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Evaluation Of Antidiabetic And Antioxidant Activity Of Root Extracts Of *Herbal Mixture (Catharanthus Roseus, Leucas Linifolia)*

SriVidya. Manthena[1*], Rajender.Arutla[2], P. Chandra Mohan[1], Dr. Bikuntha Prusty[1]

[1] *Department of Pharmacology, Tallapadmavathi college of pharmacy Urus,Warangal-Telangana*
[2] *Department of Pharmacology, Trinity college of pharmaceutical sciences, Peddapalli,-Telengana*
**Corresponding author- Sri Vidya. Manthena*

Abstract: *In the present study, administration of the extract of herbal formulation MEE and MAE at a dose of 2000mg/kg P.O produced a significant reduction in blood glucose level .The anti oxidant activity of herbal formulation is found with a significant growth of CAT and SOD levels compared with normal control and alloxan induced diabetic control animals. Because of presence of free radical scavenging activity of the secondary metabolites in the herbal formulation, MEE and MAE showed significant decrease in levels of SGOT, SGPT, and ALP. In herbal formulation mainly the MEE and MAE showed significant decrease in Cretinine, Urea, Cholesterol and significant increase in HDL-Cholesterol level.*

Keywords: *free radical scavenging activity, Catharanthus roseus, Leucas linifolia, Hypoglycemia, intraperitoneal administration*

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I.Introduction

Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by ineffectiveness of insulin produced, such a deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body systems in particular the blood vessels and nerves.

Diabetes is classified in to two

Type I or insulin dependent diabetes mellitus (IDDM) or juvanile onset diabetes

Type II or Non insulin dependent diabetes mellitus (NIDDM) or maturity onset diabetes.

Type-I

It is an insulin dependent diabetes mellitus; the majority of type 1 diabetes is of the immune mediated nature, where beta cell loss is a T-cell mediated autoimmune attack. Also viral infection may damage pancreatic beta cell and expose antigen that initiate a self perpetuating auto allergic process. Such patients regularly should take insulin otherwise they will ultimately die with diabetes ketoacidosis.

Type II

Type-II diabetes mellitus is a Non insulin dependent diabetes mellitus persist mainly in adults with obese, and is due to relative insulin deficiency and insulin resistance. This insulin resistance is may be due to the mutations of insulin receptors. Type II diabetes requires long term treatment to keep the blood glucose balance, and long term complications of the disease**.** Weight reduction, physical exercise and dietary modification will decrease insulin resistance and keep the blood glucose levels in limits.

To control of diabetes there are many allopathic drugs are available in the treatment. But these are not safe, because these drugs showing less efficacy and more adverse reactions. Thus there is a need of more effective and less toxic agents for the treatment of various secondary complications of diabetes. The ultimate attractive source is Plants formulations[1]. Herbal mixture formulations, the name itself are indicating as multiple ingredients of different herbal origin. The plant ingredients may have wide spectrum of biological activities. These formulations having different active constituents with different mechanism of actions which can produce combined action against various complications of diabetes[2]**.**

II. Material And Methods

Collection and preparation of plant extract:

Two plants ***Leucas linifolia***, ***Catharanthus roseus*** were collected from the surroundings of Kakatiya University, Warangal, Telangana, India. Collected plant roots were first washed several times with sterilized distilled water to remove the dust particles. The roots were dried at room temperature and coarsely powdered. About 10g powder of the plants was extracted using ethanol, and distilled water for 48 h in 200 ml at 120 rpm in rotary shaker at room temperature. The extraction was twice repeated and filtered through glass funnel and Whatman filter paper no. 1. Each filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. Finally the dry extracts were lyophilized and stored for further analysis.

Phytochemical analysis

The extracts obtained were subjected to preliminary phytochemical screening for the presence alkaloids, flavonoids, tannins, saponins, proteins and sugar by the methods described by Harborne (1998) and Kokate (2001) [3,4].

