

Beneficial Effect of Hydroethanolic Bark Extract of *Mallotus Philippinensis* On Serum Marker Enzymes in Streptozotocin Induced Diabetic Rats



Biochemistry

KEYWORDS : Hydroethanolic extract (HEE), *Mallotus philippinensis* (MP), Alanine Transaminase (ALT)

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ABSTRACT

*Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications. Enhanced oxidative stress and changes in antioxidants capacity observed in both clinical and experimental diabetes mellitus are thought to be the etiology of chronic diabetic complications. The present study was designed to evaluate the beneficial effect of hydroethanolic bark extract of *Mallotus philippinensis* on serum marker enzymes in streptozotocin induced diabetic rats. Results showed a significant decrease in the serum marker enzymes in the diabetic treated groups with plant extract at both the concentrations as well as in standard drug treated group when compared with a diabetic control rats (group II). This implies that the phytochemicals present in the bark extract might exert a potential which helps to suppress the activity of the enzymes as well as minimizes the membrane disruption in liver which otherwise increase the activities of enzymes in serum*

Introduction

Oxidative stress is produced during normal metabolic process in the body as well as due to various environmental factors and chemical substances. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes mellitus and its complications(1). The concentration of glucose in the blood is normally maintained within a narrow range by hormones that modulate the movement of glucose into and out of the circulation. These include insulin, which decreases blood glucose, and the counterregulatory hormones (glucagon, epinephrine, cortisol and growth hormone), which increase blood glucose concentration(2). 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. Enhanced catabolism of both liver and plasma proteins in diabetes mellitus may be due to increased amount of urea nitrogen production(3). As treatment of diabetes mellitus with insulin and other oral hypoglycaemic drugs produce undesirable side effects, people moved on to the plant based remedies. Natural compounds from medicinal plants have potential as therapeutic agents. Herbal drugs are playing an important role in health care programmes world wide and there is a resurgence of interest in herbal medicines for treatment of various ailments including hepatopathy. This highlights the need for medicinal plants.

Administration of several herbal medicines have restored the activities of certain serum enzymes when compared to normal control group as reported by Bopanna et al.,(4) and Eskander et al.,(5). Antioxidants which can mop up the free radicals are found to be present in many plants such as *Indigofera tinctoria* and *Curculigo orchiodes*. In the present study *Mallotus philippinensis* is used to evaluate the effect of serum marker enzymes in streptozotocin induced diabetic rats.

Mallotus philippinensis muell *Arg* is a tree that grows throughout tropical India and particularly along the foot of Himalaya up to a height of 100 meters. It belongs to the family euphorbiaceae.

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 phloroglucioni derivatives from *Mallotus philippinensis* exhibit anti – allergic property⁽⁶⁾.

Materials and methods

a) Procurement of animals:

Laboratory bred wistar strain male albino rats weighing approximately (140±20grms) were procured from PSG Institute of medical sciences, and research Coimbatore, Tamil Nadu, India. The study protocol was approved by Institutional Animal

ethics committee (IAEC) 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 fed with standard rat feed supplied by Hindustan lever Ltd., Bangalore, Karnataka, India and filled water ad libitum.

b) Experimental Design

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

Group –I: Normal control rats received standard pellet and sterilized water for 30 days.

Group – II: Streptozotocin was injected intraperitoneally to overnight fasting Rats.

Group – III: Streptozotocin induced diabetic rats treated with oral administration of hydroethanolic bark extract of *Mallotus philippinensis* at a concentration of 200 mg/kg body weight for 30 days.

Group – IV: Streptozotocin induced diabetic rats were treated with 400mg/kg body weight of hydroethanolic extract of *Mallotus philippinensis* orally for 30 days.

Group – V: Streptozotocin induced diabetic rats were treated with a standard drug glibenclamide at a concentration of 600mg/kg body weight orally for 30 days.

After the experimental regimen, 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.

c) Biochemical Estimations

Biochemical analysis in serum such as Alanine transaminase and aspartate transaminase was estimated according to the method of Reitman and Frankel(7). The activity of serum alkaline phosphatase was measured by the method of king and Armstrong(8) and serum acid phosphatase was determined according to the method of King(9) .

d) Statistical Analysis

Data were reported as means ± SD by using the Statistical Package of Social Sciences (SPSS, Version 10.0 for windows). Data from the replicates of each experiment conducted (n varying from 6 to 12) were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple

Range Test (DMRT). Values were considered statistically significant when $p < 0.05$ (10).

