Evaluation of Toxicity and Antidiabetic Activity of Ethanolic Extract of Flowers of *Moringa Oleifera* Against Dexamethasone Induced Hyperglycemia in Albino Wistar Rats

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Abstract-Diabetes is a defect in the ability of the body to convert glucose (sugar) to energy. Glucose is the main source of energy in our body. When food is digested it is metabolized into fats, proteins, or carbohydrates. Glucose is then transferred to the blood and is used by the cells for energy production. To investigate the antidiabetic effect ethanolic extracts of flowers of *Moringa oleifera* against dexamethasone induced insulin resistance in wistar albino rats. To study the antidiabetic effect, flowers of *Moringa oleifera* were collected and authenticated, extracted and investigated for acute toxicity and dexamethasone induced hyperglcemia. The animals treated with EEMOF at a dose of 100mg/kg and 200mg/kg prevented the development of hyperglycemia, hypercholesteremia and hypertriglyceridemia in dexamethasone induced insulin resistance models. Oral administration of *Moringa Oleifera* 100mg/kg and 200mg/kg reduces serum glucose, triglyceride, total cholesterols and LDL concentration and improve the concentration of HDL in dexamethasone administered rats. The lignin *Moringa Oleifera* showed significant anti-diabetic effect in rats after oral administration. The present study demonstrated that *Moringa Oleifera* could be useful in Management of diabetes associated with abnormalities in lipid profiles. Further study need to isolate, identify the active compounds and find out the possible mechanism of actions.

Keywords: *Moringa oleifera,* Dexamethasone, Hyperglycemia, Ethanolic Extract

Introduction

Moringa Oleifera is the most cultivated species of the genus *Moringa*, the only genus in the Moringaceae plant family. Common names include moringa, wand tree (long and thin triangular pods), horseradish (from the taste of roots, Reminiscent of horseradish) and Ben oil tree or benzoyl tree (from the oil that comes from the seeds)[6]. *M. Oleifera* is a fast-growing, drought-resistant tree native to the tropical and subtropical regions of southern Asia. It is widely cultivated for its young pods[7] and leaves used as vegetables and for traditional herbal medicine. It is also used for water purification. *M. Oleifera* is considered an invasive species[8]. t shows Pharmacognostical and Preliminary Phytochemical Studies[9], Adaptogenic Activity[10], anthelmintic activity[11], *in-vitro* antibacterial activity[12], Adaptogenic Activity[13] in Anti-Obesity Activity [14] etc.

Diabetes is a defect in the ability of the body to convert glucose (sugar) to energy. Glucose is the main source of energy in our body. When food is digested it is metabolized into fats, proteins, or carbohydrates. Glucose is then transferred to the blood and is used by the cells for energy production. For transferring of glucose, the hormone - insulin is needed which is mainly secreted by pancreatic beta cells. Diabetics mellitus is a type of metabolic disorder that is characterized by increased glucose production in the blood with disturbances in metabolism of carbohydrate, protein and fat mainly due to defects in insulin secretion, its action or both[1].

If this hyperglycemic stage of diabetes [2,3,4] persists for a long time, it is associated with long-term complications like improper functioning and failure of different organs causing deep damage to the eyes, kidneys, nerves, heart, and blood vessels [5].

Dexamethasone is an effective and greatly selective glucocorticoids used in the treatment of inflammation. Large exposure of glucocorticoids impairs insulin sensitivity, leads to the generation of metabolic syndrome as well as insulin resistance and hypertension. The mechanism which

dexamethasone induces peripheral insulin resistance is in inhibiting GLUT-4 translocation, and rising lipase activity in adipose tissue leads to cause impairment of Endothelium-dependent vasodilatation[15]. Dexamethasone increases the triglycerides levels causing an difference in lipid metabolism leads to hyperlipidemia and increases glucose levels causes to hyperglycemia Pharmacological doses of glucocorticoids induces ob gene expression in rat adipose tissue within 24 hrs and is followed by a complex metabolic changes ensuing in decrease in food consumption causing reduction in body weight and also occurred with by diabetes and generation of Insulin resistance with improved glucose and triglycerides levels. In this experiment administration of dexamethasone for 10 days resulted in improved glucose, triglycerides, cholesterol, insulin, levels[16].