Experimental animals

Healthy wister rats of either sex (200 – 225g) were used in the study. The animals were kept in polyacrylic cages and maintained under standard having conditions of temperatures (24-27 degree Celsius) and humidity (60 – 65%) within 12 hours light 12h dark cycle. They were acclimatized for 10 days. Food was provided in the form of dry pellets and water ad libitum. The experiments were performed based on animal ethics of guidelines of university animals ethics committeeStudies The animals were randomly distributed into nine different groups with six animals in each group[35]. This study was carried out with approval from the Institutional animal Ethics Committee (**1505/PO/a/11 CPCSEA 2011)**

Invivo Anti diabetic activity

Induction of diabetes:-

Diabetes was induced in rats by injecting 120 mg/kg of alloxan monohydrate intraperitoneally in 0.6% w/v CMC to overnight fasted rats. After 72 hrs of injection; fastig blood glucose was measure. The animals did not develop more than 250 mg/dl glucose lever were rejected.

Experimental Design:

The selected animals were divided into nine groups.

Group I: Animals received a single dose of 0.6 % w/v (2ml/kg) CMC, P.O of the vehicle, on 7 consecutive days

Group II: Animals received a single dose of alloxan 120mg/kg (I.P) and 0.6 % w/v (2ml/kg) CMC, P.O of the vehicle, on 7 consecutive days

Group III: Animas received a single dose of LNEE (2000 mg/kg body weight, P.O) and alloxan 120mg/kg (I.P), on 7 consecutive days

Group IV: Animals received a single dose of CREE (2000 mg/kg body weight, P.O) and alloxan 120mg/kg (I.P), on 7 consecutive days

Group V: Animals received a single dose of MEE (2000mg/kg body weight, P.O) and alloxan 120mg/kg (I.P), on 7 consecutive days

Group VI : Animals received a single dose of LLAE (2000 mg/kg body weight, P.O) and alloxan 120mg/kg (I.P), on 7 consecutive days

Group VII :Animals recieved a single dose of CRAE(2000 mg/kg body weight, P.O) and alloxan 120mg/kg (I.P), on 7 consecutive days

Group VIII : Animals received a single dose of MAE(2000 mg/kg body weight, P.O) and alloxan 120mg/kg (I.P), on7 consecutive days

Group IX: Animals received a single dose of glibenclamide (5mg/kg body weight, P.O) and alloxan 120mg/kg (I.P), on7 consecutive days

At the end of the 7th day the rats were fasted for 16h and blood parameters were determined.

Biochemical Analysis:

Fasting blood samples were collected from the retro-orbital sinus of each rat and subjected for estimation of fasting blood glucose on Day 1, 3, 7 day of the experiment. The plasma was separated by centrifugation (5min, 5000rpm) and was analysed for lipid profiles (Serum cholesterol, HDL cholesterol), Serum creatinine, serum urea, serum glutamate oxygenate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and Alkaline phosphatase (ALP), and SOD, CAT. The plasma profiles were estimated by enzymatic method using reagent kit procedural guidelines and details

Biochemical Estimations for Antioxidant Status of Plant Extracts

A. Super oxide dismutase (SOD)Assay[5,6]

1 ml of tissue sample, 0.25 ml of absolute ethanol and 0.15 ml of chloroform was taken into test tube, shaken for 15 min in a mechanical shaker. This suspension was centrifuged at 3000 rpm for 5min and the supernatant was collected. To 0.5ml supernatant, add 2ml of 0.1M tris Hcl and 0.5ml of 2m mole pyrogallol was added and volume was made upto 4ml with water. The rate of auto oxidation of pyrogallol was noted at 420nm against blank and compared with standard. The SOD levels were expressed as nM/mg protein.

B. Enzymatic assay of Catalase (CAT)[7]

Catalase activity was measured by the method of Aebi. 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (apH 7.0).Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H2O2. The rate of decomposition ofH2O2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/ mg protein.

