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Assessment of Anti-Oxidant and Anti Diabetic Activity of *Ageratum littorale Leaves* in Albino Wistar Rats

***[1]C. Bhuvaneswara Rao, [2]Basini Jyothi, [3]Kothapalli Bannoth Chandra Sekhar**

[1]Research Scholar, Krishna Teja Pharmacy College , Thirupathi, Chittor dist, Andhra Pradesh, India.517506
[2] Professor, Krishna Teja Pharmacy College , Thirupathi, Chittor dist, Andhra Pradesh, India.517506
[3]Professor, Department of Chemistry, Krishna University, Machilipatnam, Andhra Pradesh, India.521001

raopharmacy07@gmail.com

Abstract:

Antioxidants may be defined as radical scavengers, which are protecting the human body against free radicals. The main aim of this work to evaluate the antioxidant and antidiabetic activity of methanolic extract of *leaves* of *Ageratum Littorale* (Asteraceae) MEAL in alloxan induced diabetic rats. The plant extract have been proven experimentally to possess significant antioxidant activity in invitro methods. Methanolic extract of *leaves* of *Ageratum Littorale* showed effective response on 2, 2-Diphenyl-1-picryl-hydrazyl radical (DPPH) method for determining the free radical scavenging activity and 50% inhibition of DPPH radical scavenging effect (IC50) were recorded as 28µg/ml and 36µg/ml for MEAL and standard ascorbic acid. A solitary dose of alloxan monohydrate (120mg/kg, i.p.) was used to bring diabetes mellitus. The meal at the dose of 200 and 400mg/kg and the standard drug glibenclamide (2.5mg/kg) were directed orally to the diabetic rats for 15 days. The fasting blood glucose levels were estimated on 0, 5, 10 and 15th day. On 15th day, the blood was withdrawn and serum is separated for the estimation of biochemical parameters and the pancreas was isolated for histopathological studies. The results of biochemical parameters and histopathological studies advices that, the MEAL posesses significant antidiabetic property and antioxidant activity compared to ascorbic acid.

Keywords: *Ageratum Littorale,* Fasting blood glucose Antioxidants, Antidiabetic, Alloxan etc.

INTRODUCTION

Antioxidants may be defined as radical scavengers, which are protecting the human body against free radicals. Oxidation reactions can produce free radicals, may cause pathological conditions like neurodegeneration, parkinson's disease, mongolism, ageing, anemia, asthma, arthritis, inflammation, and dementia[1,2]. Free radicals are generated as part of the body's normal metabolic process[3], and free radical chain reactions are usually produced in the mitochondrial respiratory chain[4], liver mixed function oxidases, atmospheric pollutant, drugs and metal catalysts.[5] Free radicals cause oxidative damage to lipids and nucleic acids.[6] Plants and animals maintain complex systems of multiple types of antioxidants like glutathione, vitamin C, and vitamin E as well as enzymes like superoxide dismutase, catalase and various peroxidases. Low levels of antioxidants cause oxidative stress and may damage or kill cells.[7]

Diabetes mellitus is a chronic metabolic disorder, characterized by elevated blood glucose levels and disturbances in carbohydrates, fats and protein metabolism. These metabolic abnormalities resulting from defects in insulin secretion and/or insulin action[8].The level of glucose as well as other fuel molecules is increased due to a disorder in the production or function of the hormone 'insulin'. A range of health problems occurs primarily due to the damaging effects of elevated of glucose on blood vessels.[9]

The chronic hyperglycemia of diabetes is associated with damage, dysfunction and failure of various organs over the long term. It causes number of complications like retinopathy, neuropathy and peripheral vascular insufficiencies[10]a major complication of diabetes is loss of vision due to either to cataracts (excessive glucose attaches to lens proteins, causing cloudiness) or to damage blood vessels of the retina. Sever kidney problems also may results from damage to renal blood vessels.[11] Diabetic patients typically have delayed or impaired wound healing and may develop chronic ulcers.[12]

The increasing worldwide incidence of diabetes mellitus in adult constituents is a global public health burden.[13] Regions with greatest potential are Asia and Africa, where diabetes mellitus rates could rise to 2 to 3 folds than the present rates.[14] the world wide prevalence of diabetes mellitus is expected to be more than 240 million by the year 2010. The countries with the major number of diabetic people in the year 2025 will be China, India and United States. In the next 25 years diabetes is being projected as the world's main disabler and killer[15]. In India more than 30 millions peoples are with diabetes mellitus. Diabetes mellitus is the fourth foremost cause of death in the United States, mainly of its damage to the cardiovascular system.