Material and Methods

Plant Material

Plant material flower of *Moringa oleifera* was purchased from local market of Indore (M.P.).

Authentication: *Moringa oleifera* was authenticated at Department of Botany, Govt. Holkar Science College, Indore (M.P.).

Experimental Animals

Albino Wistar rats of both sexes were used in approximately the same age group after acclimatization for a week under laboratory conditions. They received a standard diet for rodent pellets (Lipton India) and *ad libitum* water. The animals had free access to food and water and were kept under a 12.12 h light and darkness cycle. All experiments were performed during the day from 9:00 am to 5:00 pm. The protocol was approved by the institutional committee on animal ethics and animal care was carried out in accordance with the guidelines of the committee for control and supervision in animal experiments (CPCSEA), representative of Animal Welfare, Government of India (Protocol No. SVCE/BIO/2019/004).

Instruments

UV-Visible Spectrophotometer (UV-1800 Shimadzu, Model, Mfg by Shimadzu Corporation), Centrifuge (Research centrifuge, Mfg by Remi Instruments Ltd, Mumbai), Tissue Homogenizer (Type: RO-127A Elect, IND.Ltd, Remi Instruments Division), Sonicator (Pci made in Mumbai) Milli pore water collector (Mfg by TKA smart pure Made in Germany), Soxhlet apparatus (Agarwal) Rotory evaporator (Medika instrument Mfg co.) UV chamber (Singhla sciences, Ambala).

Chemicals Used In this Study

Dexamethasone, glipinclamide, Heparin sodium injection I.P, Sodium lauryalsulphate L.R), Acetic acid(L.R), Thiobarbicturic acid (L.R), Tri chloro acetic acid (L.R), Phosphate buffer (K_2HPO_4) (L.R), 5,51-dithiobis(2-nitrobenzoic acid) 99% extra pure(L.R), Sulphosalicylic acid (L.R), Sodiumpyro Phosphate di basic (L.R), PhenazineMethosulphate (R & D), Nitro blue tetrazolium (L.R), Coomassie brilliant blue (Bradford Reagent) (R & D), Ethanol absolute (L.R), Sodium Phosphate dibasic (L.R),

Extraction and Identification of Phytoconstituents

Extraction of plant material

The air dried flower of *Moringa Oleifera* 500gms each were coarse powdered and extracted with ethyl alcohol. The crude extract was further filtered and evaporated by the aid of rotary evaporator. The final mass is weighed and preserved for further use[17,18].

Preliminary phytochemical screening

Preliminary phytoconstituents present in the hydroethanolic extract of *Moringa oleifera* plant were identified based on the chemical tests described by Kokate *et al.,* 1994[19].

***In-vivo* studies**

Acute oral toxicity study (OECD 423)

