ANTIDIABETIC ACTIVITY OF *MOLLUGO NUDICAULIS* AGAINST ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT: The present study was designed to investigate the in alloxan (120 mg/kg b.wt) induced diabetic rats. The ethanolic extract of the whole plant of *Mollugo nudicaulis* (200mg/kg) administered orally to the diabetic rats for 21 days, produced significant decrease in the level of blood glucose, cholesterol, triglycerides, low density lipoprotein (LDL), lipid peroxidation, liver glycogen, serum creatinine, urea, uric acid and liver marker enzymes such as AST, ALT, ALP. It also produced significant increase in High density lipoprotein (HDL), Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione-S-Transferase (GST), Reduced glutathione (GSH), Vitamin C, which clearly show the antioxidant property of extract. The effect of the ethanolic the extract of *Mollugo nudicaulis* was compared with the standard drug Glibenclamide (1.25mg/kg b.wt).

Keywords: *Mollugo nudicaulis*, Alloxan, Antidiabetic, Glibenclamide, Antioxidants.

INTRODUCTION

Diabetes mellitus is a syndrome, which is characterized by hyperglycemia, lipo protein abnormalities, raised basal metabolic rate, defect in enzymes and high oxidative stress induced damage to pancreatic beta cells (Sharma *et. al.,* 2010). Type II diabetes mellitus developed by metabolic abnormalities such as impaired insulin secretion, increased hepatic glucose production and decreased insulin-stimulate of glucose uptake in peripheral (Kakadiya *et. al.,* 2010). Several distinct types of diabetes mellitus exist and are caused by a complex interaction of genetics and environmental factors (Chitra *et. al.,* 2010). The prevalence of diabetes for all age groups world wide was estimated to be 4.4% in 2030 (Chauhan *et. al.,* 2010). In india the statistical projection that the number of diabetes will raise to 57 million in 2025, which was only 15 million in 1995 (Sikarwar and Patil, 2010).

From ancient period, people are using medicinal plants for the treatment of diabetes and WHO estimates that 80% of the populations presently use herbal medicine for primary health care (Atmakuri and Dathi, 2010). Anti diabetic plants has the ability to restore the function of damaged pancreatic tissue by increasing the insulin or inhibiting the intestinal absorption of glucose (Malviya *et.al*., 2010). Administration of appropriate antioxidants from plant source could prevent or retard the diabetic complications to some extent (Muthulingam, 2010).

Mollugo nudicaulis is a wild medicinal herb, used by traditional practitioners to cure whooping cough, jaundice and wound healing (Nagesh, 2008). It is useful mainly in preventing small ring worms in stomach, to control diabetes and to increase the life energy of blood cells (Rao, 2009). *Mollugo nudicaulis* is also used to treat wounds, cold, cough, fever, and body pain (Ragupathy *et.al.*, 2008). The present study was investigated to evaluate the anti-diabetic activity of *Mollugo nudicaulis* against alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material and extraction

The whole plant of *Mollugo nudicaulis* was collected from Pollachi, Coimbatore district and authenticated by Dr.G.V.S. Murthy, Botanical survey of India, TNAU campus, Coimbatore, Tamilnadu, India. The voucher specimen was deposited to the Botanical Survey of India (No.BSI/SRC/5/23/10-11/Tech.420).
The plant material was washed with water, dried in shade and pulverized in grinder-mixer to obtain a coarse powder. About 100 gms of the powder were continuously extracted with 500 ml of ethanol using soxhelt apparatus upto 48 hours. The residue was filtered and concentrated in rotatory evaporator at 40°C under reduced pressure until the solvent has been removed to give an extract. The obtained extract was stored in air tight container at 4°C.

Experimental animals

The female albino rats (150-180 gms) were procured from Karpagam University, Coimbatore, India. The animals were housed in group of 6 rats per cage and maintained under standard laboratory condition at 24± 2°C in a light controlled room (12 h dark/12 h light) and were provided commercial pellet diet, purified drinking water *ad libitum*. The study was approved by Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA. Govt of India.

Experimental design

The rats were divided into five groups of 6 rats each.

Group I – Normal control rats
Group II – Alloxan treated control rats (120 mg/kg b.wt)
Group III – Alloxan + ethanolic extract of *Mollugo nudicaulis* (200mg/kg b.wt)
Group IV – Normal rats + ethanolic extract of *Mollugo nudicaulis* (200mg/kg b.wt)
Group V– Alloxan + standard drug Glibenclamide (1.25mg/kg b.wt)

Induction of diabetes

Type II diabetes was induced to female albino rats by a single intraperitoneal (i.p) injection of alloxan monohydrate (120 mg/kg b.wt) in sterile normal saline (0.9%). The diabetic state was determined after 3 days of alloxination by high blood glucose level and loss of body weight. At the end of 21 days treatment, blood glucose level was estimated by one touch glucometer and rats were sacrificed under chloroform anesthesia. Blood was collected and centrifuged at 3000 rpm for 20 minutes to separate the serum. Liver was removed and washed with ice cold normal saline (0.9%) to remove the blood. About 1 g of the liver tissue was homogenized using 0.1 M Tris – Hcl buffer at pH 4.7 and the supernatant was separated. Serum and the supernatant were used to analyze the biological parameters within 24 hours of sacrifice.