Diabetes was described more than 2000 years ago. For the past 200 years, it has been featured in the history of modern medicine. Since the discovery of insulin work on diabetes at both cellular and clinical levels has expanded as fast new laboratory and diagnostic techniques allow. Several drugs like Sulfonylureas and Biguanides are currently obtainable to decrease hyperglycemia , have side effects and thus searching for a new class of compounds is crucial to overawed diabetic problems. Management of diabetes without any side effects is still a challenge to the medical community.[16]

Ageratum littorale (*Asteraceae*) plant is a fast-growing deciduous plant that grows under different climatic conditions (i.e., tropical, subtropical, and temperate).[17]

Ageratum littorale grows in beach sand and nearby thickets along the coast as well as hummocks and roadsides at elevations of less than 10 m (33 ft). It is a trailing to decumbent perennial herb up to 50 cm (19.5 in) tall, spreading by means of underground rhizomes. Stems are glabrous except at the nodes. Leaf blades are ovate to oblong, up to 4 cm (1.5 in) long. Flowers are blue, lavender or white, in cyme-like arrays. In Japan, consumption of mulberry leaf as a tea or powdered juice has been increasing. The leaf is nutritious, palatable, and non-toxic, and is stated to improve milk yield when fed to dairy animals. Reports indicate that mulberry leaves contain proteins, carbohydrates, calcium, iron, ascorbic acid, β-caroteine, vitamin B_1, folic acid, and vitamin D. The presence of rutin, quercetin, isoquercetin, and other other flavonoids in mulberry leaves also has been reported.[18] The total antioxidant activity of plant foods is the result of individual activities of each of the antioxidant compounds present such as vitamin C, tocopherols, carotenoids, and phenolic compounds, the latter being the major phytochemicals responsible for antioxidant activity of plant materials.[19] Moreover, these compounds render their effects via different mechanisms such as radical scavenging, metal chelation, inhibition of lipid peroxidation, quenching of singlet oxygen, and so on to act as antioxidants.[20]

Therefore, this work was aimed to evaluate the effect of *Ageratum littorale* (*Asteraceae*) plant on antioxidant capacities, and diabetic properties with different in-vitro and in-vivo models.

MATERIALS & METHODS

Collection and Authentication of the plant

The leaves of *Ageratum littorale* (Asteraceae) was collected from Talakona forest, Chittoor dist of Andhra Pradesh, India, during October 2019. The plant was authenticated by and was authenticated by Dr. K. Madhava Chetty, Professor and Head, Department of Botany, S.V. University, Tirupati. The voucher specimen (2021/127) of the plant was deposited at the college for further reference.

Acute Toxicity Study

The acute toxicity PECB was determined as per the OECD guideline no. 423 (Acute toxic class method). It was detected that the test extract was not mortal even at 2000mg/kg dose. So, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of the dose were selected for further study[18].

Evaluation of Antioxidant activity

DPPH Radical scavenging test

The free radical scavenging activity of the methanol extracts of *Ageratum littorale* (Asteraceae) was determined by using 2, 2 Diphenyl-1-picryl hydrazyl radical (DPPH) using UV-Spectrometry[21] at 517nm. The DPPH solution was prepared in 95% methanol. The MEAL was mixed with 95% methanol to prepare the stock solution (10mg/100ml or 100µg/ml). From the stock solution 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in five test tubes and by serial dilution with same solvent were made the final volume of each test tube up to 10ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively.

Freshly prepared DPPH solution (0.004% w/v) was added in each of their test tubes. Containing MEAL (20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) and after 10 min, the absorbance was taken at 517nm, using a spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotometer). Ascorbic acid was used as a standard. It is dissolved in distilled water to make stock solution with the same concentration of MEAL control sample was prepared without extract and reference ascorbic acid. 95% methanol was used as blank % scavenging of the DPPH free radical was measured using following equation.