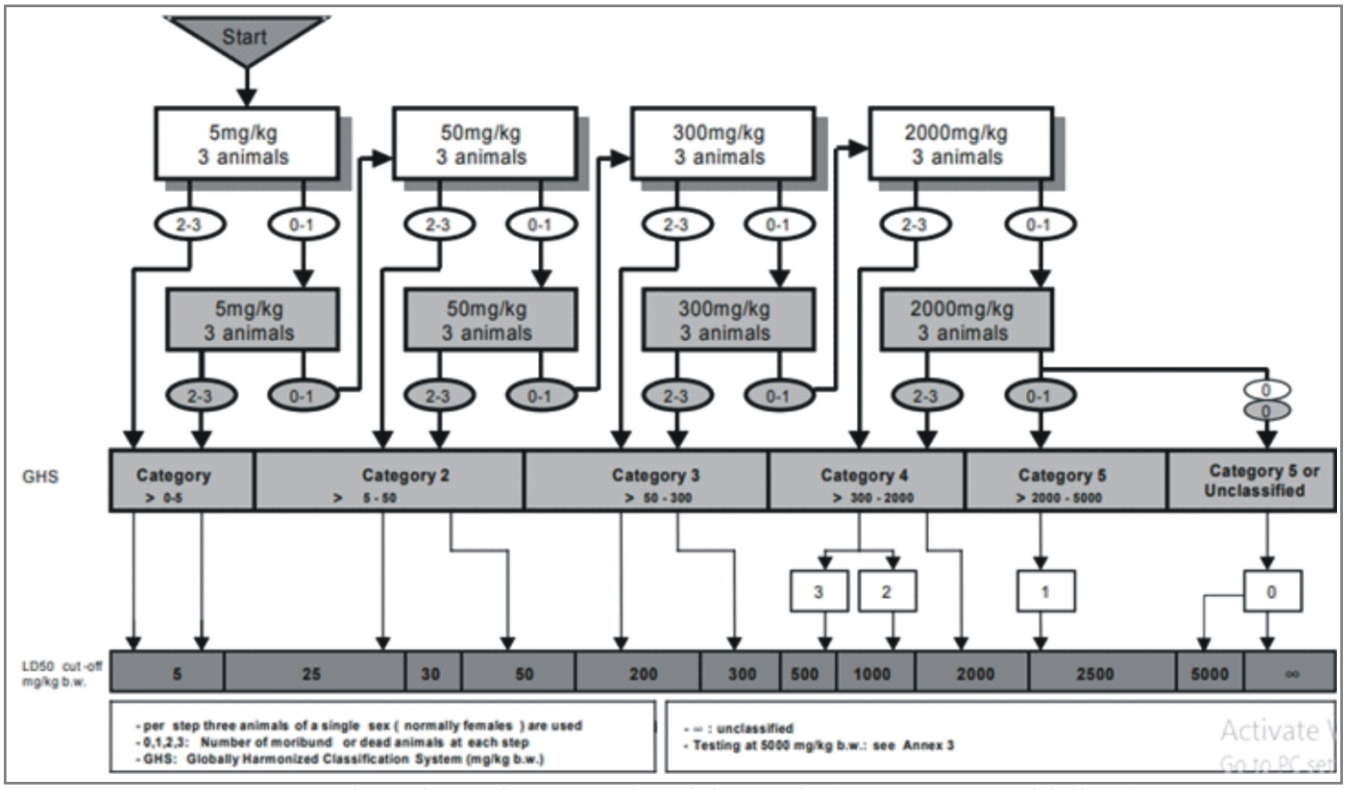

Figure-1 Flow chart of acute oral toxicity study (OECD 423 Guidelines)

To carry out the oral toxicity study, the OECD guidelines 423 were followed: it is a gradual procedure with three animals of one sex for each step. Depending on the mortality and/or 5, 50, 300, 2000 mg/kg body weight and the results allow classifying a substance as animal morbidity, some steps may be needed to judge the toxicity of the test substance. This procedure has an advantage over other methods due to the minimal use of animals and allows acceptable data. The method uses defined and classified doses according to the globally harmonized system. The initial dose for the ethanol extract was 2000 mg/kg of body weight (p.o). The dose was administered to rats that were fasting overnight with *ad libitum* water and observed for signs of toxicity. The same dose was tested once again with three other rats and was observed for 72 hours to detect symptoms such as change in skin color, salivation, diarrhea, sleep, tremors, convulsions and even respiratory, autonomous and CNS effects[20].

Sub acute toxicity study (OECD 407)

To carry out the subacute oral toxicity study, OECD 407 guidelines - A 28-day oral toxicity study was performed with repeated doses in rodents. The duration of the study was 28 days. The rat was given at a dose of 200 mg / kg. Each group is composed of ten animals (five animals / sex / group). The drug was administered orally once a day for 28 days. On the 29th the animals were anesthetized and the blood was collected by a retro-orbital puncture. The hematological parameters were evaluated. Serum was separated and biochemical parameters were estimated. The animals were sacrificed and the organs were removed and weighed. The organs were maintained in 10% formalin and used for histopathological analysis[21].

Hematological studies

The following hematological parameters were estimated by standard procedures.

Blood samples were drawn by cardiac puncture and haematological parameters were analyzed by autoanalyzer[22].