In Vitro- Anti Oxidant Activity

1) Nitric oxide (NO) scavenging [8]:

Nitric oxide scavenging activity was measured by using spectrophotometer. Sodium nitroprusside (5 mM) in phosphate buffer saline was mixed with different concentrations of MEE and MAE (25-800 µg/ml) dissolved in normal saline and incubated at 25oC for 30 min. A control without test compound but with equivalent amount of sodium nitroprusside was taken. After 30 min 1.5 ml of the incubation solution was removed and diluted with 1.5 ml of GRIESS reagent (1% Sulphanilamide, 2%phosphoric acid, and 0.1% napthyl ethylene diamine dihydrochloride). The absence of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthyl ethylene diamine dihydrochloric acid was measured at 549 nm. Vitamin-E was used as reference standard.

2) 1-1Diphenyl, 2-picryl hydrazyl(DPPH) radical scavenging activity[9]:

DPPH scavenging activity was measured by spectrophotometric method. 0.1 mM solution of DPPH was prepared in ethanol and 1 ml of this solution was added to 3ml of MEE and MAE in normal saline at different concentrations(25-800ml). Equalamount of normal saline was added to the control. The mixture was shaken well and incubated at room temperature for 30 min. the absorbance was read at 517 nm using a spectrophotometer. Vitamin-E was used as reference standard. All the assays were read at particular nm using spectrophotometer, UV-1601 schimadzu model.

III. Results And Discussion

PhytoChemical Analysis of Herbal Formulations.

Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, tannins, saponins, and phenols in herbal mixture formulation.

Acute oral toxicity of Herbal formulations in mice :

Acute toxicity studies revealed that both extracts are relatively nontoxic upto 2000mg/kg/body weight /p.o indirectly pronouncing the safety profile of extracts. Chronic toxicity studies should be instituted to rule out the toxicity profiles, if any on long time treatment.

Invitro anti oxidant methods :

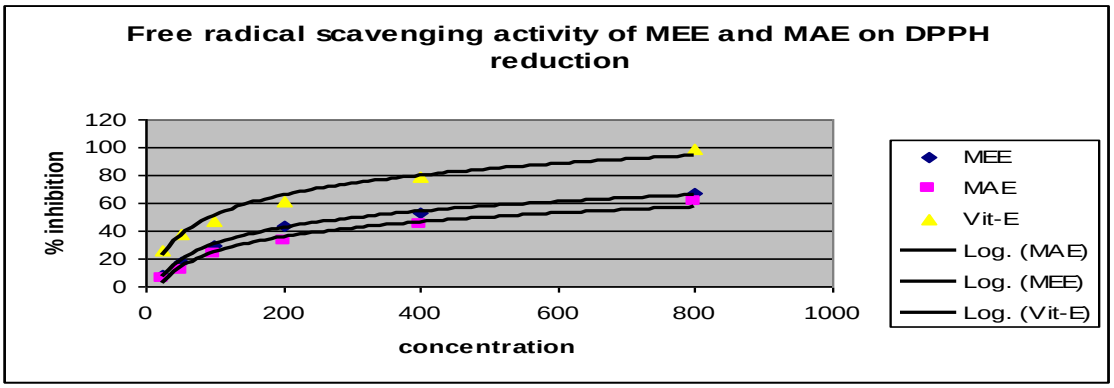
A 1-1 Diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity :

MEE and MAE showed promising free radical scavenging effect in the reduction of DPPH in concentration dependent manner at a concentration of 25-800 µg/ml. The IC_{50} values of MEE and MAE were found to be 459.69 µg/ml (r=0.915) and 568.5 (r=0.950), respectively. The IC_{50} value of vit-E was 164.6 µg/ml (r=0.954) (Table-1, Fig-1).

