ANTIDIABETIC ACTIVITY AND *INVIVO* ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *MACROTYLOMA UNIFLORUM*(Lam.) Verdc. IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The prevalence of diabetes is growing in the world's population. The epidemic of disease is rising in the population of India and other countries. Many herbal plants are in use for the management of diabetes till today. They are being advantageous with the lesser side effects and high therapeutic potential than the standard antidiabetic drugs. The present research is to investigate the antidiabetic activity of ethanolic extract of *Macrotyloma uniflorum* leaves (EEMUL)in alloxan-induced diabetic rats.

Methods: Diabetes is induced in the experimental animals by the administration of alloxan (150 mg/kg *i.p*) for a week. Blood glucose levels were tested using standard blood glucometer and treatment was carried out for 14days. This activity also includes the estimation of biochemical parameters and *in-vivo* antioxidant parameters. Histopathological studies were performed on liver tissue samples.

Results: The diabetic +EEMUL rats experienced a significant reduction in glucose, total cholesterol, triglycerides, and malondialdehyde and increase in high density lipoproteins compared to disease control.

Conclusion: These results demonstrate that *Macrotyloma uniflorum* possess antidiabetic activity, potent antioxidant activity, and can be used in the future studies for the estimation of distinct phytochemical showing antidiabetic activity.

Keywords: *Macrotyloma uniflorum*, alloxan, antidiabetic activity, antioxidant studies.

INTRODUCTION

Diabetes mellitus, one of the major health factors of the public worldwide is a metabolic disorder of various etiologies distinguished by an alteration of glucose homeostasis with disturbances in carbohydrate, fat and protein metabolism, as a result of defects in insulin secretion and/or insulin action [1]. The prevalence of diabetes is quickly rising everywhere throughout the world at a disturbing rate. In 2040, there will be in excess of 640 million individuals with diabetes around the world [2].

Medicinal plants are utilized as a source of medications for treatment of different disease around the world, from ancient times to the present day. They serve as source of crude materials for making customary and present-day medications.

Macrotyloma uniflorum is one of such herbal medicine which is having high nutritious just as ethno-restorative qualities. In the interest for vegetable protein there is an expansion request towards underutilized vegetables as new alternate protein sources. Other than healthful significance *M. uniflorum* has been known to its incredible therapeutic qualities because of essence of non-nutritive bioactive substances [3].

M.uniflorum (Lam.) Verdc. normally referred to as horse gram and as Kulthi in India. It is likewise acclaimed for its restorative uses in light of the fact that various parts of the plants are utilized for the treatment of heart conditions, asthma, bronchitis, leucoderma, urinary releases and for the treatment for kidney stones [4]. "Horse gram seeds are used for treatment of urinary stones, piles and urinary diseases, act as astringent, tonic, regulate the abnormal menstrual cycle in women" [5]. Literature studies has sown that Dolichin A and B, pyroglutaminglutamine alongside certain flavonoids were segregated from this plant [6].

Alloxan, an unstable organic compound, widely used in experimental diabetic studies. It is well known to destroy β-cells of the pancreas and hyperglycemia in mice and it also causes selective toxicity to β-cells by induction of necrosis [7].

The mechanism for alloxan induced diabetes is that it generates free radicals which initiates the damage and leads to death of β-cells [7].

Gliclazide is a second-generation sulfonyl urea oral hypoglycemic agent used in the treatment of diabetes mellitus. It stimulates insulin secretion by pancreatic β-cells by binding to its sulfonyl urea receptor (SUR1) [8].

MATERIAL AND METHODS

Animals and research protocol approval

In the present study animals of adult male Albino Wristar rats of about 150-250gm body weight (from Sainath Agencies, #1-6-197/45/D, Balaji nagar, Musheerabad, Hyderabad-500048, 282/PO/Bt/S/2000/CPCSEA, India) were selected. The animals were approved for the research with the protocol no: **CPCSEA/1657/IAEC/CMRCP/COL-18/71** and approved by IAEC. The animals were housed in a well air-conditioned room maintaining the temperature of 22 ± 2^0C and relative humidity of 45 to 55% under 12h:12h light and dark cycle. The animals were fed with commercial rat pellet feed and were given water *ad libitum*. All the experimental methodology and conventions utilized in this investigation were approved by the Institutional Animal Ethics Committee (IAEC) of CMR College of Pharmacy, Hyderabad.