I. Total R.B.C. count
ii. Total W.B.C. count
iii. Differential leukocyte count
iv. Haemoglobin (Hb) concentration

Biochemical studies

Blood samples were drawn by cardiac puncture. Blood from three animals was pooled for serum separation. Each serum sample was analyzed by auto analyzer [23].

- Aspartate Aminotransferase (ASAT)
- Alanine Aminotransferase (ALAT)
- Alkaline Phosphatase (ALP)
- Total Bilirubin (TB)
- Total protein.

Experimental Design - Anti Diabetic

Experimental Protocol

30 Rats were divided in to 5 groups (n=6) and the duration of the experiment was 21 days with overnight fasting.

Groups	Treatment
Group-I	Rats received normal distilled water for 21 days.
Group-II	Rats received Dexamethasone 10 mg/kg i.p for a period of 10 days
Group –III	Rats received Dexamethasone 10 mg/kg i.p with low dose of drug 100mg/kg p.o
Group –IV	Rats received Dexamethasone 10 mg/kg i.p with high dose of drug 200mg/kg p.o
Group –V	Rats received Dexamethasone 10 mg/kg i.p along with standard drug Glipenclamide 500mg/kg p.o

At the end of the treatment period, the rats were deprived of food during the night and sacrificed on day 22 and the day under anesthetic with ether and beheaded after recording their final body weight. Blood was taken from each rat for biochemical estimation and the pancreas was quickly isolated, immersed in ice-cold saline and weighed. The pancreas was stored in a freezer (-20 ° C) to estimate the tissue's antioxidant parameters.

Evaluation of Biochemical parameters

Estimation of various biochemical parameters like blood glucose, serum cholesterol, serum triglycerides by using Span diagnostics kit. Estimation of serum HDL-Cholesterol and LDL-Cholesterol was performed by using S.D Kit.

Evaluation of *in-vivo* antioxidant activity

TBARS levels were determined by a modified version of the method described by **Ohkawa *et al*., 1979; Sapakal, 2008.** Glutathione was estimated by the method described by Ellman, 1959. SOD levels in the hearts were determined by the method of McCord and Firdovich method (1969) and modified by Kakkar. Estimation of Catalase by Goth, 1991; Sapakal, 2008.

Results

Percentage yield

The percentage yield of ethanolic extracts of roots of *Moringa Oleifera* was 16%.

Preliminary phytochemical tests

The phytochemical analysis of ethanolic extract of *Moringa Oleifera* revealed the presence of alkaloid, glycoside, sterols, phenols, tannins, flavanoids, terpenes, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, and phytosterols.

Table-1 Preliminary Phytochemical

S. No.	Constituents	Ethanolic Extract
1	Alkaloids	+ve
2	Carbohydrates	-ve
3	Proteins	+ve
4	Phenols	+ve
5	Tannins	+ve
6	Flavonoids	+ve
7	Glycosides	+ve
8	Saponins	+ve

-ve: indicate the absence of compound, **+ve:** indicate the presence of compound

Acute Toxicity studies

Table-2 Acute Toxicity studies of ethanolic extract of the plant *Moringa Oleifera*

Parameters observed	I hr	II hr	III hr	IV hr
Aggressiveness	+	+	+	+
Alertness	-	-	-	-
Alopecia	-	-	-	-
Circling	-	-	-	-
Diarrhoea	-	-	-	-
Edema	-	-	-	-
Eye closure at touch	+	+	+	+
Grip strength	+	+	+	+
Grooming	+	+	+	+
Lacrimation	-	-	-	-
Loss of writing reflex	-	-	-	-
Mortality	-	-	-	-
Nasal sniffing	-	-	-	-
Piloerection	-	-	-	-
Rearing	-	-	-	-
Righting reflex	-	-	-	-
Seizures	-	-	-	-
Straub tail	-	-	-	-
Urine stains	-	-	-	-

4 hours observation in acute toxicity studies of 2000mg/kg of *Moringa Oleifera*

Table-3 Observation in acute toxicity studies at the dose of 2000mg/kg bw p.o dose of *Moringa Oleifera*

Parameters observed	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Sub-Acute Toxicity Studies

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into groups of 10 animals (5 male and 5 female). The suspension of ethanolic extract was administered to rats at the dose of 100 & 200 mg/kg/day for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection.