Table 1: Free radical scavenging activity of MEE and MAE by DPPH reduction (Values are mean ± SEM of six replicates)

Concentration (µg/ml)	% Inhibition		
	MEE	MAE	Vitamin-E
25	8.28 ± 0.04	5.86 ± 0.07	26.30 ± 0.109
50	17.54 ± 0.06	12.31 ± 0.12	37.66 ± 0.02
100	29.35 ± 0.07	23.74 ± 0.04	47.30 ± 0.03
200	43.03 ± 0.08	33.07 ± 0.09	60.60 ± 0.10
400	52..49 ± 0.50	44.14 ± 0.12	79.19 ± 0.08
800	66.98 ± 0.02	60.75 ± 0.05	98.77 ±0.03
IC_{50} and confidence Interval	459.7 ± 0.50	568.6 ± 0.228	164.71 ± 0.18

Figure:1



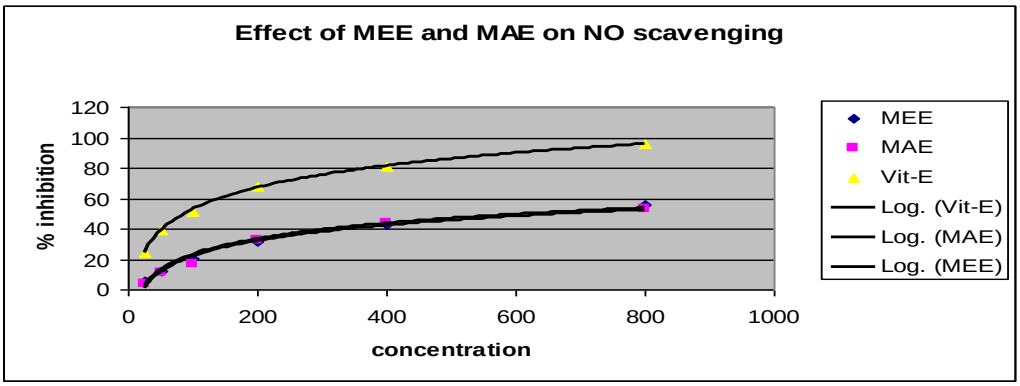
B Nitri oxide (NO) scavenging activity:

MEE and MAE showed promising free radical scavenging effect against nitric oxide induced release of free radicals in a concentration dependent manner. The IC_{50} values of MEE and MAE were found to be 638.35 µg/ml (r=0.931) and 645 µg/ml (r=0.921), respectively. The IC_{50} value of vit-E was 142.2 µg/ml (r=0.909) (Table- 2, Fig-2).

Table 2: Nitric oxide scavenging activity of MEE and MAE

Concentration (µg/ml)	% Inhibition		
	MEE	MAE	Vitamin-E
25	5.4 ± 0.04	4.16 ± 0.02	24.20 ± 0.16
50	12.4 ± 0.08	10.86 ± 0.21	39.46 ± 0.02
100	20.5 ± 0.06	16.8 ± 0.04	50.82 ± 0.01
200	31.8 ± 0.06	32.3 ± 0.08	67.82 ± 0.01
400	43.2 ± 0.07	44 ± 0.02	81.05 ± 0.01
800	56.1 ± 0.02	53.1 ± 0.01	96.18 ± 0.01
IC_{50} and confidence interval	637.8 ± 0.07	645 ± 0.04	142.19 ± 0.02

Figure:2



Screening of anti hyperglycemic activity of Herbal formulations in alloxan induced diabetic rats.

The effects of herbal formulations on fasting blood glucose level in alloxan induced diabetic rats were given in table.No-3 and Figure-3. When compared with Normal group, Diabetic group is significant, because blood glucose level are increased. When compared with Diabetic group, Standard and Treated groups(LNEE, CREE, MEE, LNAE, CRAE, MAE) are significant, because blood glucose level are significantly decreased. When compared with Standard group, Treated groups(LNEE, CREE, MEE, LNAE, CRAE, MAE) less significant, because blood glucose level are not significantly decreased. When compared with MEE group, LNEE, CREE, groups are less significant, because blood glucose level are not significantly decreased. When compared with MAE group, LNAE, CRAE, groups are less significant, because blood glucose level are not significantly decreased.