Results and Discussion

It is evident from Table 1 that there is a significant increase in the enzyme activities in diabetic control group when compared to normal control. Oral administration of HEE of MP bark showed a significant decrease in ALT and AST when compared to Group - I.

The increase in the activities of serum AST, ALT indicated that Diabetes mellitus may induce hepatic dysfunction. The enzymes directly associated with the conversion of amino acids to keto acids are AST and ALT, and are increased in the diabetic condition.

Table 1 : Effect of *Mallotus philippinensis* bark extract on serum alanine and aspartate transaminases of control and experimental rats

Groups	ALT (IU/L)	AST (IU/L)
Group - I (G1)	43.17 ± 2.76*	52.21 ± 3.25*
Group - II (G2)	81.32 ± 3.53	108.73 ± 5.83
Group - III (G3)	43.62 ± 2.35*	51.23 ± 3.73*
Group - IV (G4)	43.46 ± 1.55*	52.18 ± 3.31*
Group - V (G5)	43.73 ± 3.56*	52.36 ± 3.11*

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

Begum and Shanmugasundaram⁽¹¹⁾ also reported an increase in the activities of AST and ALT in the liver of diabetic animals. The increased protein catabolism accompanying gluconeogenesis and urea formation that are seen in the diabetic state might be responsible for the elevation of these tissue transaminases. Serum ALT, AST levels were determined to evaluate the hepatic functions. The increase in aminotransferase levels may be due to the cellular damage in the liver caused by Streptozotocin (STZ) induced diabetes.

b) Acid and alkaline phosphatases

Table 2 showed significant increase in acid and alkaline phosphatases activity in serum in diabetic control (Group - II) when compared to normal control (Group-I).

Treatment of diabetes induced rats with HEE of MP (200mg/kg and 400mg/kg) concentration showed a significant decrease in ALP and ACP levels when compared to diabetic control group. Glibenclamide treated group (Group - V) also showed a significant

decrease in the enzyme activities when compared to diabetic control (Group - II).

Table 2: Effect of *Mallotus philippinensis* bark extract on serum Acid and Alkaline phosphatases of control and experimental rats

Groups	ACP (IU/L)	ALP (IU/L)
Group - I (G1)	45.03 ± 2.88*	37.56 ± 1.75*
Group - II (G2)	88.17 ± 5.03	64.53 ± 3.71
Group - III (G3)	44.83 ± 2.39*	36.93 ± 2.13*
Group - IV (G4)	45.09 ± 2.39*	37.91 ± 2.31*
Group - V (G5)	46.18 ± 2.56*	38.15 ± 1.89*

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

Increased activities of phosphatases in diabetes may affect the transport of metabolites across the membrane due to alteration in dephosphorylation reactions. Enhanced level of phosphatases cause increased intracellular inorganic phosphate, which further affects the efficiency of ionic pumps which is reflected in the decreased activities of Na⁺ K⁺ ATPases in diabetes. Elevated activity of ALP was observed in STZ-diabetic rats. Prince et al.,⁽¹²⁾ have also reported increased ALP activity in experimentally induced diabetic rats. The increased activity of this enzyme in plasma may be a result of diabetes-induced damage to the tissues.

Alkaline phosphatase is a membrane bound glycoprotein enzyme. Plasma ALP levels are found to be raised in STZ induced diabetes. Leibovitch *et al.*,⁽¹³⁾ observed increased levels of serum ALP in pathological conditions involving the kidneys and liver. Increase in the levels of ALP in diabetic rats was also reported by Ramesh & Pugalendi⁽¹⁴⁾. Acid phosphatases are found in different organs, and their serum levels are used to evaluate the diseased state of liver and kidney. Acid phosphatases were found to be significantly increased in diseased state. Increased level of ACP may be due to increased dephosphorylation of enzymes in the liver. A significant decrease in the level of ACP in treated groups implies that the liver cells are protected from free radical damage in STZ induced diabetic rats. Alimentary hyperglycemia in rats induces increases in the activities of the acid and alkaline phosphatases of the kidney.

Conclusion

The present work implies that the hydroethanolic extract of *Mallotus philippinensis* bark showed a significant decrease in the serum marker enzymes which may be due to the phytochemicals present in the plant. Further studies may be taken for the isolation and characterization of the bioactive compounds which may exert a potent antioxidant and antidiabetic effects.

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