Table-4 Signs of toxicity in sub acute toxicity (28 days)

Parameters observed	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 22	Day 24	Day 26	Day 28
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table-5 Change in body weight after the drug treatment *Moringa Oleifera*

Treatment	0^{th} day	5^{th} day	10^{th} day	15^{th} day	20^{th} day	25^{th} day	28^{th} day	% increase
Control	175.83±6.84	179.50±6.28	181.83±6.46	184.83±6.31	187.16±6.01	190.66±6.46	193.66±5.70	10.3637
100mg/kg	177.00±4.43	180.50±4.47	182.83±5.02	186.16±5.40	190.00±6.04	192.50±5.70	195.50±5.63	10.1695
200mg/kg	177.16±8.02	178.83±8.17	182.83±8.23	186.00±7.85	189.00±8.11	192.16±8.43	194.83±8.34	09.6044

Haematological Parameter

Table-6 Haematological Parameter

Haematological parameter	Control	*Moringa Oleifera*	
		100 mg	200mg
Total R.B.C. count ($\times 10^6$ mm−3).	9.09±0.15	8.90±0.12	9.11±0.16
Total W.B.C. Count ($\times 10^3$ mm−3).	12.67±0.22	12.35±0.15	11.23±0.23
Haemoglobin (Hb) (g/dl)	15.61±0.36	14.07±0.30	15.63±0.36
Hematocrit (%).	44.21±1.01	43.61± 1.72	36.4±1.36
Platelets (×103 mm−3).	834.91±24.01	867.21±23.25	739.81±26.86
Lymphocytes(%).	84.7±1.32	81.8±1.33	72.8±1.43
Neutrophils (%).	20.6±0.65	12.6±0.52	19.2±0.91

Data are expressed as mean ± SEM

Table-7 Biochemical Parameters

Biochemical parameter	Control	*Moringa Oleifera*	
		100 mg	200mg
Creatinine (mg/dl)	0.5890±0.079	0.6600 ± 0.049	0.5540 ±0.074
Urea (mg/dl)	15.30 ± 0.47	14.50 ± 0.40	15.20 ±0.57
Triglycerides (mg/dl)	52.20±1.13	51.40±1.08	47.10±1.62
Total Cholesterol (mg/dl)	46.60±1.21	51.40±1.08	54.03±1.67
Total protein (mg/dl)	4.40±0.26	4.20±0.35	3.70±0.26
Albumin (g/dl)	3.20±0.41	3.70±0.33	3.20±0.29
AST (IU/L)	121.41±2.68	121.3±1.65	116.61±2.045
ALT (IU/L)	69.40±1.57	67.60±1.301	68.60±1.108
ALP (IU/L)	112.6±4.67	117.01±0.714	117.41±0.718
T. Bilirubin (mg/dl)	0.2569±0.32	0.267±0.029	0.254±0.023

Dexamethasone induced diabetic model

Blood was collected from each rat for biochemical estimation and pancreas was quickly isolated immersed in ice cold saline and weighed. Pancreas was stored under freezer (-20 °C) for estimation of tissue antioxidant parameters.

Effect of *Moringa oleifera* on serum biochemical parameters

Blood glucose, Total cholesterol, HDL, LDL, VLDL, Triglycerides, levels were estimated in serum. The results were presented.

Serum level of Blood glucose

There is significant (P<0.0001) increase in the level of Blood glucose at group 2 when compared with Group 1. There is significant (P<0.001) decrease in the level of Blood glucose at group 3, 4 and 5 when Compared with group 2.