Collection and authentication of plant

The leaves of *Macrotyloma uniflorum* were collected from Tirupathi. The plant was authenticated by Dr. K. Madhava Chetty (Associate professor, Department of Botany, Tirupathi-517 502, Andhra Pradesh, India).

Drugs and chemicals

Alloxan was purchased from Research Lab Fine Chem Industries (Hyderabad, India). Gliclazide was purchased from APL research center (Hyderabad, India).

The required chemicals were purchased from SD Fine chemicals (Hyderabad, India), and biochemical estimation kits were purchased from Coral Clinical Systems (Uttarakhand, India). All the chemicals and reagents used were of analytical grade.

Preparation of the extract

The leaves of *Macrotyloma uniflorum* were collected and washed thoroughly with distilled water to make sure the leaves are free of dust and are shade dried.

The dried leaves are then powdered finely using mechanical grinder. And then, required quantity of the powder is subjected to successive solvent extraction with solvents like petroleum ether, chloroform, ethyl acetate, ethanol and distilled water. The obtained ethanolic extract of *Macrotyloma uniflorum* leaves (EEMUL) is dried completely using desiccators.

Phytochemical screening

A preliminary phytochemical screening of ethanolic extract of *Macrotyloma uniflorum* was performed according to the standard procedures.

Phytochemical screening of ethanolic extract of *Macrotyloma uniflorum* has shown the presence of all the required chemical constituents mainly, flavonoids, phenols, glycosides, amino acids, tannins, carbohydrates and alkaloids. The phenolic components may be a reason for the antidiabetic activity of the plant [9]. Ferulic acid has been shown to possess antidiabetic, anti-cholesterolemic and cardio protective activities. Literature study has shown that the phenolic compounds obtained from the plant acts as potent antioxidants [10]. The results of the phytochemical screening tests performed are summarized in the **Table-1**.

Experimental induction of Diabetes

A freshly prepared solution of alloxan monohydrate (150mg/kg body weight), dissolved in CMC was injected intraperitoneally to 6-8 h fasted rats [11]. Blood glucose level was measured after five days of induction using Glucose kit. Rats showing fasting blood glucose levels of >250mg/dl were selected for the study [12].

The rats were divided into 5 groups of six animals each as follows: Group-I: Normal control- rats received vehicle 5ml/kg *p.o*; Group-II: Diabetic control- rats received Alloxan monohydrate (150mg/kg *i.p*); Group-III: Standard group- alloxan-induced diabetic rats received Gliclazide (25mg/kg *p.o*); Group-IV: alloxan-induced diabetic rats received EEMUL 200mg/kg *p.o*; Group-V: alloxan-induced diabetic rats received EEMUL 400mg/kg *p.o*. The animals were exposed to the treatment period for 14days [13].

Blood glucose determination

Blood samples from experimental rats were collected from the retro-orbital plexus after 6-8h of fasting. For the blood glucose determination blood is collected from the alloxan-induced diabetic rats on days 1st, 3rd, 7th, 14thafter initiation of treatment [14]. The blood glucose levels obtained from the alloxan-induced diabetic rats is mentioned in the **Table-2**, and graphically represented (**Fig.2**).

Biochemical parameters

On day 14, blood samples were collected from the retro-orbital plexus and centrifuged at 3000rpm for 15min using cold-centrifuge; the obtained serum was collected and stored at 2-8^{0}C.

Biochemical parameters like glucose, HDL, total cholesterol, triglycerides, total protein, albumin, SGOT, SGPT, and Bilirubin were determined by the specific kits [15]. The results of various biochemical parameters estimated from the serum of alloxan-induced diabetic rats are summarized in **Table-3**.

***In-vivo* antioxidant studies**

The isolated liver tissue homogenate was used for the estimation of effect of plant extract on Lipid peroxidation, reduced glutathione, and Catalase in the experimental animals [15]. The effect of *in-vivo* antioxidant activity of the plant extract is represented in **Table-4** and the **Fig.3** shows the graphical representation of the obtained results.

Estimation of Lipid peroxidation:

The quantitative estimation of LPO was performed by determining the concentration of TBA reactive substances (TBARS) in the liver using the method of Buege and Aust. The amount of MDA formed with TBA is quantified and used as an index of lipid peroxidation. The absorbance was measured at 532nm. The results were expressed in μM/mg tissue [10].

Estimation of Glutathione:

Glutathione was estimated in the liver homogenate using DTNB by the Ellman method. The assay was performed accordingly and absorbance was measured at 412nm. The results were expressed as μM of GSH/mg tissue [10].

