ANTIHYPERGLYCEMIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF *Glinus oppositifolius* (L.) AUG. DC.

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Summary

Glinus oppositifolius (L.) Aug. DC. belongs to family Molluginaceae is an annual or perennial sub shrub. In the present study the Methanol extract of *Glinus oppositifolius* (MEGO) and Aqueous extract of *Glinus oppositifolius* (AEGO) at the dose of 200 and 400mg/kg b.w was evaluated for its *in-vitro* and *in-vivo* antioxidant, antihyperlipidemic and antihyperglycemic activity. The various antidiabetic parameters like Body weight, Fasting glucose serum level, serum lipid profile, Glutathione levels has been analysed. On Preliminary phytochemical analysis of *Glinus oppositifolius* contains various alkaloids, carbohydrates, glycosides particularly cardiac glycosides, flavones and flavonoids, which shows antihyperglycemic, antihyperlipidemic and antioxidant activity. The MEGO and AEGO in the doses of 200 and 400mg/kg are able to produce antioxidant activity and consistent reduction in serum glucose, serum cholesterol and serum triglyceride. These plant extracts have also shown significant *in-vitro* and *in-vivo* activity.

Key Words: *Glinus oppositifolius,* Antioxidant, Antihyperlipidemic and Antihyperglycemic activity.

Introduction

Diabetes mellitus is a metabolic disorder, characterized by chronic hyperglycemia with disturbances of carbohydrate, protein and fat metabolism resulting in defects in insulin secretion, insulin action or both (WHO) [1]. Diabetes mellitus is a chronic disease characterized by high blood glucose levels due to absolute or relative deficiency of circulating insulin levels. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is increasing demand by patients to use the natural products with anti- diabetic activity. Insulin cannot be used orally and continuous use of the synthetic drugs causes side effects and toxicity. Herbal drugs are prescribed widely even when their biologically active compounds are unknown, because of their effectiveness, less side effects and relatively low cost [2].

Glinus oppositifolius belongs to family Molluginaceae [3], is an annual or perennial sub shrubs, or shrubs, rarely dioecious, glabrous or rarely hairy; Stems erect or prostrate; Leaves simple, alternate, rarely opposite; Flowers bisexual, Petals absent or few to many, white, pink, or purple. Fruit usually a loculicidal capsule rarely breaking into 2 nutlets; Seeds with embryo curved around a hard, starchy perisperm [4]. Traditionaly *Glinus oppositifolius* is used in the treatment of skin disease, increase appetite, cures vata, kapha, piles, leucoderma, tonic to intestine, urinary infections, fever, cough, liver problem and also used as antioxidant due to its excellent properties and potent phytoconstituents [5]. Activities like Free radical scavenging and Antioxidant activities of the ethanol extract [6]. Hepatoprotective effect of a methanolic extract of root [7]. Antiprotozoal activity of aerial part [8]. Immunomodulating activity of aerial part of *Glinus oppositifolius* [9] has been reported. An amino acid derivative, L-(−)-(*N-trans*-cinnamoyl)-arginine, was isolated from the whole plant of *Glinus oppositifolius* (L.) Aug. DC. along with kaempferol 3-*O* galactopyranoside, is orhamnetin3-*O*-β-D-xylopyranosyl-(1→2)-β-D-galactopyranoside,vitexin, vicenin-2, adenosine and L-phenylalanine was reported [10] . Ethno-botanical information indicates that more than 800 plants are use as traditional remedies for the treatment of diabetes and more than 200 pure compound have showed lowering blood glucose activity, but many plants do not have a scientific scrutiny [11].

Diabetes mellitus is the most common endocrine disorder which is complicated by hyperlipidemia and oxidation of micro and macro vascular tissues [12]. From the available literature survey there is no work reported on the Antihyperglycemic, Antihyperlipidemic and Antioxidant activity of the leaves of *Glinus oppositifolius* (L.) Aug. DC. Hence the present investigation was undertaken to study the antihyperglycemic, antihyperlipidemic and antioxidant effects of MEGO and AEGO on STZ induced diabetic rat.

