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ANTIDIABETIC EFFECT OF *MALLOTUS PHILIPPINENSIS* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

In the present work the antidiabetic activity of the *Mallotus philippinensis* was evaluated. The hydroethanolic bark extract showed significant increase in the levels of body weight, insulin and significant decrease in blood glucose, and glycosylated haemoglobin when administered orally for 30 days to STZ induced diabetic rats at a dose of 200 and 400mg/kg body weight. Phytochemical studies also revealed the presence of phenolics in the bark extract which may be responsible to exert antidiabetic activity. Hence it can be used as a drug for diabetes mellitus.

KEYWORDS:Antidiabetic,*Mallotusphilippinensis*(MP),Hydroethanolicextract(HEE),treptozotocin (STZ), Insulin, Glycosylated haemoglobin

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INTRODUCTION

Diabetes mellitus is the most common metabolic disorder. It is characterized by hyperglycaemia that results from an absolute or relative insulin deficiency and is associated with long term complications affecting the eyes, kidneys, hearts and nerves[1]. Diabetes represents a spectrum of metabolic disorders, which has become a major health challenge worldwide[2]. The unprecedented economic development and rapid urbanization in Asian countries, particularly in India had lead to a shift in the health problems from communicable to non – communicable diseases. Of all the non – communicable diseases, diabetes and cardiovascular diseases lead the list. Diabetes mellitus is characterized by abnormalities in the metabolism of carbohydrate, protein and fat, primarily due to deficiency in the synthesis, secretion or function of insulin. The disease is associated with microvascular, macrovascular and metabolic complications such as diabetic retinopathy, neuropathy, nephropathy etc., Numerous drugs are available to treat Diabetes mellitus but providing drugs is expensive and the side effects need careful monitoring of it[3]. Hence plant based medicines play an important role in health care programmes worldwide with minimal side effects. World Health Organization (WHO) also approves the use of plant drugs for different diseases, including diabetes mellitus. *Mallotus philippinensis muell Arg* is a tree grows throughout tropical India and particularly along the foot of Himalaya up to a height of 100m[4]. During the month of February to March its fruits ripen, becoming red in colour. The glands and hairs of the fruits are used to remove intestinal worms and also as a purgative. Reports indicate that new phloroglucionl derivatives from *Mallotus phillippinensis* exhibit anti – allergic property[5]. *Mallotus philippinensis,* a plant with very high medicinal value has been in use since 1000 BC. It is stated to be effective in non –healing or infectious wounds, dermal problems etc., Medicinally, the bark decoction is used for typhoid and meningitis[6] stomach disorders such as diarrhoea, dysentery[7] and stomachic effect[8]. Since no reports are available on antidiabetic activity, the present study is focused to evaluate the antidiabetic activity of the bark of *Mallotus philippinensis*.

MATERIALS AND METHODS

PROCUREMENT OF ANIMAL

Laboratory bred Wistar strain male albino rats weighing approximately (140 ± 20 grams) were procured from PSG laboratory Institute of Medical Sciences, and Research, Coimbatore, Tamil Nadu, India. The study protocol was approved by Institutional Animal ethics committee (IAEX) constituted for the purpose and care of laboratory animals as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India (CPCSEA No : 158/1999/ CPCSEA). The animals were housed in polypropylene cages inside a well ventilated room. They were maintained under standard laboratory conditions. They were acclamatized to animal house conditions with controlled temperature and humidity and they are fed with standard rat feed supplied by Hindustan Lever ltd., Bangalore, Karnataka, India and filtered water *ad libitum*.

INDUCTION OF DIABETES

Diabetes mellitus was induced by a single intraperitoneal injection of Streptozotocin (STZ) which is dissolved in ice cold 0.01M citrate buffer, pH 4.5 at a concentration of 50 mg/kg to overnight fasting rats. The doses were determined according to the body weight of animals. After 72 hours of injection, the blood samples were collected from rats through retino-orbital plexus and the serum was separated by centrifugation at 2500 rpm for 15 minutes. The serum was then used for the determination of blood glucose level.