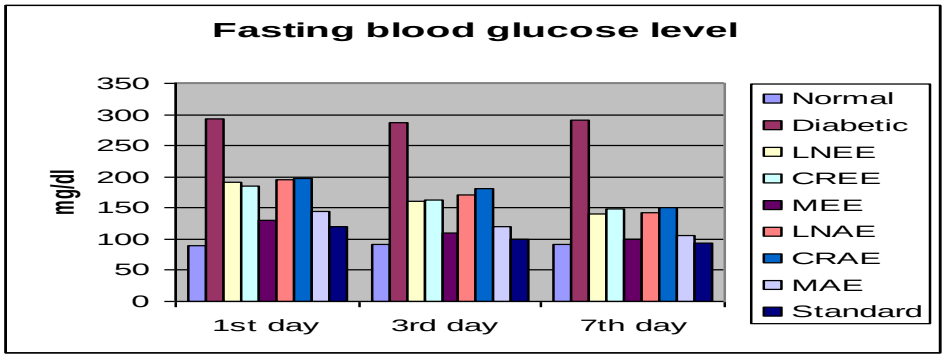
Table No-3. The effect of herbal 6-different Formulations on fasting blood glucose level in Diabetic induced Rats.

Fasting Blood Glucose level (mg/dl)

TREATMENT	1st DAY	3rd DAY	7th DAY
Normal (CMC 0.6%)	90.46±0.36	92.44±0.39	92.48±0.43
Diabetic control (alloxan 120mg/kg)	293.55±0.44[a]	286.5±0.41[a]	291.58±0.43[a]
LNEE (2000mg/kg)	190.3±0.43 [b,c,d]	160.3±0.36[b,c,d]	140.26±0.37[b,c,d]
CREE (2000mg/kg)	185.35±0.35[b,c,d]	162.25±0.70[b,c,d]	148.01±0.70[b,c,d]
MEE (2000mg/kg)	130.2±0.40[b,c]	110.3±2.23[b,c]	100.26±0.36[b,c]
LNAE (2000mg/kg)	195.3±0.43[b,c,e]	170.2±0.43[b,c,e]	142.3±0.41[b,c,e]
CRAE (2000mg/kg)	198.5±0.37[b,c,e]	180.26±0.43[b,c,e]	150.3±0.41[b,c,e]
MAE (2000mg/kg)	145.20±0.43[b,c]	120.2±0.42[b,c]	105.2±0.40[b,c]
Standard (glibenclamide5mg/kg)	120.3±0.39[b]	100.23±0.36[b]	93.31±0.37[b]

The represented data was in Mean±SEM(n=6) (a= ***, b= ###, c= **, d= ++, e= ##)
***(p< 0.001) vs Normal, ### (p< 0.001) vs Diabetic, **(p< 0.01) vs Standard, ++(p< 0.01) vs MEE, ##(P< 0.01) MAE

Figure.3



Effect of Herbal formulations in anti oxidant study.

The effect of Herbal formulations (MEE,MAE,LNEE,CREE,LNAE and CRAE) on Pancreatic antioxidant enzymes were given in table. No-4 and figure -4.

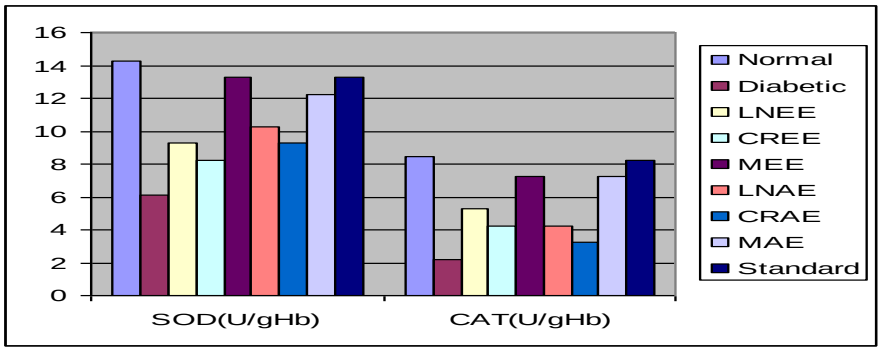
When compared with Normal group, Diabetic group is significant, because SOD,CAT level are decreased. When compared with Diabetic group, Standard and Treated groups(LNEE, CREE, MEE, LNAE, CRAE, MAE) are significant, because SOD,CAT level are significantly increased. When compared with Standard group, Tteated groups(LNEE, CREE, MEE, LNAE, CRAE, MAE) are less significant, because SOD,CAT level are not significantly increased. When compared with MEE group, LNEE, CREE, groups are less significant, because SOD,CAT level are not significantly increased. When compared with MAE group, LNAE, CRAE, groups are less significant, because SOD,CAT level are not significantly increased.