% DPPH radicals-scavenging = [(Absorbance of control – Absorbance of test Sample) / (Absorbance of control)] x 100.

Reducing Power Method

The assay of reducing power methodis one to determine the antioxidant activity. [22, 23] In this 1 ml of plant extract of MEAL solution mixed with 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml Potassium Ferricyanide [K_3Fe (CN6)] (10g/l), the mixture was incubated at 50°C for 20 minutes. 2.5 ml of Tri chloroacetic acid (100g/l) was added to mixture.

This was centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (lg/L) and absorbance measured at 700nm in UV-visible spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotometer). Ascorbic acid was used as standard and phosphate buffer used as blank.

Evaluation of Antidiabetic activity

Animals used

Male Wistar albino rats (150-200g) were obtained from the animal house in Krishna Teja Pharmacy College, Tirupati, Andhra Pradesh. The animals were preserved in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard pellet feed (Hindustan Lever Limited., Bangalore) and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref. No.18/KTPC/IAEC/2018).

Induction of diabetes mellitus

Diabetes Mellitus was brought in overnight fasted adult male Wistar albino rats weighing 150-200 g by a single intraperitoneal injection of 120 mg/kg alloxan monohydrate. Hyperglycemia was confirmed by the elevated glucose levels determined at 72h. This model has been used in earlier studies to induce diabetes in rats.[24] Animals with blood glucose level more than 250 mg/dl were considered as diabetic. Rats found with permanent Diabetes were used for the antidibetic study.

Experimental design

Animals were divided into five groups, each consisting of six rats. The extracts were administered for 15 days.

Group-I: Normal control rats administered normal saline (0.9% w/v);

Group-II: Diabetic control rats administered normal saline (0.9% w/v);

Group-III: Diabetic rats administered standard drug glibenclamide (2.5 mg/kg);
Group-IV: Diabetic rats administered MEAL (200 mg/kg);
Group-V: Diabetic rats administered MEAL (400 mg/kg) daily for 15 days.

Antidiabetic study

The effects of administration of MEAL extracts in diabetic rats were observed by measuring fasting blood glucose levels and serum lipid profile. Fasting blood glucose was estimated on days 0, 5, 10 and 15 of extracts administration. The biochemical parameters (TG, TC, HDL, LDL, and VLDL) and the histopathalogical studies of the pancreas were determined on day 15 after the animals were sacrificed by decapitation.

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA). The test followed by Tukey-Kramer multiple comparison tests, the p values less than 0.05 were considered as significance.

RESULTS

Table 1: Antioxidant activity by DPPH method

S. No.	Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of MEAL	% scavenging DPPH of Ascorbic acid	%scavenging DPPH of MEAL
1	20µg/ml	0.142	0.128	35.15	41.55
2	40µg/ml	0.102	0.089	53.42	59.36
3	60µg/ml	0.086	0.067	60.73	69.40
4	80µg/ml	0.058	0.039	73.51	82.19
5	100µg/ml	0.032	0.011	85.38	94.97

Table 2: Antioxidant activity by reducing power method

S. No.	Concentration (mg/ml)	Absorbance of Ascorbic acid	Absorbance of MEAL
1	0.1	0.16	0.11
2	0.2	0.22	0.20
3	0.3	0.31	0.28
4	0.4	0.39	0.35
5	0.5	0.48	0.41