Materials and Methods

Plant material

Glinus oppositifolius were collected in the month of October 2009 from village and sea side river areas of Ramnagar, Dist-Kendrapara, Orissa. They were identified and authenticated by Dr. S.K. Dash department of Biotechnology of College of Pharmaceutical sciences, Mohuda, Berhampur, India. A voucher specimen No- GCP 231 of the collected sample was deposited in our institutional herbarium for the further reference.

Preparation of Plant Extract

They plant material were mechanically powered and sieved through sieve no-10. 600 gm of powdered plant material were taken and subjected for successive soxhlet extraction. Solvents were chosen depending upon their increase in polarity like methanol and water. The extraction was carried out for 72hours for each solvent.

Preliminary phytochemical analysis

Different extracts obtained from the above extraction process were analyzed for presence of phytochemical constituents by the method of qualitative phytochemical analysis. The extract were subjected for test for Alkaloids, Carbohydrates, Glycosides, Gums and Mucilage, Proteins, Amino Acids, Tannins, Phenolic Compounds, Saponins, Steroids and sterols, Triterpenoids and Flavonoids [13].

Animals

Thirty six numbers of healthy male Sprague-Dawley rats weighing between 150-200 grams and twenty four numbers of Swiss mice weighing between 25-30 grams, of either sex, were selected for the study and obtained from the animal house of Gautham College of Pharmacy (GCP), Bangalore. All the animals were housed in polypropylene cages with clean sterilized husk bedding with a 12:12 light: dark cycle. Bedding was changed every alternate day to maintain proper hygienic condition. The animals were fed with standard diet recommended by National institute of nutrition and pure drinking water ad libitum.

Determination Invitro Antioxidant Activity

Evaluation of Antioxidant Activity by Inhibition of Autoxidation in a Linoleic Acid System

2ml of 200mg/lit concentration of different extracts of plant were taken to this 2 ml 2.5%w/v of linoleic acid in ethanol 95%v/v, 4ml of 0.05M phosphate buffer (pH 7.0), 2 ml of distilled water were added and mixed in 10 ml test tube covered with aluminum foil. The test tubes were kept at 37^{o}c and kept in dark cup board for oxidation. Then add 0.1 ml of above mixture to 9.7 ml of 75% ethanol and 0.1 ml of 30%w/v ammonium thiocyanate. After 5 mins, add 0.1 ml of o.02 M $Fecl_3$. Absorbances were taken at 500nm. Blank- As above in place of plant extract distilled water taken. Here Standard was Tocopherol [14].

Percentage inhibition= AC-AS/AC

Where AC is absorbance of control and AS is absorbance of standard or sample.

DPPH (1, 1-Diphenyl-2-Picrylhydrozyl) Radical Quenching Assay

500 µl of different extracts of plant were taken in a test tube to this 5ml of 0.1 mM methanol sol. of DPPH were added and vertexed. Then these samples are incubated at 27^{o} c for 20 min in dark and after 20mins the samples are measured spectrophotometically at 517nm [15].

Control:-as above without plant extract
Standard:-BHT (Butylated hydroxy toluene)
Parameters:-inhibition of DPPH radicals

Percentage inhibition= AC-AS/AC

Where AC is absorbance of control and AS is absorbance of standard or sample

Non Enzymatic Glycosylation of Hemoglobin by Colorimetry

1 ml of glucose solution mixed with 1 ml of hemoglobin solution (60 mg in 100 ml of 0.01 M phosphate buffer pH 7.4) and 1ml of gentamycin (20 mg in 100 ml of 0.01 M phosphate buffer pH 7.4). The mixtures were incubated in dark at room temperature for 72 hours. The degree of Glycosylation in the presence of different conc. of extracts &their absence were measured at 520 nm. Here standard is ascorbic acid and blank is as above except plant extract [16].