Table No-4. The effect of herbal formulation on pancreatic anti oxidant enzymes in Alloxan intoxicated rats

No of Groups	Treatment	SOD (units/min/mg protein)	CAT N mol H2O2 (consumed/min/mg protein)
I	Normal (CMC0.6%)	14.23±0.43	8.45±0.30
II	Diabetic Alloxan (120mg/kg)	6.1±0.28[a]	2.2±0.26[a]
III	LNEE (2000mg/kg)	9.25±0.33[b,c,d]	5.3±0.28[b,c,d]
IV	CREE (2000mg/kg)	8.3±0.27[b,c,d]	4.25±0.28[b,c,d]
V	MEE (2000mg/kg)	13.2±0.27[b,c]	7.3±0.56[b,c]
VI	LNAE (2000mg/kg)	10.25±0.28[b,c,e]	4.2±0.28[b,c,e]
VII	CRAE (2000mg/kg)	9.3±0.33[b,c,e]	3.25±0.34[b,c,e]
VIII	MAE (2000mg/kg)	12.2±0.28[b,c]	7.3±0.17[b,c]
IX	Standard Glibenclamide(5mg/kg)	13.25±0.37[b]	8.2±0.40[b]

The represented data was in (Mean±SEM). (a= ***, b= ###, c= **, d= ++, e= ##)
***(p< 0.001) vs Normal, ### (p< 0.001) vs Diabetic, **(p< 0.01) vs Standard, ++(p< 0.01) vs MEE, ##(P< 0.01) MAE

Fig.4,5 Effect of herbal formulations on Pancreatic antioxidant enzymes, SOD, and CAT in alloxon treated rats.



The Effect of Herbal formulations on Pancreatic function test parameters.

In table No-5 and 6 the represented data belongings to changing of pancreatic test parameters due to administration of herbal formulations. When compared with Normal group, Diabetic group is significant, because SGOT, SGPT, ALP, level are increased and When compared with Diabetic group, Standard and Treated groups(LNEE, CREE, MEE, LNAE, CRAE, MAE) are significant, because SGOT, SGPT, ALP, level are significantly decreased . When compared with Standard group, Tteated groups(LNEE, CREE, MEE, LNAE, CRAE, MAE) less significant, because SGPT,SGOT,ALP level are not significantly decreased. When compared with MEE group, LNEE, CREE, groups are less significant, because SGOT,SGPT,ALP level are not significantly decreased. When compared with MAE group, LNAE, CRAE, groups are less significant, because SGOT,SGPT,ALP level are not significantly decreased (figure-5).

When compared with Normal group, Diabetic group is significant, because Creatinine, Urea, Cholesterol level are increased and HDL level decreased. When compared with Diabetic group, Standard and Treated groups(LNEE, CREE, MEE, LNAE, CRAE, MAE) are significant, because Creatinine, Urea, Cholesterol level are significantly decreased and HDL level significantly increased. When compared with Standard group, Tteated groups less significant, because Creatinine, Urea, Cholesterol level are not significantly decreased and HDL level not significantly increased. When compared with MEE group, LNEE, CREE, groups are less significant, because Creatinine,Urea,Cholesterol level are not significantly decreased and HDL level not significantly increased. When compared with MAE group, LNAE, CRAE, groups are less significant, because Creatinine,Urea,Cholesterol level are not significantly decreased and HDL level not significantly increased (figure-6,7).