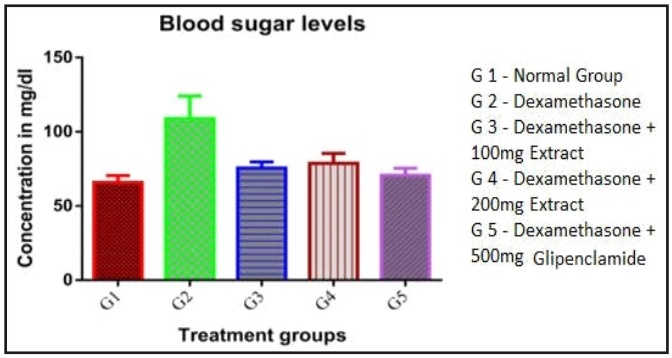

Figure-2: Effect of *Moringa Oleifera* on Blood Glucose Levels

Serum level of Total cholesterol

There is no significant increase in the level of Total cholesterol at group 2 when compared with group 1. There is significant increase (P<0.001) in the level of Total cholesterol at group 3 when compared to group 2. Group 4 showed significant increase (P<0.0001) in the level of total cholesterol compared to group2. There is significant(p<0.001) increase in the level of total cholesterol in group 5 compared to group.

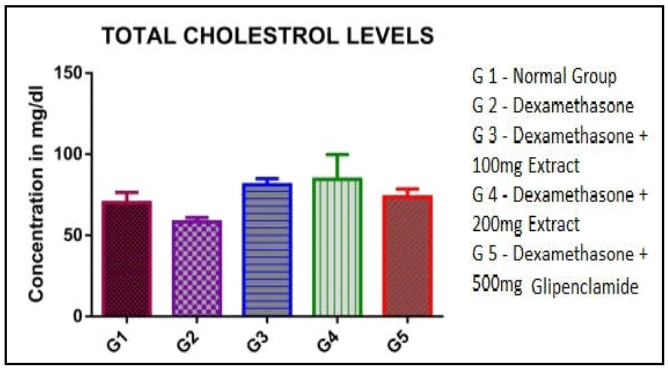

Figure-3: Effect of *Moringa Oleifera* on Total Cholesterol Levels

Effect of *Moringa Oleifera* on HDL

There is significance (p<0.01) increase in the level of ALP at group 2 when compared with group 1. There is significant (p<0.0001) decrease in the level of ALP at group 3 when compared to group 2. There is significance (p<0.01) increase in the level of ALP at group 4 when compared to group 2. There is no significance increase in the level of ALP at group 5 when compared to group 2.

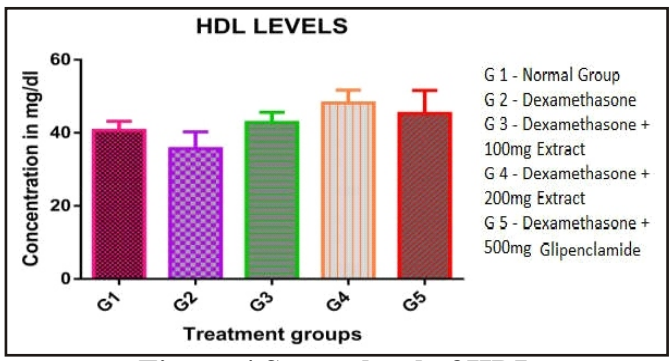

Figure-4 Serum level of HDL

Serum level of LDL

There is no significance increase in the level of LDL at group 2 when compared with group 1. There is significant increase (p<0.0001) in the level of LDL at group 3 when compared to group 2. There is significance (p<0.01) increase in the level of LDL at group 4, when compared to group 2. There is significance (p<0.0001) increase in the level of LDL at group 5 when compared to group 2.

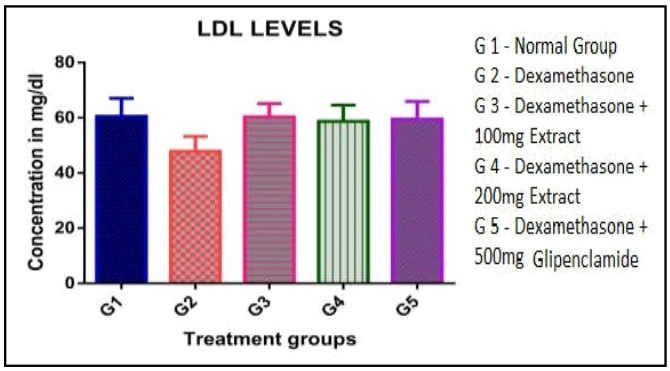

Figure-5 Serum level of LDL

Serum level of VLDL

There is no significance increase in the level of VLDL at group 2 when compared with group 1. There is significant ($p<0.0001$) increase in the level of VLDL at group 3, 4 and 5 when compared with group 2.