Percentage inhibition= AC-AS/AC

Where AC is absorbance of control and AS is absorbance of standard or sample.

In-Vivo Studies

Acute Toxicity Studies

For acute toxicity study the animal model chosen was Swiss albino mice of body weight 20-30 gm. Each group contains at least 3mice. The initial dose given was 10,100, and 1000mg/kg. They were in an observation period of 24-72 hour. If there is no toxic effect then the final three doses were given i.e. 2000, 3000 and 4000mg/kg [17].

Experimentally induced diabetes mellitus

All the rats were allocated into dietary regimens consisting of HFD (58% fat, 25% protein, and 17% carbohydrate, as a percentage of total kcal) *ad libitum,* respectively, for the initial period of 3 weeks except the control group animals. After 3 weeks of dietary manipulation, low dose of STZ (L-STZ) (45mg/kg) was injected intra-peritoneally (i.p), while the respective control rats were given vehicle i.e. citrate buffer (pH 4.5).

Sprague-drawly rats (130–200 g) were fasted for 14 h before challenging with single injection of STZ (45mg/kg), freshly prepared and injected within 5 min of preparation to prevent degradation at a dose of 45 mg/kg, i.p. After administration of STZ the animals had

free access to feed and water *ad libitum*. The development of hyperglycemia in rats was confirmed by estimation of fasting serum glucose after 96 h post STZ injection wherein the animals were fasted again for 14 h before blood withdrawal from retro orbital plexus. The rats with fasting serum glucose level of above 200 mg/dl at 96 h after STZ injection were considered diabetic and included in the study.

Experimental design

Albino Sprague-drawly rats were divided into eleven groups of six animals in each group as follows: Group-I: Normal control; Group-II: STZ induced diabetic rats (Diabetic control); Group-III: Diabetic rats given metformin, orally once daily (dose: 10mg/kg); Group-IV: Diabetic rats given MEGO, orally once daily (dose: 200 mg/kg); Group-V: Diabetic rats given MEGO, orally once daily (dose: 400 mg/kg); Group-VI: Diabetic rats given AEGO, orally once daily (dose: 200 mg/kg); Group-VII: Diabetic rats given AEGO, orally once daily (dose: 400 mg/kg).

Collection of serum samples

The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anesthesia on different occasions, i.e., day 0, day 14, and day 28. The blood samples were allowed to clot for 30 mins at room temperature and then they were centrifuged at 3000 rpm for 10 mins. The resulting upper serum layer was collected in properly labeled, clean and dry micro-centrifuge tubes. The serum samples were stored at 2-8^{o}C, analyzed immediately for glucose values and stored at -80^{o}C for two weeks for the estimation of lipid profiles.

Parameters analyzed

Body Weight

The body weight of each animal was recorded weekly and the data on day 0, day 14 and day 28, i.e. the days corresponding to other parameters analyzed and tabulated.

Biochemical parameters

Fasting Serum Glucose Estimation was carried out by the GOD/POD (Glucose oxidase and peroxidase) method using the GLUCOSE–GOD/POD kit (Coral/clinical systems). *Serum* Cholesterol was estimated by the colorimetric, End point CHOD-POD (Cholesterol oxidase and peroxidase) method using the CHOLESTEROL kit (Coral/clinical systems). Serum Triglyceride was estimated by the Enzymatic, End point, colorimetric, GPO-POD (Glycerol-3-phosphate oxidase-peroxidase) method using the TRIGLYCERIDE kit (Coral/clinical systems). Estimation of HDL-Cholesterol (HDL-C) in Serum was analysed by using HDL-Cholesterol kit from (Coral/clinical systems). Biochemical systems International Srl [Model-3000 evolution, Cod-RM 4030, S.L. NO-34508] (Made in Italy) on day 0, day14 and 28.