Biochemical estimations:

Determination of Non-Protein nitrogenous compounds

Non-protein nitrogenous compounds such as urea (Natelson *et.al*, 1951), uric acid (Caraway, 1963), creatinine (Brod, 1948) were assayed in serum.

Determination of Lipid profile

Lipid profile such as cholesterol, triglycerides, HDL and LDL were estimated in serum by diagnostic kit method.

Determination of Transaminase

ALP, AST and ALT (King, 1965) were estimated in serum.

Determination of protein

Protein (Lowry *et.al*, 1951) was estimated in the homogenate of liver.

Estimation of tissue lipid peroxidation

Lipid peroxidation was estimated (Hogberg *et.al*, 1974) and it was calculated on the basis of the molar extinction coefficient of malondialdehyde (MDA) and expressed in terms nanomolar of MDA/mg protein.

Antioxidant assays

The enzymic antioxidants such as superoxide dismutase (Misra and Fridovich, 1972) catalase (Lueck, 1965) glutathione peroxidase (Rotruck *et.al*, 1973) glutathione-S- transferase (Habig *et.al*, 1974) and non enzymatic antioxidants activity such as vitamin C (Omaye *et.al*, 1979) glutathione (Moran *et.al*, 1979) were evaluated in liver tissue homogenate.

Statistical analysis

The results were expressed as mean ± SD. The significant of the data was calculated in SPSS software package (10.0) followed by one way ANOVA and were considered statistically significant when $p<0.05$.

RESULTS

Table 1 shows the blood glucose level of diabetic and control rats. The diabetic rats treated with *Mollugo nudicaulis* and glibenclamide which was significantly reduced the level of blood glucose compared with Group II diabetic rats.

Table 2 summarizes the concentration of serum creatinine, urea and uric acid were found to be significantly increased in Group II diabetic control rats when compared to Group I normal control rats. When *Mollugo nudicaulis* was administered to Group III rats, the above parameters were reversed.

Table 1. Effect of ethanolic extract of *Mollugo nudicaulis* on the levels of glucose in serum of control and experimental animals

Groups	Glucose
Normal control	110.57 ± 0.87^{a}
Diabetic control	300.78 ± 0.71^{c}
Diabetic control + PE (200mg/kg)	197.74 ± 0.78^{bc}
Diabetic control + Glibenclamide	154.51 ± 1.07^{b}
PE alone (200mg/kg)	120.574 ± 1.42^{a}

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Units: Glucose: mg/dl.

Table 2. Effect of ethanolic extract of *Mollugo nudicaulis* on serum biochemical parameters in control and experimental animals

Groups	Urea(mg/dl)	Uric acid(mg/dl)	Creatinine(mg/dl)
Normal control	29.85±0.57[a]	8.54±0.48[a]	1.27±0.27[a]
Diabetic control	60.24±0.57[c]	19.2±0.24[e]	2.87±0.48[d]
Diabetic Control + PE (200mg/kg)	47.41±1.21[b]	10.4±0.21[d]	1.88±0.61[c]
Diabetic Control + Glibenclamide	37±0.85[b]	9.0±0.273[c]	1.54±0.87[b]
PE alone (200mg/kg)	33.12±0.84[a]	8.78±0.27[b]	1.34±0.47[a]

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Table 3 shows that there was a significant increase in the level of cholesterol, triglycerides, LDL, and decrease in the level of HDL Cholesterol in Group II diabetic control rats when compared to Group I normal control rats. The above parameters were significantly reversed on Group III diabetic rats treated with *Mollugo nudicaulis*.