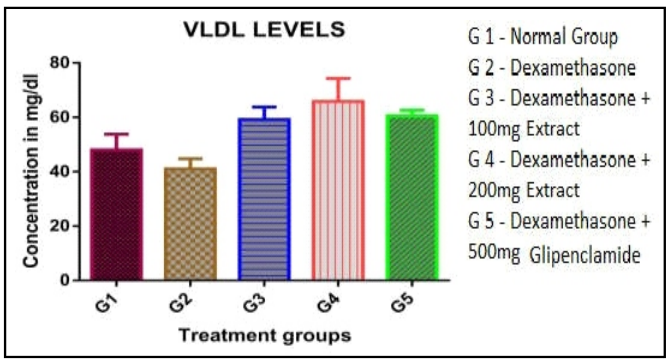

Figure-6 Effect of *Moringa oleifera* on VLDL level

Serum level of Triglycerides

There is no significance increase in the level of Triglycerides at group 2 when compared with group 1. There is significant ($p<0.0001$) increase in the level of cholesterol at group 3, 4 and 5 when compared with group 2.

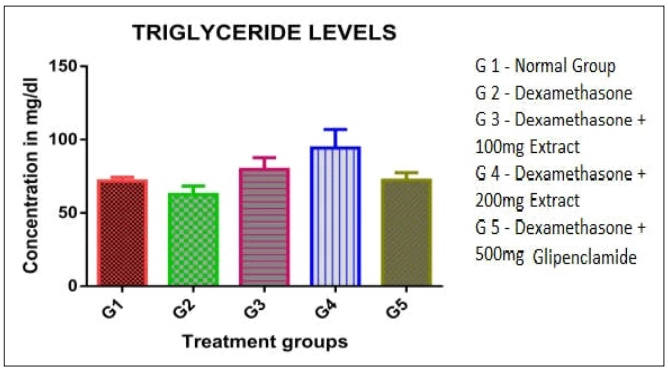

Figure-7 Serum level of Triglycerides

Effect of *Moringa oleifera* on tissue parameters

Pancreas are homogenized and TBARS, GSH, SOD, Catalase, levels were estimated.

The results were below.

Effect on TBARS

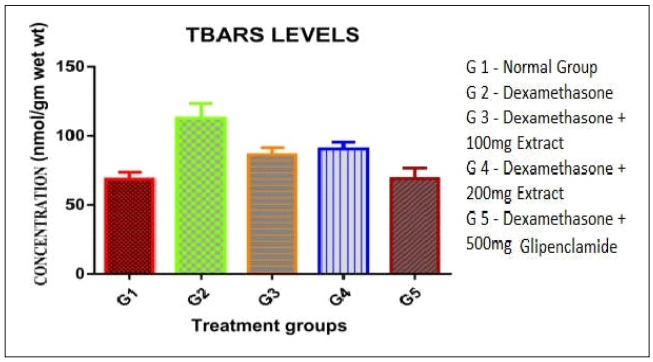

Figure-8 Effect of *Moringa oleifera* on TBARS levels

There is significance ($P<0.0001$) increase in the level of TBARS at group 2 when compared with group1. There is significance ($P<0.01$) decrease in the level of TBARS at group 3, 4, and5 when compared with group 2.

Effect on reduced glutathione (GSH)

There is significance decrease in the level of GSH at group 2 when compared to group There is significance ($P<0.0001$) increase in the level of GSH at group 3 when compared to group 2. There is significance ($P<0.0001$) increase level of GSH at group 4, 5 when compared with group 2.