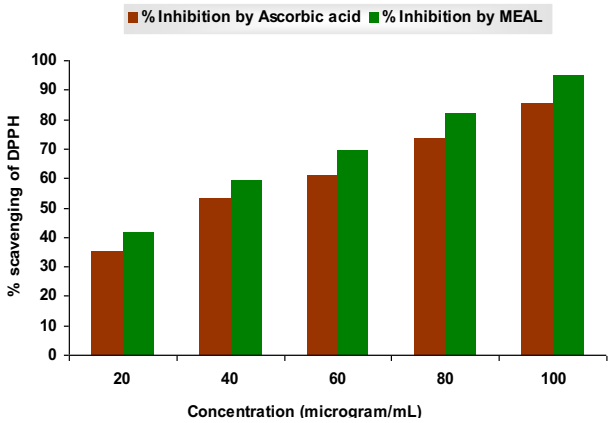


Figure 1: Antioxidant activity by DPPH method

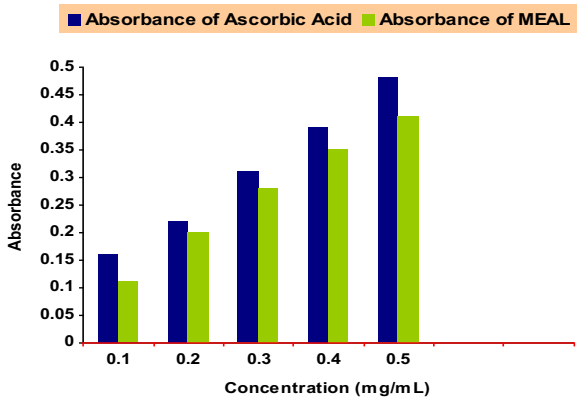


Figure 2: Antioxidant activity by reducing power method

Figure 1: DPPH radical scavenging activity of methanol extracts of *Ageratum littorale L.*(Asteraceae) (MEAL) added to methanol solution of DPPH and radical scavenging activity was measured as 517 nm as compared to standard Ascorbic acid. Values are the average of triplicate experiments.

Fig. 2: Reducing power of methanol extract of *Ageratum littorale* (Asteraceae) (MEAL) of as compared to Ascorbic acid. Values are the average of triplicate experiments.

Alloxan has been observed to cause a massive reduction in the number of the β-cells of the islets of langerhans and induce hyper glycemia. The diabetogenic action of alloxan is mediated by reactive oxygen speciese, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of pancreatic β-cells. It reduces the synthesis and the release of insulin. It will decrease anti- oxidant enzymes levels and enhanced lipid peroxidation observed in alloxan-induced diabetes.

Table 3: Effect of MEAL on fasting blood glucose levels of alloxan induced diabetic rats

Groups (n=6)	Fasting Blood Glucose Levels (mg/dl)			
	0th Day	5th Day	10th Day	15th Day
Group-I (Normal Control)	96 ± 1.15***	95.66 ± 1.14***	95.16 ± 0.79***	95.5 ± 0.76***
Group-II (Diabetic Control)	289.5 ± 4.47	292.33 ± 4.19	292.33 ± 4.04	293.66 ± 3.96
Group-III (Glibenclamide - 2.5 mg/kg)	271.5 ± 5.05	216.33± 3.24***	141.16 ± 5.56***	100 ± 0.66***
Group-IV(MEAL-200mg/kg)	271.5 ± 6.75	241.5± 10.97***	173.66 ± 9.46***	127.16± 4.41***
Group-V (MEAL-400 mg/kg)	288.16 ± 7.89	239 ± 7.44***	181.83 ± 19.04***	120.5 ± 1.28***

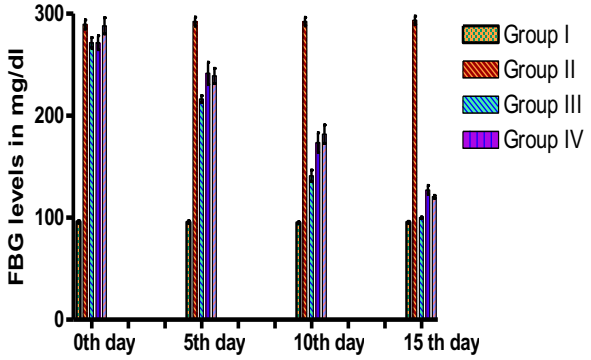
Values are expressed as mean ± SEM of 6 rats in each group.*** p<0.0001, as compared to diabetic control group.