Estimation of Glutathione

Estimation of Glutathione was done using the Ellman's reagent (DTNB). The sulfhydryl groups present in glutathione forms a colored complex with DTNB which was measured colorimetrically at 412 nm[18] .

Statistical Analysis

The values are expressed as mean ± SEM. The data was analyzed by using one way ANOVA. Statistical significance was set as per analysis.

Results

Phytochemical Study

The various phytoconstituents found in the MEGO and AEGO are illustrated in the Table -1

TABLE-1. Preliminary phytochemical screening of *Glinus oppositifolius*

SL. NO	PHYTOCONSTITUENTS	Methanolic extract of G.o.	Aqueous extract of G.o.
1	ALKALOID	+ ++ +	+ - + +
2	CARBOHYDRATE	+ - -	+ - -
3	GLYCOSIDE (cardiac glycoside)	+	+
4	TANINS	+ + -	+ +
5	PROTEIN &AMINO ACID	- - +	- ++
6	GUM AND MUCILAGE	+++	+ + +
7	FLAVONES & FLAVONOIDS	++	+ +
8	SAPONINS	+	+
9	STEROIDS & STEROLS	+	+
10	TRITERPINOIDS	+	+

In-Vitro Evaluation of Antioxidant Activity

Ammonium Thiocyanate System or Inhibition of Autoxidation in a Linoleic Acid System

TABLE-2. *In- vitro* Antioxidant Activity of *Glinus oppositifolius*

SL.NO	Type of solution	%inhibition (0 hr)	%inhibition (24hr)	%inhibition (48hr)	%inhibition (60hr)
1	MEGO	54.073± 0.712	56.107±0.614	64.057±1.608	69.393±0.7258*
2	AEGO	39.46±0.515	43.33±0.452	51.98±1.011	55.267±0.783*
3	STD	60.333± 0.882	66.333±0.882	71.667±0.882	80±1.55*
4	BLANK	0	0	0	0

*Values are significant. Values are mean ±SEM, (n=3) Statistical data was carried out by One way analysis of variance P value $p<0.0001$for methanolic extract of *Glinus oppositifolius* in comparison with standard.

From the Table -2, it was found that the MEGO and AEGO show significant percentage of inhibition when compared with the standard α-tocopherol. Where as in case of blank there was zero percentage of inhibition so there is no comparison with blank.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging Method

TABLE-3. **Absorbance and Percentage inhibition of DPPH by *Glinus oppositifolius***

SL.NO	Name of Preparation	Absorbance	Percentage inhibition
1	MEGO	0.102±0.005	72.577±0.153*
2	AEGO	0.122±.001	67.1±0.391*
3	STANDARD	0.063±0.008	82.970±2.370*
4	BLANK	0.372	0

* Values are significant with standard. (n=3) $P<0.05$) as compared to standard and other group and standard drug treated group.

From the Table -3, it was found that MEGO and AEGO shows significant percentage of inhibition when compared with the standard Butylated hydroxy toluene.

Hemoglobin glycosylation method

TABLE-4a. **Absorbance and Percentage inhibition of hemoglobin Glycosylation by methanolic extract of *Glinus oppositifolius***

SL.NO	Name of Preparation	Absorbance	Percentage inhibition
1	MEGO 2000µg/ml	0.470±0.005	84.298±0.364*
2	MEGO 1000µg/ml	0.653±0.008	78.427±0.358*
3	MEGO 500µg/ml	0.827±0.008	73.063±0.073*
4	MEGO 250µg/ml	1.007±0.008	66.55±0.425*
5	STANDARD	0.35±0.005	88.787±0.212*
6	BLANK	3.063±0.003	0

Values are mean ±SEM, (n=3) Statistical data was carried out by One way analysis of variance P value ($P<0.05$) as compared to standard

From the Table -4a, it was found that MEGO with different concentration shows significant percentage of inhibition when compared with the standard ascorbic acid.