Estimation of catalase:

The tissue homogenate of 50μl, phosphate buffer (0.05M p^H-7.0) 1.95ml, and freshly prepared 1ml of Hydrogen peroxide (0.019M) are mixed well. Blank and standard are prepared similarly but without addition of tissue homogenate and with CAT replacing tissue homogenate respectively. Absorption against blank was measured at 240nm. A standard curve was constructed using different concentrations of CAT standard and linear regression equation was used to measure the CAT activity in units per gram tissue [16].

Histopathology of liver tissue

At the end of the treatment, animals were sacrificed by the spinal-cord dislocation technique, and liver was isolated immediately. The isolated liver is stored in a 10% formalin solution for the histopathology studies.

The organ is homogenized at 4 ℃ for 1min and centrifuged at14000* g at 4 ℃ for 15min; the supernatant is collected and used for the *in-vivo* antioxidant studies [17]. **Fig-4** shows the photographs of the liver tissue which represents the effect of the plant extract.

Statistical analysis

The results were expressed as mean±S.D (Standard error of the mean (SEM)). Statistical analysis was calculated using One-way ANOVA followed by post hoc Dunnett's test for multiple comparisons and statistical significance was set at $p<0.05$ [18].

RESULTS

Preliminary phytochemical screening:

Table-1: Phytochemical screening of ethanolic extract of *Macrotyloma uniflorum*

Tests	Reagents	Inferences
Test for Amino acids	Ninhydrin	+
	Tyrosine	+
Test for Flavonoids	Shinoda test	+
	Lead-acetate	+
Test for Tannins and Phenolic compounds	5% $FeCl_3$	+
	Lead acetate	-
	Acetic acid	-
	Dil.Iodine	+
	Bromine water	+
Test for Glycosides	Legal's test	+
	Keller-Killiani test	-
	Test for saponin	+
Alkaloids	Dragendroff's	+
	Mayer's	+
Test for Steroids	Salkowski	-

Effect on body weight of experimental animals:

The initial day body weights are compared with final day body weights, and changes are reported in a graph (**Fig.1**). The normal control animals do not differ in the change of body weights.

Diabetic rats have shown slight increase in the body weight, and standard group animals also have shown significant increase in the body weight as gliclazide causes weight gain. Extract treated rats have shown slight increase in the body weights by the end of the treatment period.

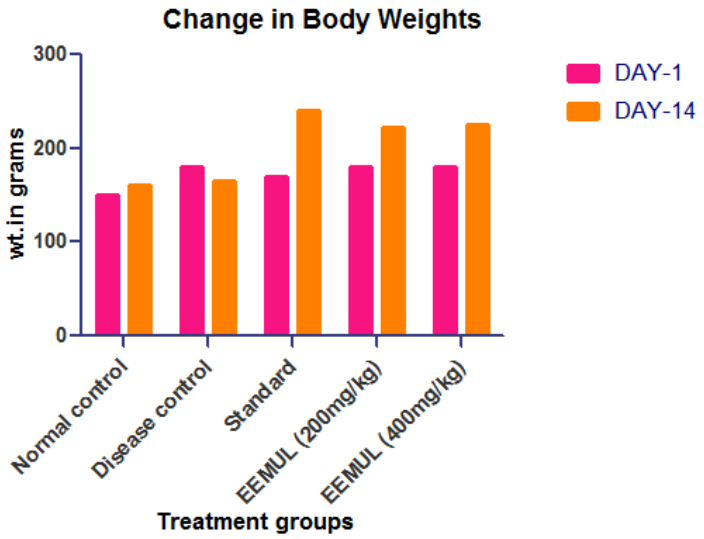

FIG. 1: Effect of EEMUL on body weights of the experimental animals

***In-vivo* studies**

The effect of EEMUL on serum glucose in normal and alloxan-induced diabetic rats:

In the experimental animals, daily administration of EEMUL (200mg/kg, 400mg/kg *p.o*), to alloxan induced diabetic rats once a day for 14 days has shown a significant reduction in glucose levels as compared to diabetic control with $p<0.0001$.