Table 4: Effect of MEAL on lipid profile in alloxan induced diabetic rats

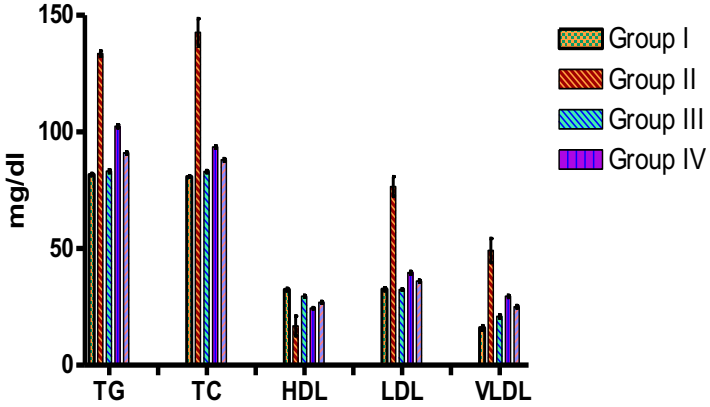
Groups (n=6)	Biochemical Parameters				
	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Vldl (mg/dl)
Group-I (Normal Control)	81.72 ± 0.43***	80.86 ±0.17***	32.39 ± 0.50***	32.54 ± 0.63***	16.01 ± 0.90***
Group-II (Diabetic Control)	133.57 ± 1.10	142.72 ± 5.89	16.87 ± 4.19	76.57 ± 4.19	49.2 ± 5.12
Group-III (Glibenclamide - 2.5 mg/kg)	83.14 ± 0.61***	83.00 ±0.49***	29.61 ± 0.34***	32.47 ± 0.25***	20.92 ± 0.58***
Group-IV (MEAL-200mg/kg)	102.4 ± 0.59***	93.58 ±0.44***	24.39 ± 0.25***	39.65 ± 0.59***	29.52 ± 0.44***
Group-V (MEAL-400 mg/kg)	91.00 ± 0.47***	88.04 ±0.49***	26.93 ± 0.39***	36.07 ± 0.35***	25.07 ± 0.47***

Values are expressed as mean ± SEM of 6 rats in each group.*** p<0.0001, as compared to diabetic control group. TG=Triglycerides, TC=Total cholesterol, HDL=High density lipoproteins, LDL=Low density lipoproteins, VLDL = Very low density lipoproteins.

Effect of MEAL on fasting blood glucose levels of alloxan induced diabetic rats



Effect of MEAL on lipid profile in alloxan induced diabetic rats



Effect of MEAL on fasting blood glucose levels of alloxan induced diabetic rats

The MEAL at the dose of 200 and 400mg/kg, p.o. produces a dose-dependent fall in fasting blood glucose level (FBG). After 15 days of treatment, the maximum reduction in FBG was observed in the treated group rats (Groups- IV and V) as compared with untreated group (Group-II). On the progression of treatment with MEAL, FBG reduced from 5th day. At the end of experiment (15th day) FBG levels was127.16 ± 4.41and 120.5 ± 1.28mg/dl in the doses of MEAL 200 and 400mg/kg respectively. The changes in FBG of all groups were represented in Table-1.

Effect of MEAL on lipid profile in alloxan induced diabetic rats

The levels of serum lipid profile such as triglycerides (TG), total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins (VLDL) of the all groups are shown in Table-2. The levels of TG, TC, LDL and VLDL were significantly higher as well as levels of HDL significantly lower in diabetic **control rats as compared to** normal rats. The MEAL at the dose of 200 and 400mg/kg were significantly decreases in the levels TG, TC, LDL, and VLDL as well as increases in the level HDL as compared to diabetic control rats.

Effects MEAL on pancreas histopathological changes

Architecturally, pancreatic islet of diabetic control rats (group-II) shown significantly destruction as compared to normal control animals (group-I). Pancreatic islets of rat which are treated with MEAL 200 and 400mg/kg (groups-IV and V) also showed architectural destruction but to a lesser extent as compared to diabetic control rats. The architectural of pancreatic islets of animals treated with standard drug glibenclamide showed similar to normal animal group.