PREPARATION OF PLANT EXTRACT

The shade dried bark was grounded and subjected to successive solvent extraction in different solvents. It was then distilled,

evaporated and vacuum dried as per standard procedure.

a) Acute toxicity study

To study any possible toxic effects and/or changes in behavioural pattern, five rats were treated with a graded doses of plant extract (2,000 mg/kg body weight / rat / day was assigned as the upper limit dose) as assigned by the British toxicology society in 1984. Fixed dose procedure (FDP) rats were kept under close observation for 3 days.Dose identification was done when the substance produced toxicity but no mortality. When the first animal was dosed with the upper limit dose and survived, the second animal received the same dose. When a total of three animals had been dosed with the limit dose and no deaths had occurred, the test was terminated. The larger therapeutic index of the plant extract can be obtained when there is no death of rats even at a maximum dose of 2000 mg/kg body weight. Tests were repeated until it showed no lethality. The starting doses of 200 and 400 mg/kg body weight of plant extract were recommended as per OECD guideline[9].

b) EXPERIMENTAL DESIGN

The animals were divided into five groups each comprising a minimum of six animals as detailed below.

Group I-Normal control rats received standard pellet diet and distilled water for 30 days.

Group II- The rats were made hyperglycemic by an intraperitoneal injection of Streptozotocin at a concentration of 50 mg/kg body weight to overnight fasting rats.

Group III - Diabetic rats treated with hydroethanolic bark extract of *Mallotus philippinensis* at a concentration of 200 mg/kg body weight orally for 30 days.

Group IV- Diabetic rats treated with hydroethanolic bark extract of *Mallotus philippinensis* at a concentration of 400 mg/kg body weight orally for 30 days.

Group V- Diabetic rats treated with a standard drug Glibenclamide at a concentration 600 µg/kg body weight orally for 30 days.

COLLECTION OF SERUM

After the experimental regimen (30 days), the animals were fasted overnight and sacrificed by cervical dislocation under mild anaesthesia. Blood was collected through Cardiac puncture and serum was separated by centrifugation at 2500 rpm for 15 minutes. The serum collected was then used for biochemical estimations.

EXPERIMENTAL PROTOCOL

Blood glucose was estimated by GOD-POD method[10]. Concentrations of Glycosylated haemoglobin and insulin assay were determined by Saibene[11] and Clark and Hales[12] method respectively.

STATISTICAL EVALUATION

Data were reported as mean ± SD by using the Statistical Package of Social Sciences (SPSS, Version 10.0 for windows). The evaluation was done using one way analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when $p < 0.05$[13].

RESULTS

Effect of *Mallotus philippinensis* on body weight, blood glucose, insulin and glycosylated haemoglobin

A significant decrease and increase in body weight and blood glucose level respectively were seen in diabetic control rats (Group II) when compared to normal control rats (Group I). Administration of *Mallotus philippinensis* bark extract at 200 mg/kg and 400 mg/kg body weight significantly lowered the serum glucose level and significantly increased the body weight. Also treatment with Glibenclamide at a concentration of 600 µg/kg reverted the body weight and blood glucose level to almost normal (Table : 1).

Table 1
Effect of Mallotus philippinensis on Blood glucose and body weight of control and experimental treated rats

Groups	Blood Glucose mg/dl	Body weight (gms)
Group – I	124.67 ± 7.07	146.12 ± 6.92
Group – II	383.31 ± 18.00	120.57 ±7.29
Group – III	125.26 ± 6.24	152.18 ± 7.17
Group –IV	124.83 ± 5.48	154.91 ± 7.72
Group – V	127.45 ±6.76	160.85 ± 11.56

Values are expressed as mean ± SD (n=6) (p<0.05)
Group Comparison : G2 vs G1, G3,G4 and G5
Statistical Significance : * - P< 0.05, ns = not significant