Absorbance and Percentage inhibition of hemoglobin Glycosylation by AEGO

TABLE-4b. Absorbance and Percentage inhibition of hemoglobin Glycosylation by aqueous extract of *Glinus oppositifolius*

SL.NO	Name of Preparation	Absorbance	Percentage inhibition
1	AEGO 2000µg/ml	0.57±0.005	81.720±0.167*
2	AEGO 1000µg/ml	0.72±0.005	76.033±0.261*
3	AEGO 500µg/ml	0.95±0.020	69.753±0.357*
4	AEGO 250µg/ml	1.213±0.008	60.690±0.299*
5	STANDARD	0.39±0.005	87.937±0.325
6	BLANK	3.070±0.005	0

Values are mean ±SEM; Statistical data was carried out by One way ANOVA, * indicates $P<0.05$, n=3

From the Table -4b, it was found that AEGO with different concentration shows significant percentage of inhibition when compared with the standard ascorbic acid.

Acute Toxicity Studies

Up to 4000 mg/kg the MEGO and AEGO do not show any toxic effect. As per the ranking system European Economic Community (EEC) for acute oral toxicity, the LD_{50} dose of 2000 mg/kg and above is categorized as unclassified (EC Directive 83/467/EEC, 1983).

In-Vivo Experimental Method

Body Weight TABLE-5.

SL. NO	GROUPINGS (n =6)	DOSE (mg/kg)	Body weight on different day		
			0	**14th**	**28th**
1	Normal control	Vehicle, 10 ml/ kg	209.80 ± 1.98	232.20 ± 5.92	243.00 ± 13.43*
2	Diabetic control	Vehicle10 ml/ kg	183.00 ± 3.78	177.00 ± 3.12	150.17 ± 5.69
3	Standard group	Metformin 10mg/ kg	187.67 ± 4.04	180.00 ± 4.59	168.83 ± 7.14*
4	MEGO 200	200 mg/ kg	186.00 ± 4.89	186.50 ± 6.68	173.00 ± 4.12*
5	MEGO 400.	400 mg/ kg	185.00±4 .12	180.21± 3.45	179.23±4.32 *
6	AEGO 200	200mg/ kg	185.33 ± 3.29	174.50 ± 4.14	177.17 ± 5.29*
7	AEGO 400.	400mg/ kg	191.67 ± 1.38	186.50 ± 4.14	186.83 ± 5.67*

From Table -5, it was found that the animals treated with MEGO and AEGO shows significant increase in body weight when compared with control and standard group. As we know in diabetes, the reduction in body weight is one complicated factor.

Fasting Serum Glucose

TABLE-6. Effect of Different extracts of *Glinus oppositifolius* on fasting serum glucose levels in rats

SL. NO.	GROUPS (n =6)	DOSE (mg/kg)	Values of serum glucose level in different days				
			0	**7th**	**14th**	**21st**	**28th**
1	Normal control	Vehicle, 10 ml/ kg	73.16 ±0.601	73.667±0.667	73.16 ±0.477	73.667±0.333*	73.5±1.057*
2	Diabetic control	Vehicle 10 ml/ kg	234.83±5.082	243.167± 5.412	212.333±4.417	210.5 ±2.012*	206±1.732*
3	Standard group	Metformin 10mg/ kg	259.5 ±5.396	223.667± 2.824	177.833±2.496	138.833±1.701*	119.5± 0.764*
4	MEGO 200	200 mg/ kg	247.167±5.642	226.5±5.156	216.333±5.852	198.667±6.438*	134.333±1.687*
5	MEGO 400	400 mg/ kg	242±8.132	291± 17.307	187± 3.907	146± 3.347*	119.333±1.944*
6	AEGO 200	200mg/ kg	229.333±4.153	214.167± 1.721	198.167±0.703	188.167±2.548*	165.167±1.973*
7	AEGO 400.	400mg/ kg	221±3.347	205.167± 3.291	195.333±1.647	183.5 ±3.49*	151.833±2.810*

Values are mean ±SEM; Statistical data was carried out by One way ANOVA, * indicates P<0.05, n=6, Fvalue-1.218 and df is 10.