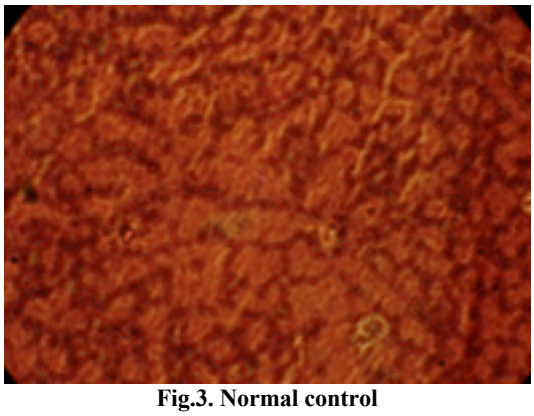


Fig.3. Normal control

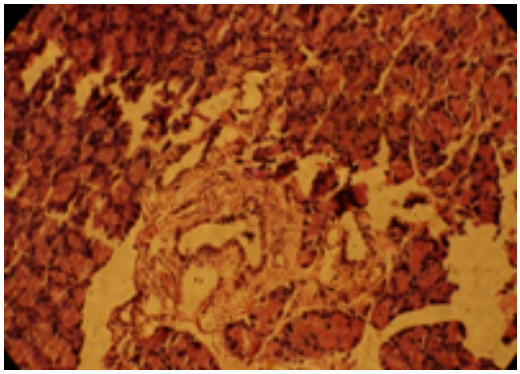


Fig.4. Diabetic control

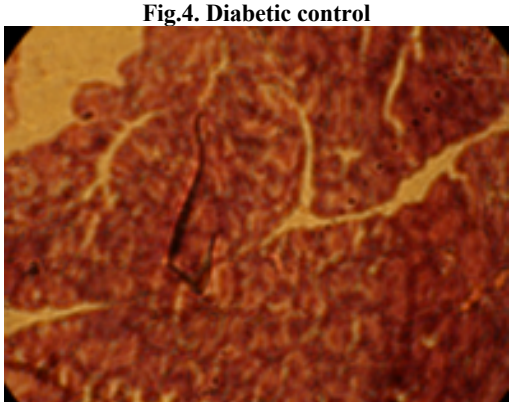


Fig.5. MEAL-200mg/kg

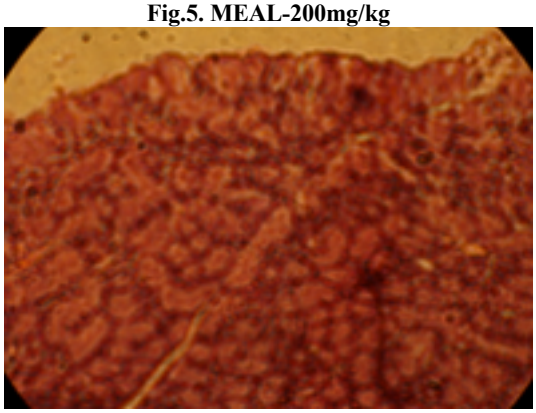


Fig.6. MEAL-400mg/kg

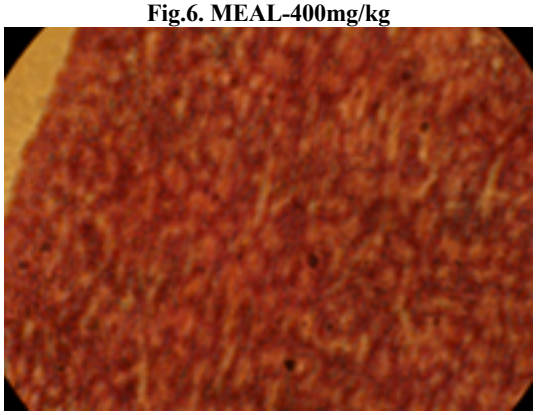


Fig.7.Glibenclamide-2.5mg/kg

DISCUSSION

In this present study the methanol extract of *leaves* of *Ageratum littorale L.*(Asteraceae) were investigated by using DPPH scavenging test and reducing power method. The whole plant of MEAL showed by their two methods effectively when compared with reference standard ascorbic acid. In the DPPH scavenging method is based on the capability of DPPH radical to decolorize in the presence of antioxidants. The DPPH radical is considered to be model of a stable lipophilic radical a chain reaction. In lipophilic radicals was initiated by the lipid autooxidation antioxidants react with DPPH reducing a number of DPPH molecules equal to number of their hydroxyl groups. Therefore, the absorption at 517 nm was proportional to the amount of residual DPPH.[25] In Figure-16, The MEAL exhibited a significant dose dependent inhibition of DPPH activity, the IC50 value of the MEAL and reference standard ascorbic acid were found to be 28 μg/mL and 35 μg/mL respectively.