Table.5 The effect of herbal formulation on Pancreatic function test parameters

No of Groups	Treatment	Serum SGOT (IU/L)	Serum SGPT (IU/L)	Serum ALP (IU/L)
I	Normal (CMC 0.6%)	38.05±0.68	26.2±0.60	118.1±0.65
I I	Diabetic Alloxan(120mg/kg)	93.1± 0.68[a]	81.2± 0.60[a]	315.2± 0.17[a]
III	LLEE (2000mg/kg)	80.25± 0.66[b,c,d]	72.3±0.60[b,c,d]	260.3± 0.66[b,c,d]
IV	CREE (2000mg/kg)	85.3 ± 0.60[b,c,d]	70.2± 0.60[b,c,d]	250.25±0.60[b,c,d]
V	MEE (2000mg/kg)	44.2± 0.60[b,c]	31.3± 0.60[b,c]	132.3±0.66[b,c]
VI	LLAE (2000mg/kg)	82.3± 0.60[b,c,e]	75.25± 0.60[b,c,e]	270±0.60[b,c,e]
VII	CRAE (2000mg/kg)	84.25± 0.57[b,c,e]	72.5± 0.66[b,c,e]	275.3± 0.66[b,c,e]
VIII	MAE (2000mg/kg)	45.2± 0.66[b,c]	32.2± 0.60[b,c]	140.5 ± 0.60[b,c]
IX	Standard (glbenclamide 5mg/kg)	41.1± 0.60[b]	30.1± 0.66 [b]	125.3 ±0.66 [b]

The represented data was in Mean±SEM. (n=6). (a= ***, b= ###, c= **, d= ++, e= ##) ***(p< 0.001) vs Normal, ### (p< 0.001) vs Diabetic, **(p< 0.01) vs Standard, ++(p< 0.01) vs MEE, ##(P< 0.01) MAE

Table No-6. The Effect of herbal formulation on Pancreatic function test parameters in rats.

No. of gps	Treatment	Urea	HDL	Cholesterol	Creatinine
I	Normal (CMC0.6%)	20.3±0.54	24.5±0.65	34.04±0.57	0.42±0.03
II	Diabetic(Alloxan 120(mg/kg)	65.2±0.66[a]	7.23± 0.60[a]	110.25±0.57[a]	3.25±0.03[a]
III	LLEE (2000mg/kg)	55.25± 0.57[b,c,d]	12.8±0.60[b,c,d]	80.2±0.60[b,c,d]	.83±0.03[b,c,d]
IV	CREE (2000mg/kg)	60±0.60[b,c,d]	11.3±0.60[b,c,d,]	85.3±0.60[b,c,d]	.61±0.02[b,c,d]
V	MEE (2000mg/kg)	25.50± 0.95[b,c]	20.25±0.54[b,c]	40.25±0.66[b,c]	.825±0.04[b,c]
VI	LLAE (2000mg/kg)	57.8± 0.60[b,c,e]	10.5±0.60[b,c,e]	80.5±0.66[b,c,e]	.82±0.03[b,c,e]
VII	CRAE(2000mg/kg)	64.3 ±0.60[b,c,e]	11.8±0.66[b,c,e]	83.3±0.66[b,c,e]	1.73±0.07[b,c,e]
VIII	MAE (2000mg/kg)	28.25±0.60[b,c]	18.4±0.66[b,c]	42.25±0.66[b,c]	6.75±0.02[b,c]
IX	Standard (Glibenclamide5mg/kg)	23.3±0.83[b]	23.33±0.66 [b]	36.2 ±0.66 [b]	3.74±0.06 [b]