From Table -6, it was found that the diabetic animals have higher level of fasting serum glucose, the animals treated with MEGO and AEGO for a period of 28days shows significant reduction in fasting serum glucose level when compared with the standard group.

Lipid profile

The serum cholesterol and triglyceride levels of all groups of animals were recorded during the study period on day 0, 14 and day 28. The results were as follows.

Cholesterol

TABLE-7. Effect of Different extracts of *Glinus oppositifolius* on serum Cholesterol levels in rats

SL. NO	GROUPINGS (n =6)	DOSE (mg/kg)	Values of cholesterol on different day		
			0	**14th**	**28th**
1	Normal control	Vehicle, 10 ml/ kg	63.667±1.256	62.167±1.662*	64.833±1.740*
2	Diabetic control	Vehicle10 ml/ kg	100.333±0.843	107±0.577*	115.667±0.760*
3	Standard group	Metformin 10mg/ kg	100.333±0.667	79.167±0.872*	64.667±1.022*
4	MEGO 200	200 mg/ kg	101.833±0.601	81.333±0.558*	72.333±0.558*
5	MEGO 400.	400 mg/ kg	102.667±1.085	79.333±0.494*	70±0.258*
6	AEGO 200	200mg/ kg	101.833±0.601	84.333±1.202*	73.167±1.6*
7	AEGO 400.	400mg/ kg	102.667±0.882	87.833±0.477*	71.667±0.333*

Values are mean ±SEM; Statistical data was carried out by One way ANOVA, * indicates P<0.05, n=6, Fvalue-1.458 and df is 10.
From Table -7, it was found that the diabetic animals have higher level of fasting serum glucose, the animals treated with MEGO and AEGO for a period of 28days shows significant reduction in fasting serum cholesterol level when compared with the standard and control group.

TABLE-8. Effect of Different extracts of *Glinus oppositifolius* on serum Triglyceride levels in rats
Triglyceride

SL.NO	GROUPINGS (n =6)	DOSE (mg/kg)	0day	14day	28day
1	Normal control	Vehicle, 10 ml/ kg	65.167± 0.477	65.333±0.751*	65.833±1.167*
2	Diabetic control	Vehicle10 ml/ kg	167.167 ±1.195	169.833±2.613*	170.667± 1.430*
3	Standard group	Metformin 10mg/kg	164.833 ±1.078	120.167±2.482*	78.833±0.307*
4	MEGO 200	200 mg/ kg	164.5±0.764	132.167±1.195*	96.5±1.455*
5	MEGO 400	400 mg/ kg	164.5±0.764	130.167±0.477*	88±0.365*
6	AEGO 200	200mg/ kg	163.5±0.764	138.833±0.601*	100.333± 0.333*
7	AEGO 400.	400mg/ kg	164.167 ±0.601	129.167±0.477*	93±1.211*

Values are mean ±SEM; Statistical data was carried out by One way ANOVA, * indicates P<0.05, n=6, Fvalue-1.458 and df is 10.

Fvalue-1.68481 and df is 10.

From Table -8, it was found that the diabetic animals have higher level of serum triglyceride, the animals treated with MEGO and AEGO for a period of 28days shows significant reduction in serum triglyceride level when compared with the standard and control group.