Table-2: Effect of EEMUL on glucose levels of experimental rats

Days / Groups	1	3	7	14
Normal control	55.46±5.92	56.82±5.90	59.15±5.86	56.90±4.96
Disease control	186.1±10.73	208.7±11.11	250.5±11.11	344.7±15.06
Standard	254.3±10.34^{a}	240.2±10.37^{ns}	188.2±8.25^{a}	95.10±4.30^{a}
EEMUL 200mg/kg	269.3±10.86^{a}	260.4±9.30^{b}	211.1±8.0^{b}	118.1± 5.54^{a}
EEMUL 400mg/kg	260.2±8.52^{a}	253.6±9.08^{b}	202.00±7.70^{b}	111.0±5.39^{a}

Values are represented as Mean ±SEM (n=6). Statistical analysis was done by one-way ANOVA followed by post hoc Dunnett's multiple comparison tests, a (***) - $p<0.0001$, b (**) - $p<0.001$ and c (*) - $p<0.05$ Vs Disease control. Glucose level is significantly decreased in 400mg/kg EEMUL treated animals than 200mg/kg EEMUL treated animals.

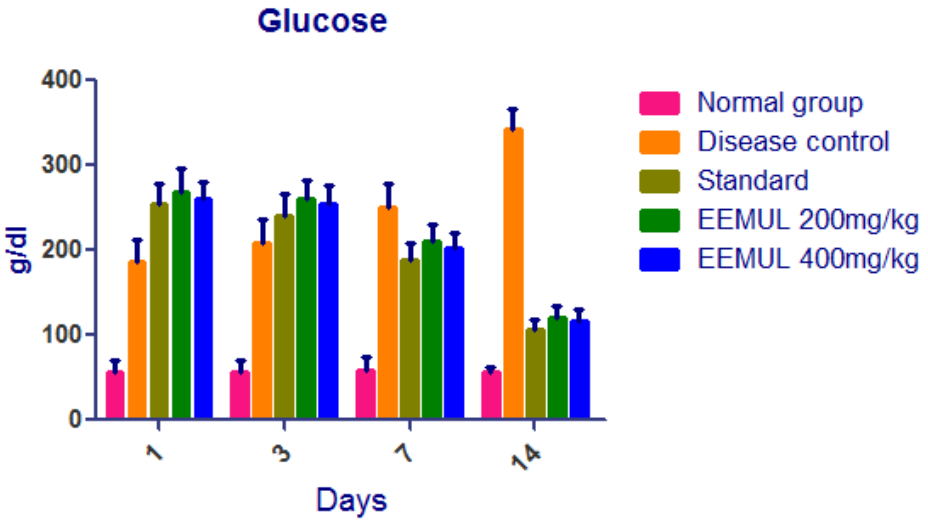

FIG.2: Effect of EEMUL on Glucose levels of alloxan-induced diabetic animals on Days 1, 3,7,14

Table-3: Effect of EEMUL on serum profile and lipid profile in normal and alloxan-induced diabetic rats

Groups	HDL (mg/dl)	TG's (mg/dl)	TC (mg/dl)	Albumin (g/dl)	Total protein (g/dl)	D.Bil (mg/dl)	T. Bil (mg/dl)	SGOT (U/L)	SGPT (U/L)
Normal control	24.34± 1.22	29.76± 8.73	58.93± 8.73	5.24±1.06	4.76± 0.23	0.368± 0.04	0.48±0.09	23.45± 4.25	21.36± 4.73
Disease control	51.03± 2.81	267.0± 1.07	295.6±10.46	2.63±0.14	3.26± 0.34	9.33±0.60	3.90±0.21	49.28± 5.09	58.08± 7.62
Standard	84.01± 4.05[a]	117.7± 9.14[a]	177.9±14.34[a]	7.27±0.33[a]	12.34± 0.36[a]	0.56±0.11[a]	0.84±0.17[a]	355.3± 16.45[a]	393.3± 15.03[a]
EEMUL (200mg/kg)	90.86± 3.39[a]	165.3± 9.37[a]	192.0±8.41[a]	4.58± 0.29[a]	10.40± 0.29[a]	1.02±0.12[a]	1.91±0.13[a]	229.8± 20.10[a]	260.2± 23.06[a]
EEMUL (400mg/kg)	110.6± 4.71[a]	158.6± 9.74[a]	194.7± 9.73[a]	6.24±0.15[a]	10.82± 0.28[a]	0.82±0.10[a]	1.33±0.21[a]	254.2± 22.47[a]	288.9± 12.05[a]

HDL-High density lipoproteins; TG's-Triglycerides; TC-Total Cholesterol; D. Bil- D. Bilirubin; T. Bil-T. Bilirubin;

Values are represented as Mean±SEM (n=6); Statistical analysis was done by using One-way ANOVA followed by post hoc Dunnett's multiple comparisons test, a (***)-p<0.0001, b (**)- p<0.001, c (*)-p<0.05 Vs Disease control.

***In-vivo* antioxidant studies:**

Table-4: Effect of EEMUL on *in-vivo* antioxidant parameters

Groups / Parameters	Normal group	Disease control	Standard	EEMUL 200mg/kg	EEMUL 400mg/kg
Lipid peroxidation	2.146 ±0.10	1.594 ± 0.24	0.674 ±0.04[a]	0.754 ±0.02[a]	0.692 ±0.05[a]
Reduced Glutathione	6.186 0.185	0.889 0.150	3.230 0.301[a]	2.036 0.237[b]	2.108 0.221[b]
Catalase	1.326 ±0.16	0.273 ±0.65	0.844 ±0.05[a]	0.673±0.06[c]	0.732 ±0.01[b]

Values are represented as Mean ±SEM (n=6). Statistical analysis was done by one-way ANOVA followed by post hoc Dunnett's multiple comparison tests, a (***)-p<0.0001, b (**)-p<0.001, c (*)-p<0.05, ns- not significant Vs disease control.

Estimation of Lipid peroxidation, GSH, Catalase:

The levels of Lipid peroxidation have significantly increased in Disease control group whereas decreased in extract treated animals with significance (p<0.0001).

In alloxan-induced diabetic rats, Reduced glutathione levels significantly (p<0.001) increased in extract treated groups.

The levels of Catalase have been significantly decreased in alloxan-induced diabetic rats, whereas in extract treated groups, levels were increased with the significance p<0.05, p<0.001 respectively.

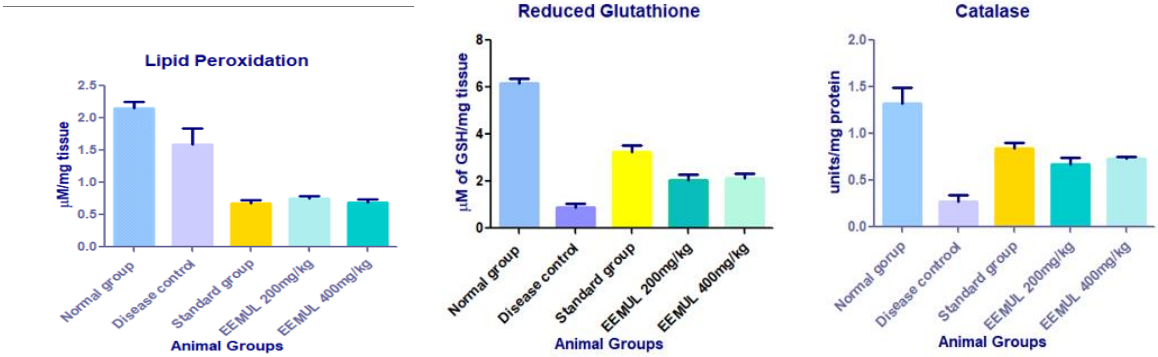

FIG.3: Effects of EEMUL on LPO, GSH and Catalase

Histopathology of liver tissue:

Non-diabetic rats have shown normal structure of the liver tissue. In diabetic control rats, the organ is seen with inflammation and tissue damage. In standard and extract treated groups, liver is seen with mild lobular inflammation and the tissue recovery is seen which is shown in the figures. Following are the photomicrographs of histopathological changes in the rat liver.

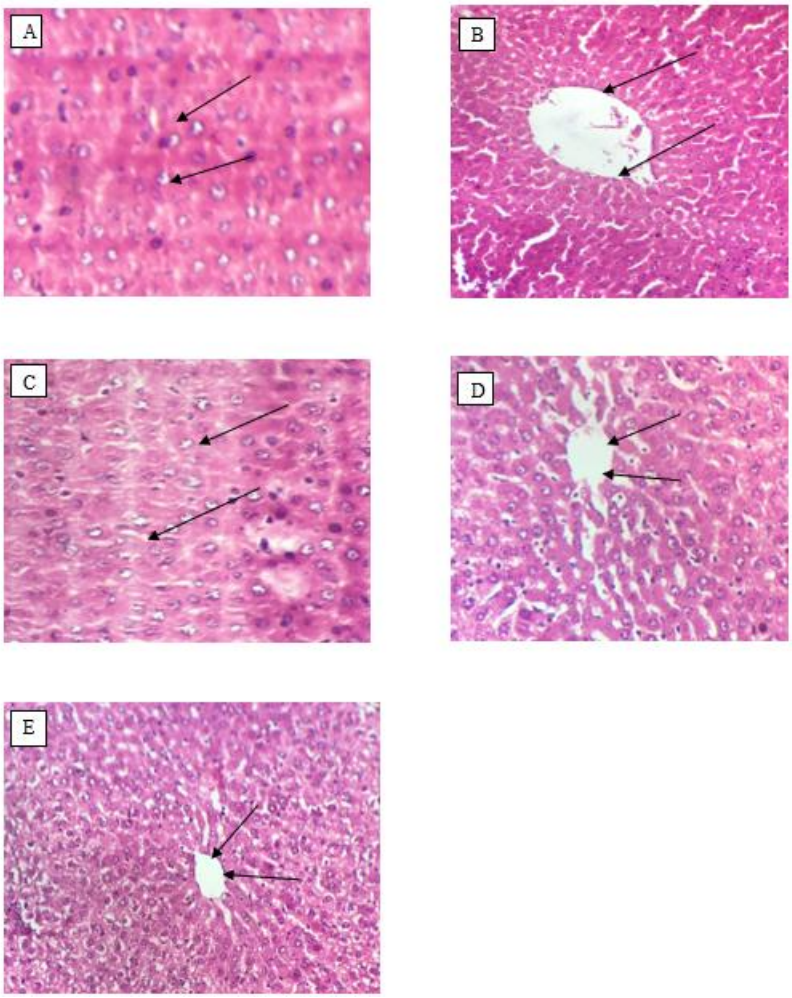

FIG:4 Photomicrographs of Histopathological studies of liver tissue. A: Normal group, B: Diabetic control, C: Standard group, D: EEMUL (200mg/kg), E: EEMUL (400mg/kg)

DISCUSSION

Irrespective of the type of diabetes, b-cell mass preservation and/or increase are known to be important targets in management of diabetes as long as it reduces chronic microvascular complications in the eyes, kidneys and nerves[19]. The plant *Macrotyloma uniflorum* has vast medicinal properties, which can be widely used in various disease conditions. Diabetes is a most common disorders in the population these days, so the treatment protocols finding its new pathway. With the increasing incidence of diabetes mellitus in urban and rural populations throughout the world, there is an urgent need to develop safe and effective anti-diabetic indigenous and cost-effective botanical remedies[20]. Herbal plants are the primary choice of medicines rather than drugs with the advantages being lesser side effects and effective treatment. So, in the present study *M. uniflorum* has been used as an antidiabetic agent.

The glucose levels have been significantly decreased in the alloxan-induced diabetic rats when administered with the doses of EEMUL 200mg/kg and 400mg/kg *p.o* for 14 days. The phytochemical screening of ethanolic extract of *M. uniflorum* reveals the presence of phenols and flavonoids. These could be the principal constituents for antidiabetic activity of the plant. However, flavonoids are the primary phytoconstituents for any medicinal plant to have hypoglycemic and antidiabetic activity. The literature study has also indicated the medicinal uses of the plant in various ailments.

It is observed that, there is an increase in lipid profile and Bilirubin levels in alloxan treated group whereas decrease in HDL, albumin, total protein, SGOT, SGPT levels. The standard (Gliclazide-25mg/kg *p.o*) and extract treated rats have shown significant increase in albumin, total protein, HDL, SGOT, SGPT levels where as Triglycerides, Total cholesterol, and Bilirubin levels have been decreased. The higher dose of EEMUL (400mg/kg *p.o*) treated groups has shown more significant values and the results than the lower dose (EEMUL 200mg/kg *p.o*) treated groups.

The alloxan-induced diabetic rats have shown decrease in the body weight which may be due to the loss of tissue proteins. Gliclazide, a standard drug treated group of animals have shown increase in body weight which is a side effect of the drug molecule. Extract treated animals have shown slight increase in the body weights by the end of the treatment.

The effects of extract of *Macrotyloma uniflorum* on LPO, GSH, ad Catalase is significant. The lipid peroxidation which was increased in diabetic control has been decreased with extract treatment. And also, the levels of GSH and Catalase have been restored in treatment groups significantly.

The histopathological effects of EEMUL on liver were studied and it is observed that the extract treated group of animals have restored the damaged tissue.

CONCLUSION

The study shows that *Macrotyloma uniflorum* has a significant effect on glucose reduction and can be utilized as an antidiabetic agent. It also possesses potent antioxidant properties which finds use in different ailments. The research can be helpful in the future for the study of molecular mechanisms and can be extended for the estimation of site and mechanism of action of phytoconstituents responsible for the antidiabetic activity.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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