Table 3. Effect of ethanolic extract of *Mollugo nudicaulis* on lipid profile in control and experimental animals

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL(mg/dl)	LDL(mg/dl)
Normal control	65.18±1.27[e]	85.27±0.89[e]	22.87±0.58[d]	16.57±0.78[d]
Diabetic control	28.42±1.47[a]	32.48±1.57[a]	12.67±1.15[a]	9.12±0.87[a]
Diabetic Control + PE (200mg/kg)	41.48±1.57[b]	52.48±0.89[b]	14.37±0.87[b]	11.48±0.99[b]
Diabetic Control + Glibenclamide	51.89±0.85[c]	68.49±0.89[c]	17.59±0.89[c]	13.48±1.07[c]
PE alone (200mg/kg)	63.47±0.98[d]	81.29±0.95[d]	22.98±0.78[d]	16.98±0.89[d]

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Table 4 shows the level of liver marker enzymes such as ALP, AST and ALT which were found to be significantly increased in Group II rats. On the other hand, the levels of enzymes were observed to have decreased markedly in the diabetic animals treated with *Mollugo nudicaulis* and glibenclamide.

Table 5 and Table 6 shows the level of liver glycogen and total protein which was found to be decreased significantly in Group II diabetic rats, whereas the levels were in Group III rats administered with the ethanolic extract of *Mollugo nudicaulis*.

Table 4. Effect of ethanolic extract of *Mollugo nudicaulis* on liver marker enzymes in serum of control and experimental animals

Groups	ALP	AST	ALT
Normal control	108.45±1.24[a]	16.87±1.41[a]	22.47±0.87[a]
Diabetic control	248.49±2.78[c]	30.47±0.57[c]	41.48±0.98[d]
Diabetic Control + PE (200mg/kg)	150.87±1.47[b]	22.75±0.48[b]	29.47±0.87[ab]
Diabetic Control + Glibenclamide	121.5±1.89[ab]	20.47±0.19[b]	25.12±1.25[ab]
PE alone (200mg/kg)	110.48±1.89[a]	18.51±0.76[a]	24.87±0.89[a]

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT). Units: AST, ALT, - µmoles of pyruvate liberated/l; ALP - µmoles of phenol liberated/l

Table 5. Effect of ethanolic extract of *Mollugo nudicaulis* on the levels of glycogen in liver of control and experimental animals

Groups	Glycogen
Normal control	19.45±0.89[b]
Diabetic control	5.02±0.45[a]
Diabetic control + PE (200mg/kg)	12.07±0.12[ab]
Diabetic control + Glibenclamide	16.48±0.52[b]
PE alone (200mg/kg)	18.48±0.78[b]

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).Units: Glycogen: mg/g tissue.

Table 6. Effect of ethanolic extract of *Mollugo nudicaulis* on protein level of liver in control and experimental animals

Groups	Protein(mg/dl)
Normal control	158.89±0.91[c]
Diabetic control	69.71±0.87[a]
Diabetic Control + PE (200mg/kg)	127.29±1.78[b]
Diabetic Control + Glibenclamide	136.87±2.78[b]
PE alone (200mg/kg)	152.17±0.17[c]

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Table 7 shows the concentration of LPO of normal and experimental rats. It was found to be significant elevation in Group II diabetic rats. A significant decrease was observed in Group III rats treated with *Mollugo nudicaulis*

Table 8 shows the non enzymatic antioxidants such as GSH and Vitamin C which was found to be significantly decreased in Group II diabetic rats. In contrast, Group III animals treated with *Mollugo nudicaulis* showed significant increase in both GSH and Vitamin C levels

Table 7. Effect of ethanolic extract of *Mollugo nudicaulis* on the levels of lipid peroxide liver of control and experimental animals

Groups	LPO
Normal control	2.82±0.12[ab]
Diabetic control	4.14±0.25[c]
Diabetic Control + PE (200mg/kg)	2.76±0.01[a]
Diabetic Control + Glibenclamide	2.81±0.21[ab]
PE alone (200mg/kg)	2.86±0.08[b]

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Units: LPO - nM/mg protein.

Table 8. Effect of ethanolic extract of *Mollugo nudicaulis* on the activities of enzymic antioxidants in liver of control and experimental animals

Groups	SOD	CAT	GPx	GST
Normal control	6.81±0.27^{d}	1.83±0.48^{d}	4.30±0.57^{e}	100.32±0.89^{c}
Diabetic control	3.47±0.57^{a}	0.53±0.57^{a}	1.02±0.27^{a}	60.67±0.81^{a}
Diabetic Control + PE (200mg/kg)	5.17±0.78^{b}	1.28±0.37^{b}	2.83±0.42^{b}	87.51±0.97^{b}
Diabetic Control + Glibenclamide	5.78±0.91^{c}	1.56±0.89^{c}	3.14±0.28^{c}	92.47±0.87^{b}
PE alone (200mg/kg)	6.12±0.58^{d}	1.81±0.27^{d}	4.14±0.57^{d}	97.91±0.78^{c}

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Units -SOD - inhibition of 50% nitri formation/min/mg protein; CAT - µmol of H_2O_2 consumed/min/mg protein; GPx - µmol of glutathione oxidized/min/mg protein;GST- µmole of glutathione utilized/min/ mg protein.

