Sumia Fatima MS. *et al.* / *International Journal of Biological & Pharmaceutical Research.* 2013; 4(5): 340-348.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

ANTIDIABETIC ACTIVITY OF POLYHERBAL FORMULATION CONTAINING *MOMORDICA CHARANTIA, CARICA PAPAYA, TRIGONELLA FOENUM* AND *CURCUMA LONGA* (MCTC) IN ALLOXAN INDUCED DIABETIC RATS

Ms Sumia Fatima[1], Saheel Qureshi[1], G N Pramodini[1], Mahinder Singh Rathour[2], Vikram sharma[3], Jayesh Mehta[*3]

[1]Shadan College of Pharmacy, Peerancheru, Hyderabad, Andhra Pradesh -500008, India.
[2]Sanjeevan College of Pharmacy, Dausa (Raj.) -303303, India.
[3]Sri Balaji College of Pharmacy, Jaipur (Raj.) – 302013, India.

ABSTRACT

The aim of the present study was to evaluate the antidiabetic activity of polyherbal formulation containing *Momordica charantia, Carica papaya, Trigonella foenum and Curcuma longa* in alloxan induced diabetic rats. The polyherbal MCTC, is one of such herbal remedies prepared from the fruit of *Momordica charantia*, seeds of *Carica papaya*, seeds of *Trigonella foenum* and rhizome of *Curcuma longa* used to evaluate antidiabetic activity. The dose of the formulation was determined from acute toxicity study. The polyherbal formulation of MCTC had shown significant protection and lowered the blood glucose levels to normal in glucose tolerance test. The antidiabetic effect of polyherbal formulation was studied in Alloxan (120mg/kg b.w., i.p.) induced diabetes in male wistar rats for doses 200 mg/kg b.w. and 400 mg/kg b.w. (p.o.) daily for 21 days, and the effect was compared with oral dose of 5mg/kg, b.w. glibenclamide. The administration of extracts were continued for 21 days was evaluated through the estimation of HDL, LDL, VLDL levels, SGPT and SGOT levels, Total cholesterol levels and Total triglycerides levels. Blood samples were collected through the tail vein on days 1, 7, 14 and 21 after drug administration and the blood glucose levels were estimated using Accu-check glucometer. Diabetes caused by Alloxan treatment increases the level of glucose and biochemical parameter in blood sample but treatment with polyherbal formulation, protects from diabetes and significant decrease the elevated glucose, LDL, VLDL levels, SGPT and SGOT levels, Total cholesterol levels and Total triglycerides levels, increased in HDL level.

Key Words: Polyherbal formulation, Alloxan, Glibenclamide, Tween 80 (5%), Ethanol, Hexane, 5% glucose.

INTRODUCTION

Diabetes mellitus is a disease in which the body doesn't produce or properly use insulin. Insulin is a hormone produced in the pancreas, an organ near the stomach. Insulin is needed to turn sugar and other food into energy. When diabetes occurs, body can't make enough insulin or can't use its own insulin as it should, or both. This causes sugars to build up too high in the blood. (International Diabetes Federation) The body has to maintain the blood glucose levels at a very narrow range, which is done with insulin and glucagons. Insulin is a hormone produced by special cells called beta cells in the pancreas. (Anonymous 1).

Many different agents and strategies have been reported to ameliorate Alloxan induced diabetic in

Corresponding Author

Jayesh Mehta
Email: j.mehta17@yahoo.com

experimental animal (Maske H and Weinges K, 1957). Among them protection against diabetes was mainly focused on use of various antioxidants agents including the extract from medicinal plants with antioxidant properties.(Ramirez-Tortosa MC et al., 1999) However, none of these strategies have been found to be suitable/safe for clinical practices. We have observed that alloxan administration caused marked alteration in biochemical parameter in rat body (Anonymous 2). Various activities of polyherbal formulation containing components have been common/similar reported activity like Antioxidant (Anonymous 1) activity (Anonymous 3), Anti-viral activity (Anonymous 4), Anti-microbial activity (Anonymous 5), