*International Journal of Advanced Research in Engineering and Applied Sciences*

**ISSN: 2278-6252**
**Impact Factor: 7.358**

# ANTIDIABETIC ACTIVITY OF ARTEMISIA ABSINTHIUMVERSUS METFORMIN IN STZ INDUCED DIABETIC RATS

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**Abstract:** *The objective of this two months study is to evaluate antidiabetic activity of Artemisia absinthium (methanolic leaf extract of Artemisia absinthium - MLEAA) in Streptozotocin induced experimental diabetes mellitus in normal adult male Wistar rats via comparing with reference anti-diabetic drug Metformin by measuring the changes in body weight , levels of plasma glucose and insulin , among the empirical groups. About 40 experimental male rats were divided into five groups, eight in each group; Normal (N), Normal rats treated with MLEAA (NA), Diabetic rats (D), Diabeticrats treated with MLEAA (DA) and Diabeticrats treated with metformin (DM).Diabetes mellitus was induced by a single dose intraperitoneal injection of Streptozotocin (55 mg/kg body weight) in respective groups meant for inducing diabetes - D, DA and DM rats. Relevant groups meant for treatment (NA and DA) were administered with MLEAA at a standard dose of 500 mg/kg body weight/day and the DM group was treated with metformin 100mg//kg body weight/day by forced feeding (gavage) for 60 days. D group rats have shown elevation in glucose levels (hyperglycemia) and decline in insulin levels (hypoinsulinemia) but upon treatment with MLEAA for 60 days have rectified these signs of disease in DA group like in DM group. This long term study evidenced that, the methanolic leaf extract of Artemisia absinthium (MLEAA) is a potential antidiabetic agent against hyperglycemia and hypoinsulinemia in STZ induced experimental diabetic rats alike the reference antidiabetic drug metformin.*

***Keywords:*** *Diabetes, MLEAA, Metformin, Streptozotocin*

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## INTRODUCTION

Diabetes is anincessant issue of demented body metabolism of carbohydrate, fat, and protein [1, 2]. Diabetes mellitus (DM) is a lifetime condition caused by deficiency or diminished effectiveness of endogenous insulin that can be either inherited or acquired [3]. Diabetic symptoms include increased urine output (polyuria), excessive thirst (polydipsia), excessive hunger (polyphagia), and fatigue [4]. It is characterized by acute complications like hyperglycemia (high blood sugar), hypoglycemia (low blood sugar) and chronic complications like indelible damage, debilitation, and failure of various organs, notably the kidneys, eyes, heart, nerves, and blood vessels due to hyperglycemia [5]. The prevalence of diabetes mellitus has increasingly seen in all ages of the population because of change in food habits and lifestyle changes associated with rapid urbanization [6]. Along with insulin there are several oral hypoglycemic drugs are available nowadays to decrease elevated glucose levels in the body, but with unavoidable side effects, that is why still this disease is a big challenge to the medical community. So it is essential to investigate and discover alternative drugs to overthrow the diabetic problems without any side effects. Herbal formulations and native plant derivatives are being used as another choice to treat the disease, where blood sugar swings wildly and its difficulties. Indian conventional medicinal entities like Ayurveda, Siddha and Unani system distinctly claim to cure the hyperglycemia and its symptoms with the use of natural drugs [7]. Moreover, diabetes, complications and management had become an astronomically immense economic load for patients, community and the world society.

*Artemisia absinthium*, a perennial shrubby plant that belongs to the family Asteraceae, the species that is wide spreading Kashmir valley and have global distribution from Europe to North Asia, an ingredient in the liquor absinthe [8].In the current study, we fixated on folk medicine in archaic history from the time of Greek (i.e. *Artemisia absinthium*), for screening its antidiabetic activity with a standard dose(500mg/kg body weight) in STZ induced diabetic rats in comparison with commercial antidiabetic agent Metformin.

## MATERIALS AND METHODS

### Chemicals

All the chemicals, drugs and solvents used in this investigation were procured from Sigma Chemical Company (USA) and SISCO Research laboratory Pvt. Ltd, India).

**Plant**

The plant *Artemisia absinthium* used for current investigation was procured from Mahaks Herbal & Aromatic Agro Products, Srinagar, Jammu & Kashmir and the plant *Artemisia absinthium* was identified and authenticated by Dr. B. K. Chikkaswamy (Taxonomist), Sigma Bioscience Research Centre, Bengaluru, Karnataka, India and voucher specimen of the plant has been be deposited in the research center.

**Extract of *Artemisia absinthium***

Methanol leaf extract of *Artemisia absinthium* (dry powder) was purchased from Mahaks Herbal & Aromatic Agro Products, Srinagar, Jammu & Kashmir. A voucher specimen was deposited in the Sigma Biosciences Research Centre, a recognized research centre of Tumkur University, Tumkur. Herb to product ratio was 8:1, the necessary extract was suspended in 5% Tween-80 in distilled water prior to utilize. The extraction process followed by the company is; leaves of *Artemisia absinthium* were air dried under the shade for a week. 500g of dried leaves were powdered, sieved with mesh and extracted with 1.5L of methanol (80%) using soxhlet apparatus at 70°C for 5 hours. The extract was filtered and the filtrate was evaporated to dryness under reduced pressure at 60°C and stored at 4 °C until used for oral administration.

**Exploratory animals**

Male Wistar albinorats 2-3 months of age and weighing about 150-200g were used for the present study. Animals were acclimatized for a week in the animal house, maintained at a temperature of 24-28$^{O}$C. The light source in the animal room was regulated with 12hour day and night schedule cycle. The animals were fed with a commercial rodent pellet diet and water *ad libitum* under strict hygienic conditions by changing the bedding and cleaning the cage with a disinfectant and a detergent regularly. The long term study(60 days) of animal experimentation was performed in the Post Graduate Department of Pharmacology Laboratory, Sree Siddaganga College of Pharmacy, Tumkur, with due permission from the Institutional Animal Ethics Committee (IAEC) with registration number: 123/PO/C/99/CPCSEA.

**Design of the study**

In the present study, a total of 40 rats were used. The rats were divided into five groups of eight rats in each.

Group 1: Normal rats (N)

Group 2: Normal rats treated with 500mg/ kg body weight of MLEAA (NA)

Group 3: Diabetic rats (D)

Group 4: Diabetic rats treated with 500mg/ kg body weight of MLEAA (DA)

Group 5: Diabetic rats treated with 100mg/kg body weight of Metformin (DM)

**Induction of diabetes mellitus in rats**

After one week of acclimatization, the rats were subjected to a 16 h fast. Diabetes was induced in D, DA and DM marked rat groups by a single intraperitoneal injection of freshly prepared STZ with a dosage of 55 mg /kg body weight [18] in 0.05 M citrate buffer pH 4.5 at a volume of 0.1 ml. STZ was first weighed individually in eppendorff tubes for each animal according to the weight and then solubilized in the buffer, just 15 to 20 minutes prior to injection [11]. Plasma glucose level of each rat was determined after 72 h of STZ administration for confirmation of diabetes. Rats with fasting plasma glucose greater than 300 mg/100 ml were considered diabetic and used for further studies in the present investigation. After confirmation of induction of diabetes, they were sanctioned for a time frame of 10 days before the commencement of the treatment.

**Treatment with MLEAA and metformin**

In the present study the dose of MLEAA (500 mg/kg body weight) used for the treatment in the NA and DA groups is fixed based on the reports of maximum anti-hyperglycemic action shown by MLEAA dose of 500 mg/kg body weight in the earlier investigation conducted on hypoglycemic effects of this plant extract with different doses in STZ induced diabetic rats in 2011 [19]. The short-term (24 h) hypoglycemic activity of *Artemisia absinthium* was found to produce significant hypoglycemic action in diabetic rats, which could be compared to metformin. The results of continuous administration of *Artemisia absinthium* up to 6 weeks showed that the MLEAA is also effective in long term treatment [19]. In the current experimentation the NA and DA groups were treated daily with MLEAA, orally by gastric intubation with a dose of 500 mg/kg body weight in 5%Tween-80 in distilled water per rat once a day for 60 days. The DM group rats were treated with metformin hydrochloride (100mg/kg body weight) in distilled water once a day for 60 days orally by gastric intubation. Normal (N) and diabetic (D) rats were given distilled water instead of MLEAA. Body weight,

fasting plasma glucose and levels of insulin were monitored at 15-day intervals till the end of the study.

## ANALYSIS OF SERUM BIOCHEMICAL PARAMETERS

### Body weight changes

During the study period of 60 days body weights of all the five groups were checked by using a compression spring balance at an interval of every 15 days and the results were tabulated for statistical analysis.

### Blood collection, plasma separation

At a time period of every 15 days blood was collected from over-night fasted rats of all the five groups individually from retro orbital plexus of rats by using a capillary tube and plasma was separated by centrifugation. Collected plasma was used for estimation of fasting glucose assay of insulin.

### Estimation of glucose

Glucose was estimated by GOD-POD enzymatic method using Span Diagnostic Kit as per the manufacturer's instructions (Trinder, 1969) [20]. Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide so formed reacts under catalysis of peroxidase with phenol and 4-aminoantipyrine to form a pinkish red colored quinoneimine compound which is measured at 505nm.Ten µl of plasma was added to 1.0 ml of liquid gold glucose mono reagent (200 mM phosphate buffer, pH 7.5, 15 kU/L Glucose oxidase, 0.3 mM 4-Aminoantipyrine, 5 mM phenol and 3 kU/L peroxidase) and incubated at 37$^{o}$C for 10 min and 2.0 ml of water was added. A series of glucose standards from 0.2 to 1.0 ml was pipetted out into test tubes, made up to 1.0 ml with water. One ml liquid gold glucose mono reagent was added to standards and incubated at 37$^{o}$C for 10 min. After incubation, 1.0 ml of water was added to standards and pinkish red color formed, was read at 505 nm against water blank and values were expressed as mg/dl.

### Assay of plasma insulin

Insulin levels of plasma were assayed by the modified method of Herbert *et al* [21] of a radio-immunoassay method using kit obtained from BARC, Mumbai, India.

### Statistical data analysis

The results were expressed as mean ± S.E.M. Research data was analyzed for significant difference using Duncan's Multiple Range (DMR) test ($P < 0.05$) (Duncan, 1955) [22].

## RESULTS

Normal (N) and normal treated with *Artemisia absinthium* (NA) groups have not demonstrated any obvious behavioral changes that are observable amid the whole time of test study. Distinctive symptoms of the disease (polyphagia, polydipsia, and polyuria), that were noticed after induction of diabetes in diabetic (D), diabetic treated with *Artemisiaabsinthium* (DA) and diabetic treated with metformin(DM) groups were amended at the end of the study.

**1. Body weight changes**

Summarized mean body weights in the table.1 explain the variance in weights of the different experimental groups starting from N to DM during the period of study. No significant variation in initial weight was observed between N and NA groups, whereas, D, DA and DM groups showed a slight but significantly lesser initial weight compared to N and NA groups. During the experimental period of 60 days, D group displayed a gradual decrease in body weight, whereas, N, NA, DA and DM groups showed a trend of gradual increase in body weight (Fig 1).

**2. Plasma Glucose levels in mg /dl**

The data presented in table 2 and figure 2 indicates the fasting plasma glucose levels of 5 experimental groups during the experimental period. During the 60 days of experimental period the N and NA rats remained persistently euglycemic. The initial plasma glucose levels in D, DA and DM groups were around 4 folds greater than N and NA groups approximately. In D group, the plasma glucose level increased gradually during the experimental period from 325.47±4.16 to 368.84±3.72 mg/dl. The percent increase in plasma glucose level of D group was 2, 5.5, 9.39, and 13.3 % in 15, 30, 45, and 60 days intervals compared to initial levels, whereas in *Artemisia absinthium* treated diabetic group (DA), a significant antihyperglycemic effect was evident from the $15^{th}$ day onwards like in metformin treated diabetic group (DM) group and the decrease in the glucose was maximized and reached near normal values by 60 days of treatment.

**3. Plasma insulin levels**

Initial plasma insulin levels of D, DA and DM groups were around 2.5 to 2.7 folds lower than N and NA groups. N rats exposed no significant variation in insulin levels during the experimental period. By the end of the experimental period, D group rat plasma insulin

levels decreased by 23.7 %, i.e., 14.51±1.12 to 11.07±0.24 µunits/ml. Diabetic treated rats with MLEAA (DA) represented a significant increase in insulin concentration and percent increase was 11.8, 40.64, 83.23, and 132.12% in 15, 30, 45, and 60 days respectively compared to the initial level. Thus, at the end of the experimental period, the insulin level of DA and DM rats were 212.6 % and 297.1% more than D group. The DA group rats that has undergone treatment with *Artemisia absinthium* has shown similarity with DM group rats in enhancing insulin levels during the exploratory period.

**Table 1: Body weight changes in STZ incited diabetic rats upon MLEAA treatment**

| Body weight in grams | | | | | |
|---|---|---|---|---|---|
| **Group** | **Initial day** | **15th day** | **30th day** | **45th day** | **60th day** |
| **N** | 185.56±1.95$^{a}$ | 201.01±2.06$^{c}$ | 223.22±1.21$^{c}$ | 251.35±3.94$^{c}$ | 281.75±1.97$^{c}$ |
| **NA** | 184.75±1.97$^{a}$ | 200.25±1.08$^{c}$ | 221.18±1.58$^{c}$ | 248.29±1.32$^{c}$ | 273.52±1.01$^{c}$ |
| **D** | 170.68±1.29$^{b}$ | 162.35±2.16$^{a}$ | 155.47±2.01$^{a}$ | 144.85±1.66$^{a}$ | 132.34±1.42$^{a}$ |
| **DA** | 171.52±1.36$^{b}$ | 182.43±1.14$^{b}$ | 191.27±2.82$^{b}$ | 203.91±2.67$^{b}$ | 221.71±2.83$^{b}$ |
| **DM** | 168.37±1.25$^{c}$ | 179.61±1.15$^{c}$ | 197.56±1.21$^{c}$ | 217.68±2.11$^{c}$ | 249.13±1.57$^{c}$ |

Communicated results are mean ± S.E.M (n=8).Means with various superscripts inside the segment are fundamentally different at P<0.05 (Duncan's multiple range test).

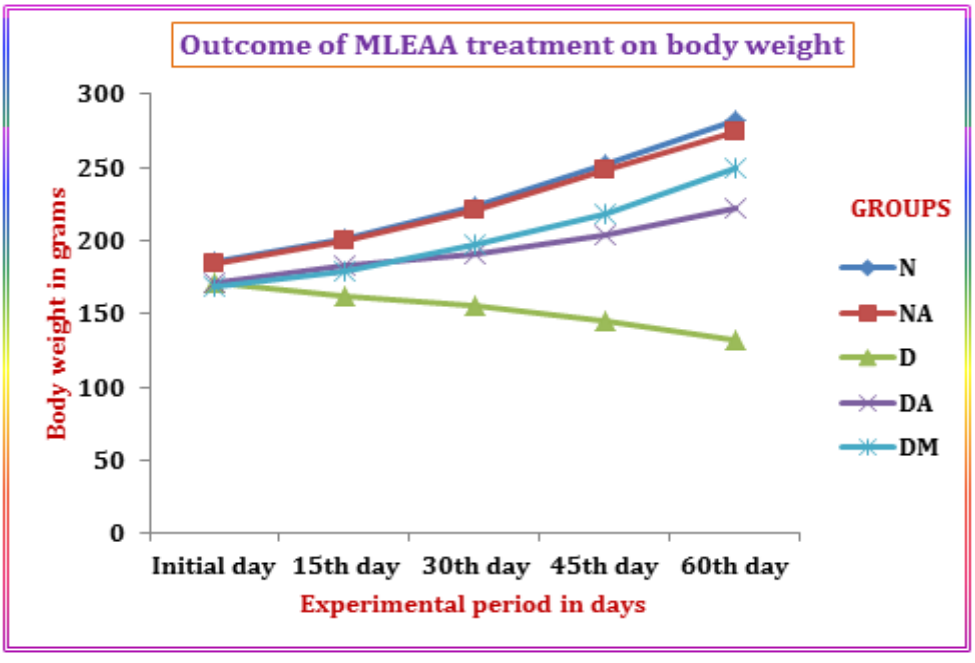


**Figure.1: Body weight changes in STZ incited diabetic rats upon MLEAA treatment**

**Table 2: Levels of glucose (mg/dl) in STZ lured diabetic rats upon MLEAA treatment**

| Plasma Glucose levels in mg /dl | | | | | |
|---|---|---|---|---|---|
| Group | Initial day | 15th day | 30th day | 45th day | 60th day |
| N | 82.23±1.21[a] | 83.93±0.52[a] | 85.82±0.61[a] | 84.14±0.78[a] | 85.80±1.28[a] |
| NA | 84.52±0.67[a] | 83.22±1.20[a] | 88.23±0.68[a] | 85.60±1.04[a] | 83.33±1.54[a] |
| D | 325.47±4.16[b] | 331.7±28.43[b] | 343.41±1.05[c] | 356.04±2.73[c] | 368.84±3.72[b] |
| DA | 339.64±4.60[b] | 280.11±9.15[c] | 181.57±4.90[b] | 120.52±3.16[b] | 90.30±1.34[a] |
| DM | 345.17±0.59[c] | 256.49±1.21[c] | 201.28±1.50[c] | 126.14±0.22[c] | 81.31±1.23[c] |

Communicated results are mean ± S.E.M (n=8).Means with various superscripts inside the segment are fundamentally different at P<0.05 (Duncan's multiple range test).

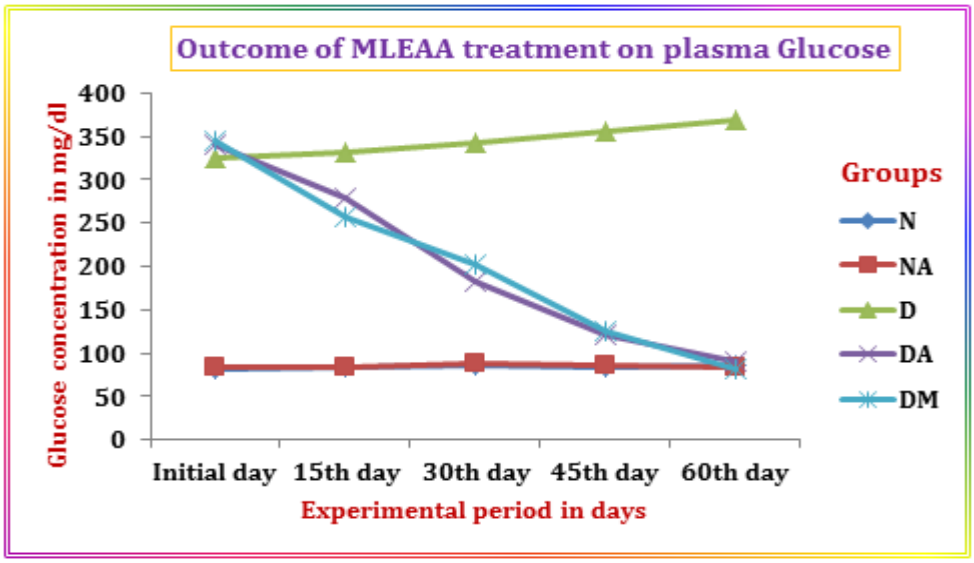


**Figure 2: Levels of glucose (mg/dl) in STZ lured diabetic rats upon MLEAA treatment**

**Table 3: Levels of Insulin (µUnits/ml) in STZ lured diabetic rats upon MLEAA treatment**

| Plasma insulin levels in µUnits/ml | | | | | |
|---|---|---|---|---|---|
| Group | Initial day | 15th day | 30th day | 45th day | 60th day |
| N | 37.97±0.46[a] | 38.21±0.34[a] | 39.38±0.87[a] | 40.55±1.65[a] | 42.27±1.59[a] |
| NA | 36.1±0.36[a] | 37.19±0.68[a] | 38.47±0.51[a] | 40.22±0.47[a] | 41.1±1.05[a] |
| D | 14.51±1.12[b] | 13.27±0.08[b] | 12.52±0.72[b] | 11.91±0.36[b] | 11.07±0.24[b] |
| DA | 14.91±0.34[b] | 16.67±0.66[c] | 20.97±0.56[c] | 27.32±0.89[c] | 34.61±0.88[c] |
| DM | 13.31±0.33[C] | 18.87±1.24[c] | 28.18±1.25[c] | 34.41±0.59[c] | 43.97±0.51[c] |

Communicated results are mean ± S.E.M (n=8).Means with various superscripts inside the segment are fundamentally different at P<0.05 (Duncan's multiple range test).

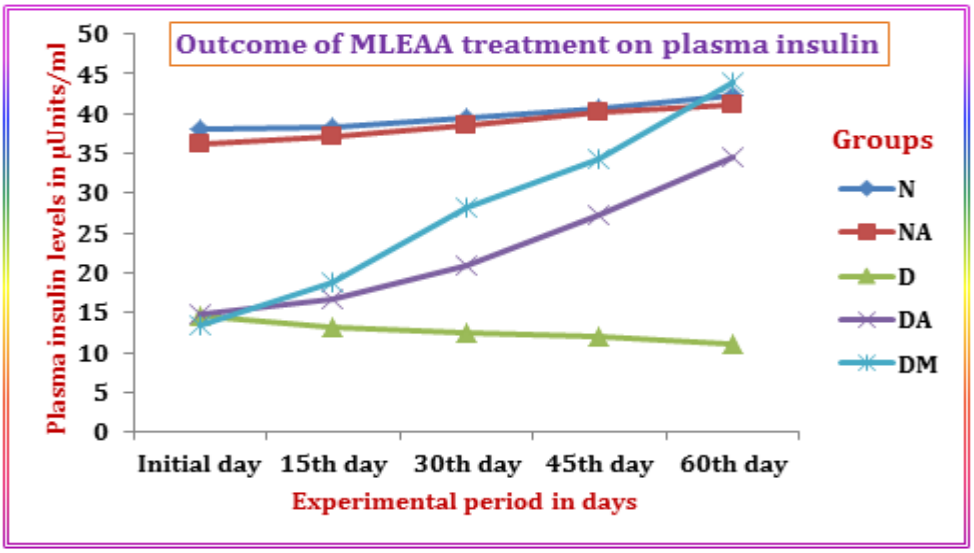


**Figure 3: Levels of Insulin (µUnits/ml) in STZ lured diabetic rats upon MLEAA treatment**

## DISCUSSION

Streptozotocin incites diabetes in exploratory rats by obliterating insulin secreting β-cells of Islets of Langerhans [9, 10]. STZ is a noxious glucose twin that heaps up in pancreatic beta cells by means of glucose transporter GLUT2 [11]. Once STZ goes into the cell; it stifles the glucose utilization system and insulin discharge from the beta cells and disables the pancreas [12].This prompts to changes in body weight, tremendous rise in blood sugar levels and progressive fall in the insulin levels of the test animals [13, 14].Body weight diminishes in the diabetic rats because of imperfection in glucose utilization system and increment in muscle squandering [15].Despite the fact that DA alike DM rats have demonstrated a steady increment in body weight at the end of the test time frame, the body weight of DA gathering was fundamentally lower than N group rats however essentially higher than D group rats. Along these lines, MLEAA therapy for 60 days obstructed the weight reduction in the DA group rat's alike metformin medication in DM group rats contrasted with D group rats; this might be because of a recovery in insulin levels. [16, 17]. The diminished plasma glucose levels of the DA group rats in 60 days are a result of *Artemisia absinthium* treatment correspondingly. In this manner, the present study shows that incessant treatment of diabetic rats for 2 months with *Artemisia absinthium* (MLEAA) diminished the plasma glucose level close to the typical values of N group rats. The plasma glucose levels of NA and DA group rats amid the trial time frame precisely demonstrates that *Artemisia absinthium* does not only display hypoglycemic activity; rather, it demonstrates the antihyperglycemic

action by diminishing overabundance of glucose levels, which was compared with metformin (reference antidiabetic drug) treated DA group rats.

At the end of the test time frame, the insulin levels of DA group rats alike in DM group rats, were more than D group rats. Consequently, *Artemisia absinthium* treatment has given sponsorship against STZ initiated exhaustion in plasma insulin levels. Long haul explorations have revealed that the recovering of body weights, decrement in enhanced glucose levels expansion insulin levels are by the medication of MLEAA like the antidiabetic agent metformin. These impacts are because of the insulin level sustainment by the antidiabetic movement of *Artemisia absinthium*, which may have restored the desolated pancreatic cell that has corrected the glucose utilization.

## CONCLUSION

It is presumed that the valuable activities of MLEAA are strikingly optimistic in deterring hyperglycemia and hypoinsulinemia in diabetic treated rats (DA) like DM group rats that have been treated with metformin a reference antidiabetic drug. The current long haul exploration on STZ prompted diabetic rats has obviously affirmed that *Artemisia absinthium* (MLEAA 500mg/kg bodyweight) treatment has antidiabetic activity like metformin. Along these lines, development and advancement of *Artemisia absinthium* as phytomedicine for diabetes mellitus treatment is moderately favored as it is economical and it is more appropriate to financial conditions [8].Anyhow, it is assertive that more clinical and pharmacological assessment studies ought to be led in support of this preclinical examination of this plant. The present exploration shows that *Artemisia absinthium* leaves to have powerful antidiabetic action and henceforth can be used as a supplement in the management of diabetes.

## ACKNOWLEDGEMENTS

Authors are grateful to Dr. Vijay Kumar S, Dept. of Pharmacology, Sree Siddaganga College of Pharmacy, Tumkur for providing facilities to carry out this long term evaluation study. The authors are appreciative to Mrs. Haritha Busineni for her cooperation in the manuscript design and preparation. The authors additionally extend their gratitude to Dr. Nagamma, Director, Sigma Bioscience Research Centre for her constant encouragement and support.

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