Table 9 shows the concentration of LPO of normal and experimental rats. It was found to be significant elevation in Group II diabetic rats. A significant decrease was observed in Group III rats treated with *Mollugo nudicaulis*.

Table 9. Effect of ethanolic extract of *Mollugo nudicaulis* on the activities of non-enzymic antioxidants in liver of control and experimental animals

Groups	GSH	Vit C
Normal control	12.48±0.15^{c}	1.48±0.12^{d}
Diabetic control	5.57±0.48^{a}	0.84±0.47^{a}
Diabetic Control + PE (200mg/kg)	7.08±0.87^{b}	1.15±0.87^{bc}
Diabetic Control + Glibenclamide	8.97±1.58^{b}	1.28±0.94^{c}
PE alone (200mg/kg)	11.41±0.84^{c}	1.45±0.89^{d}

Values are expressed as mean ± SD for six animals. Values not sharing common superscript letters (a-e) differ significantly at $p < 0.05$ (DMRT).

Units: Units : GSH, vitamin C

The result shows that there was no significant difference between Group III diabetic rats administered with *Mollugo nudicaulis* and Group V diabetic rats treated with the standard drug Glibenclamide. The level of the above biochemical parameters in Group IV normal rats treated with *Mollugo nudicaulis* shows that there is no significant difference when compared to Group I normal rats.

DISCUSSION

The result of the present study confirms that the administration of the ethanolic extract of *Mollugo nudicaulis* possesses antidiabetic activity against alloxan induced diabetic rats. The anti-diabetic effect of *Mollugo nudicaulis* may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration.

Glibenclamide is a known sulfonylurea drug which is effective in moderate diabetic state, and ineffective in severe diabetic animals where pancreatic β-cells are almost totally destroyed (Tatiya *et.al.* 2010).

Diabetic dyslipidaemia is marked by alloxan in diabetic rats by elevated triglycerides, cholesterol, low density lipoprotein (LDL) and decreased high density lipoprotein (HDL), constitutes an important cardiovascular risk factors (Okoli *et.al.,* 2010) and were reversed in *Mollugo nudicaulis* treated rats. The elevation of liver biomarker enzymes, such as AST, ALT and ALP in diabetic rats indicates the hepatic damage (Rathod *et.al.,* 2009). Rats treated with *Mollugo nudicaulis*, showed its ability to restore the normal functional status of the damaged liver.

As seen in the present study the level of renal function markers such as serum creatinine, urea and uric acid which was increased in diabetic rats indicates the dysfunction of kidney (Kakadiya, 2010), were inhibited by the treatment with *Mollugo nudicaulis* revealing that it exhibits potent antidiabetic activity. Administration of alloxan causes decrease in glycogen content due to enhanced glycogenolysis, which is due to insulin deficiency. So the normal capacity of the liver to synthesize glycogen is impaired (Dheer, 2010). A significant increase in the liver glycogen by administration of *Mollugo nudicaulis*, may be due to an increase level of insulin by it.

Lipid peroxidation was induced by glucose through activation of lipoxygenase enzymes (Kaimal, 2010). Free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies such as ageing, wound healing, oxygen toxicity, liver disorders, inflammation, etc (Gupta, 2010). The increased level of lipid peroxidation induced tissue injury was observed in diabetic rats. The free radical scavenging activity of Mollugo *nudicaulis inhibited* the lipid peroxidation.

The antioxidant enzymes, such as SOD, CAT, GST and GPx constitute a mutually supportive team of defense against ROS. GST eliminating toxic compounds in liver by conjugating them with GSH, an intracellular thiol antioxidant (Vijayakumar *et.al*., 2010). Many studies observed that vitamin C reducing plasma lipid peroxide levels, increasing GSH and enhance enzymic antioxidants (Osman *et.al*, 2010). Significant recovery in the level of antioxidant enzymes in diabetic rats administered with *Mollugo nudicaulis* may be due to correction in plasma insulin through beta cells stimulating effect of phyto ingredients present in it.

Earlier studies indicated that glibenclamide possess direct mechanism to enhance the level of antioxidant enzymes besides reducing the lipid peroxidation in diabetic animals (Rabbani *et.al.,* 2010). In present study also, glibenclamide significantly increased the level of antioxidant enzymes and decreased the lipid peroxidation by its antioxidant activity.

CONCLUSION

The study showed that the anti-diabetic activity of the ethanolic extract of *Mollugo nudicaulis* against alloxan induced diabetic rats. Pharmacological studies are required to evaluate the exact mechanism of action and components present in it. Further work is in progress for isolation and identification of the components from *Mollugo nudicaulis*.

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