sensitizing activity of PME extract is thought to be synergistic effects of various phytochemical compounds present in the drug.

CONCLUSION

It is concluded that the heartwood of *P. marsupium* has more effective insulin-sensitizing property compared with that of pioglitazone. Further studies are required to identify the active chemical compounds, which are responsible for the insulin-sensitizing property.

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