It was evident from the Table 2 that levels of insulin significantly decreased in diabetic control rats (Group II) when compared with the normal control rats (Group I). There was a significant increase in the insulin level upon administration of *Mallotus philippinensis* and glibenclamide at a concentration of 200, 400 mg/kg and 600 µg/kg body weight respectively when compared with the diabetic control rats (Group II). From the Table 2 it was evident that the levels of glycosylated haemoglobin significantly increased in diabetic control (Group II) rats when compared to normal rats (Group I). Oral administration of MP extract at 200 and 400 mg/kg and glibenclamide (600 µg/kg) decreased the levels of glycosylated Haemoglobin in group III, IV, and V when compared with diabetic control rats (Group II).

Table 2
Effect of Mallotus philippinensis on Insulin and Glycosylated hemoglobin of control and experimental treated rats

Groups	Insulin (µmol/ml)	Glycosylatedhaemoglobin (mg%)
Group – I	10.18 ± 0.46*	4.62 ± 0.21*
Group – II	3.72 ± 0.21	8.31 ± 0.52
Group – III	9.97 ± 0.79*	4.76 ± 0.25*
Group – IV	10.11 ± 0.45*	4.67 ± 0.24*
Group – V	10.22 ± 0.32*	4.84 ± 0.21*

Values are expressed as mean ± SD (n=6) (p<0.05)
Group Comparison : G2 vs G1, G3,G4 and G5
Statistical Significance : * - P< 0.05, ns = not significant

DISCUSSIONS

Increase in blood glucose levels in diabetes leads to overproduction of free radicals, defined as an imbalance between oxidants and antioxidants. Glucose autooxidises in the presence of transition metal ions generating oxygen – free radicals that make the membrane vulnerable to oxidative damage[14]. Hypoglycaemic action of Hydroethanolic extract of *Mallotus philippinensis* in diabetic rats (Group III – IV) may be due to Insulin mimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production, or elevation of gluconeogenesis in liver and muscle [15]. In Diabetes mellitus, body cells are unable to utilize glucose as a source of energy due to which proteins are spared as an energy source. This leads to decrease in protein storage which in turn reduces body weight[16]. In the present study, Streptozotocin induced diabetic rats (Group II) show decrease in body weight throughout the experimental period. Diabetes mellitus is found to be associated with body weight reduction and dehydration. Degradation of fats leads to decrease in body weight and increased catabolism of fats leads to increase in bodyweight. Decreased Insulin level in

Diabetic control group depends on the degree of β– cell destruction which in turn increases the activity of fatty acyl coenzyme A oxidase and initiates β– oxidation[17]. Increased insulin secretion may be due to the insulin stimulation from remnant β- cells which enhance utilization of glucose by hepatic and extra hepatic tissues of diabetic rats[18]. Glibenclamide a standard antidiabetic drug used to compare the antihyperglycemic activity in experimental animals, exerts the action of insulin stimulation by inhibiting ATP sensitive potassium ATP channel in the plasma membrane[19]. In Diabetes mellitus, due to persistent hyperglycemia, the excess blood glucose reacts with haemoglobin in a nonenzymatic process to form glycosylated haemoglobin. The increased level of glycosylated haemoglobin in diabetic control rats indicates that the erythrocytes are more prone to oxidative stress in diabetes[20]. Prolonged high blood sugar level enhances glycosylated haemoglobin in Chronic Diabetes[21]. Emmanuel *et al.,* 2010 reported that *Cassia occidentalis Linn*. leaf extract decreased the glycosylated haemoglobin level to near normal levels[22].

CONCLUSIONS

The present study revealed that hydroethanolic bark extract of *Mallotus philippinensis* exerts antidiabetic effect by lowering blood glucose and glycosylated haemoglobin and increase in insulin level in Streptozotocin induced diabetic rats. The phytochemical constituents present in the plant may be responsible to exert such medicinal properties. Future study that involves the isolation and purification of the bioactive component may elucidate its mechanism of action and pharmacological effects.

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