The represented data was in Mean±SEM. (n=6). (a= ***, b= ###, c= **, d= ++, e= ##)
***(p< 0.001) vs Normal, ### (p< 0.001) vs Diabetic, **(p< 0.01) vs Standard, ++(p< 0.01) vs MEE, ##(P< 0.01) MAE

Fig.6,7. The Effect of herbal formulation on Pancreatic function test parameters in rats

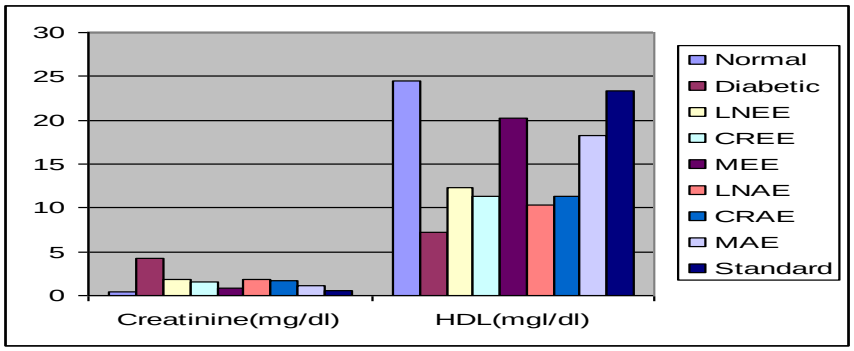
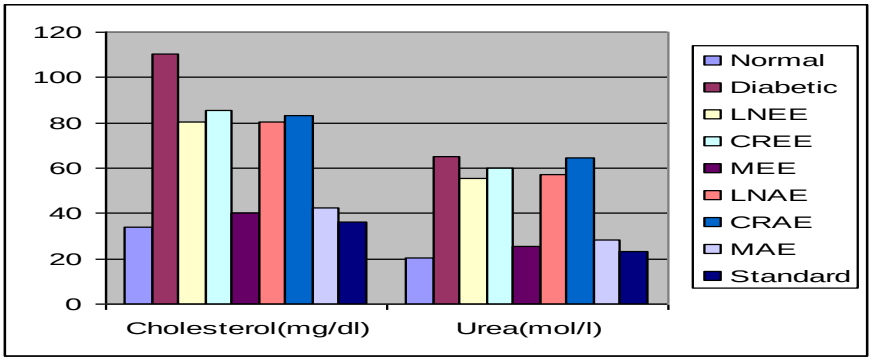


Fig.7. The Effect of herbal formulation on Pancreatic function test parameters in rats



IV. Discussion

Acute toxicity studies revealed that both extracts are relatively nontoxic upto 2000mg/kg/body weight /p.o indirectly pronouncing the safety profile of extracts. In the present study, the extract of herbal formulation MEE and MAE at a dose of 2000mg/kg P.O produced a significant reduction in fasting blood glucose level. The pronounced anti hyperglycemic effect may because of the synergistic effect of various active principles in the ingredients of herbal formulation.

The Pancreas exhibits a characteristic pattern of changes during diabetes. The increase in oxygen free radicals primarily due to increase in blood glucose levels, some of free radicals actively destroy β cells of pancreas, it leads to diabetes. The enzymatic antioxidant system CAT (Catalase) and SOD (Super Oxide Dismutase) counteract the free radicals generation and reduce the oxidative stress. The anti oxidant activity of herbal formulation is found with a significant growth of CAT and SOD levels compared with normal control and diabetic control animals. After treatment with herbal formulations the levels of SOD and CAT activities brought near to normal.

Because of presence of free radical scavenging activity of the secondary metabolites in the herbal formulation, MEE and MAE showed significant decrease in levels of SGOT, SGPT, ALP, Cretinine, Urea, Cholesterol and significant increase in HDL-Cholesterol level.

V. Conclusion

On the basis of above results it could be concluded that extract of herbal mixture formulation exerts significant hypoglycemic and anti oxidant activities. The mixture of herbal formulation MEE, MAE showing significantly more anti diabetic and anti oxidant activity compared with that of single herbal formulation.

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