HDL Cholesterol

TABLE-9. **Effect of Different extracts of *Glinus oppositifolius* on serum HDL Cholesterol levels in rats**

Sl.No	GROUPINGS (n =6)	DOSE (mg/kg)	0day	14day	28day
1	Normal control	Vehicle, 10 ml/ kg	1.365±0.007	1.358±0.008*	1.362±0.006*
2	Diabetic control	Vehicle10 ml/ kg	0.783±0.014	0.795±0.011*	0.802±0.007*
3	Standard group	Metformin 10mg/ kg	1.810±0.010	2.07±0.020*	2.312±0.025*
4	MEGO 200	200 mg/kg	1.807±0.007	1.915±0.013	2.018±0.007
5	MEGO 400.	400 mg/kg	1.813±0.008	1.983±0.003*	2.110±0.003*
6	AEGO 200	200mg/ kg	1.8±0.08	1.888±0.006	1.99±0.002
7	AEGO 400.	400mg/ kg	1.803±0.008	1.948±0.024*	2.035±0.014*

Values are mean ±SEM; Statistical data was carried out by One way ANOVA, * indicates P<0.05, n=6, F value-6.441 and df is 10.

From Table -9, it was found that the diabetic animals have lower HDL cholesterol level, the animals treated with MEGO and AEGO for a period of 28days shows significant increase in HDL cholesterol level when compared with the standard and control group.

Estimation of Reduced Glutathione

TABLE-10. Effect of Different extracts of *Glinus oppositifolius* on reduced glutathione levels in rats different organs.

SL.NO	GROUPINGS (n =6)	Liver	Kidney	Heart
1	Normal control	0.96±0.004*	0.87±0.006*	0.77±0.003*
2	Diabetic control	0.17±0.003*	0.21±0.003*	0.30±0.004*
3	Standard group	0.89±0.020*	0.715±0.009*	0.575±0.004*
4	MEGO 200	0.69±0.007*	0.58±0.006*	0.53±0.006*
5	MEGO 400	0.80±0.005*	0.64±0.008*	0.56±0.018*
6	AEGO 200	0.62±0.013	0.51±0.006	0.44±0.013
7	AEGO 400.	0.71±0.005*	0.56±0.01*	0.52±0.007*

Values are mean ±SEM; Statistical data was carried out by One way ANOVA, * indicates P<0.05, n=6, Fvalue-2.891 and df value is 10 and 22.

From Table -10, it was found that the diabetic animals have lower reduced glutathione level, the animals treated with MEGO and AEGO for a period of 28days shows significant increase in reduced glutathione level when compared with the standard and control group.

Discussion

From the preliminary phytochemical analysis it was found that MEGO and AEGO contains significant amount of various alkaloids, carbohydrates, cardiac glycosides particularly flavones and flavonoids, steroids and sterols. So MEGO and AEGO subjected for determination of biological activity.

The mean percentage inhibition of MEGO (± SEM) in in-vitro antioxidant activity was found to be 54.073±0.712 on 0 hour, 56.107±0.614 on 24 hour, 64.057±1.608 on 48 hour and 69.393±0.725 on 60 hour. Which was found to be significant (p <

0.05) effect when compared with the standard alpha-tocopherol. The α-tocopherol show a percentage inhibition mean (± SEM) of 60.333±0.882 on 0hour, 66.333±0.882 on 24hour, 71.667±0.882 on 48 hour and 80±1.55 on 60 hour. The mean percentage inhibition of AEGO was 39.46±0.515 on 0 hour, 43.33±0.452 on 24 hour, 51.98±1.011 on 48 hour and 55.267±0.783 on 60 hour which is very significant when compared with standard α-tocopherol. Percentage inhibition of DPPH radicals for MEGO was 72.577±0.153. The mean percentage inhibition of standard was 82.970±2.370, and the mean percentage inhibition of AEGO was 67.1±0.391. From the above result it was concluded that MEGO and AEGO possesses antioxidant activity, but the activity shown by methanolic fraction has more significant compare to standard.