The reducing power method based on the capability of a reducing the compound due to presence of reductants which are breaking the free radical chain by donating hydrogen atom. The whole plant of MEAL exhibited the antioxidant activity due to presence of reductants (i.e., antioxidants). The reduction of Fe^{3+}/Ferricyanide complex to ferrous form, in this main principle is increasing the absorbance of the reaction mixture indicates the antioxidant activity that leads to reducing power of the samples. In Figure-17 MEAL was very potent and the power of extract was increased with quantity of sample. By comparing the reference standard Ascorbic acid, the MEAL showed potent antioxidant activity.

Diabetes mellitus is a complex metabolic disorder, causes the hyperglycemia and severe alterations of glucose and lipid metabolism. This metabolic abnormalities leads to an increased generation of reactive oxygen species.[26] Ethanobotanical information indicates that more than 800 plants are used for the treatment of diabetes in traditional remedies.[27] *Ageratum Littorale* (Asteraceae) is climbing vine, distributed throughout India. Traditionally it is used to treat diabetes.[28] The antidiabetic activity of aerial parts methonolic extract of *Ageratum Littorale* (Asteraceae) Hook & Arn was evaluated in alloxan induced diabetic rats to support its claims in folklore practice.

Alloxan has been observed to cause a massive reduction in the number of the β-cells of the islets of langerhans and induce hyper glycemia.[29] The diabetogenic action of alloxan is mediated by reactive oxygen speciese, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of pancreatic β-cells which reduces the synthesis and the release of insulin. It is well documented that, decreased anti- oxidant enzymes leaves and enhanced lipid peroxidation observed in alloxan-induced diabetes.[30]

In this study effect of MEAL on hyperglycemia is evaluated in alloxam-induced diabetic rats. It was found that the fasting blood glucose levels (FBG) of the animals which are treated with MEAL (Groups-IV & V) and the standard drug glibenclamide (Group-III), significantly reduces when compared with diabetic control (Group-II) group. The FBG of all groups were observed on 0^{th}, 5^{th}, 10^{th}, and 15^{th} day. The diabetic rats treated with MEAL and glibenclamide displayed a noteworthy decrease in blood glucose level on 5^{th}, 10^{th} and 15^{th} day. On 15^{th} day FBG of Group-III decreases nearly too normal range. In the case of Group-IV & V animals FBG suggestively reduces, but it was some less when equated to Group-III. The MEAL at the dose of 200 & 400 mg/kg significantly reduces the hyperglycemia when equated with untreated group. These results give us the suggestion that MEAL having significant hypoglycemic effect.

Lipids play an important role in the pathogenesis of diabetes. The levels of serum lipids are usually increased in diabetes mellitus and such elevation contribute to coronary heart disease[31]. It is well documented that untreated diabetes mellitus; there will be increase in total cholesterol (TC), triglycerides (TG), VLDL and LDL cholesterol associated with decrease in HDL cholesterol[32] In the present investigation the TG, TC, LDL and VLDL cholesterol was increased in diabetic control animals (untreated group) and it was decreased in 15 days treatment with MEAL as well the HDL cholesterol level was significantly increased. The total lipid profile in serum (TG, TC, HDL, LDL, VLDL) of the alloxan induced diabetes animals treated with MEAL (200 and 400 mg/kg) was substantially improved, as compared to diabetic control group. These results suggest that MEAL may constrain the cholesterol synthesis and augmented HDL/LDL ratio may be due to the initiation of LDL receptors in hepatocyte, which is accountable for taken up LDL into the liver and diminish the serum LDL level.[33]

CONCLUSION

This study provisions the traditional privileges and the methanolic extract of this plant could be in preparations for the ailment of numerous diabetes related complications. It is concluded from the data, that the methanolic extract of leaves of *Ageratum Littorale* (*Asteraceae*). Possesses significant antioxidant and antidiabetic activity and may prove to be effective for the treatment of diabetes mellitus and for the treatment of various diseases caused by free radicals. Still, extensive studies on chronic models are obligatory to clarify the particular mechanism of action so as to develop it as a effective antidiabetic drug.

CONSENT: It is not applicable.

COMPETING INTERESTS: Authors have declared that no competing interests exits.

ETHICAL APPROVAL:

The protocol of the study was approved by the institutional animal ethics committee and experiments were conducted according to guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) bearing the registration number: CPCSEA (Ref. No.18/KTPC/IAEC/2018).

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