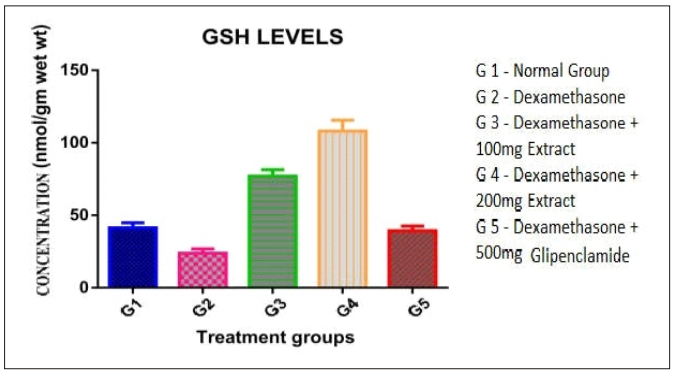

Figure-9 Effect of *Moringa oleifera* on GSH levels

Effect on superoxide dismutase (SOD)

There is significance decrease in the level of SOD at group 2 when compared with group 1. There is significance (P<0.0001) increase in the level of SOD at group 3,4 and 5 when compared with group 2.

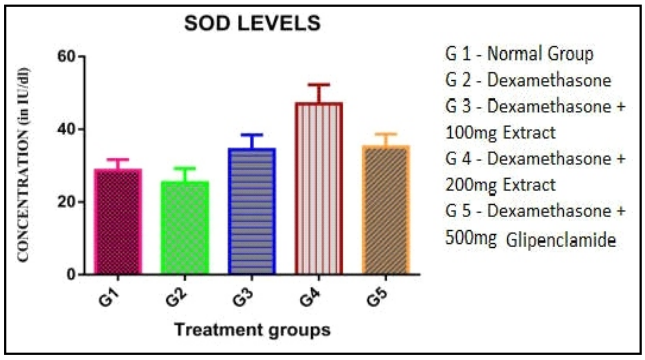

Figure-10 Effect of *Moringa oleifera* on SOD levels

Effect on Catalase (CAT)

There is significance decrease in the level of catalase at group 2 when compared to group 1. There is significance (p<0.0001) increase in the level of catalase at group 3 when compared with group 2. There is significance (P<0.0001) increase level of catalase at group 4, 5 when compared with group 2.

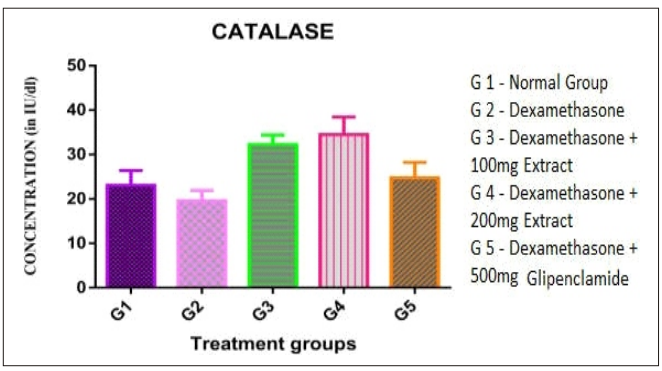

Figure-11 Effect of *Moringa oleifera* on Catalas

Conclusion

In current research, we investigated the effect of *Moringa Oleifera* on dexamethasone-induced insulin resistance models. Various biochemical estimates such as glucose, cholesterol. Triglycerides, LDL and HDL levels and antioxidant estimates such as TBARS SOD, CAT and GSH were estimated in two different doses of *Moringa Oleifera* (100 and 200 mg / kg p.o) and then compared with the standard and induced compound.

Moringa Oleifera at a dose of 100mg/kg and 200mg/kg prevented the development of hyperglycemia, hypercholesteremia and hypertriglyceridemia in dexamethasone induced insulin resistance models. Oral administration of *Moringa Oleifera* 100mg/kg and 200mg/kg reduces serum glucose, triglyceride, total cholesterols and LDL concentration and improve the concentration of HDL in dexamethasone administered rats. The lignin *Moringa Oleifera* showed significant anti-diabetic effect in rats after oral administration.

The present study demonstrated that *Moringa Oleifera* could be useful in Management of diabetes associated with abnormalities in lipid profiles. Further study needs to isolate, identify the active compounds and find out the possible mechanism of actions.

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