The inhibition of hemoglobin glycosylation for MEGO 2000, 1000,500 and 250µgm/ml were 84.298±0.364, 78.427±0.358, 69.753±0.357 and 60.690±0.299 percent. The mean percentage inhibition of standard was 87.937±0.325. And the mean percentage inhibition of AEGO 2000, 1000,500 and 250µgm/ml were 81.720±0.167, 76.033±0.261, 69.753±0.357, and 60.690±0.299.From the above result it was concluded that both methanolic and aqueous extract possess Antioxidant activity. MEGO and AEGO treated diabetic animals sustains their bodyweight when compared with standard and control group. The values shown in the Table-6 reveals the fasting serum glucose in the normal group of rats was maintained within the normal range throughout the period of study.

The MEGO (200mg and 400mg/ kg extract) treated diabetic rats show a mean (± SEM) serum cholesterol of 101.833±0.601 mg/dl and 102.667±1.085 mg/dl on 0 day, 81.333±0.558 mg/dl and 79.333±0.494 mg/dl on day 14, 72.333±0.558 and 71.667±0.333 on 28^{th} day respectively, which was found to be significantly ($p < 0.05$) reduced as against the serum cholesterol of untreated diabetic rats during the entire study period. The AEGO (200mg and 400mg/kg extract) treated diabetic rats show a mean (± SEM) serum cholesterol of 101.833±0.601mg/dl and 102.667±0.882 mg/dl on day 0; 84.333±1.202 mg/dl and 87.833±0.477 mg/dl on day 14; and 73.167±1.6 and 71.667±0.333 on 28^{th} day respectively. These changes in serum cholesterol values illustrate that the diabetic rats treated with of methanolic and aqueous extract (all the doses) show a significant ($p \leq 0.05$) reduction in serum cholesterol during the treatment period in comparison to the untreated diabetic group of

rats. In these groups MEGO (400 mg/kg), AEGO (200mg/kg) the serum cholesterol is not only reduced significantly when compared with untreated diabetic rats but the values are also comparable with those of normal rats and the metformin treated diabetic rats. The above observations indicate that the treatment of diabetic rats with the MEGO reduces the serum cholesterol of diabetic rats at all the tested dose levels during the treatment period.

The serum triglyceride of different groups of animals during the period of study is given in Table No. 8 and observation reveals that the treatment of diabetic rats with the MEGO 400mg/kg reduces the serum triglyceride of diabetic rats at all the tested dose levels during treatment period.

The changes in serum HDL Cholesterol values (table-9) illustrate that the diabetic rats treated with *Glinus oppositifolius* show a significant increase in serum HDL Cholesterol in treatment period in comparison to the untreated diabetic group of rats at all dose level. In these groups i.e. MEGO (400mg/kg) the serum HDL Cholesterol is not only increased significantly when compared with the untreated diabetic rats but the values are also comparable with those of normal rats and metformin treated diabetic rats. These above observations indicate that the treatment of diabetic rats with the MEGO 400mg/kg increases the serum HDL Cholesterol of diabetic rats at all the tested period.

Liver, kidney and heart tested for their GSH content of animal groups treated with metformin and the extracts show the glutathione values which are comparable with normal group of rats. In the above we found that MEGO and AEGO have significant effect when compared with diabetic and normal control group.

Conclusion

The present study shows that methanolic and aqueous extract of Glinus oppositifolius in the doses of 200 and 400mg/kg are able to produce a consistent reduction in serum glucose, serum cholesterol and serum triglyceride. The extracts have also shown presence of active constituents responsible for various biological activities. These plant extracts have also shown significant *in-vitro* and *in-vivo* antioxidant activity.

From the above findings, it is suggested that the methanolic and aqueous extracts of *Glinus oppositifolius* can be chosen as primary antihyperglycemic, antihyperlipidemic and antioxidant supplement. Further investigation is expected to isolate and characterize the active principle of these extracts. Clinical evaluation will throw more light on clinical usefulness, safety, and efficacy of this plant extract.

Acknowledgement

I am sincerely thankful to Principal and management of Gautham College of Pharmacy for providing such a wonderful facilities to carry out this research work.

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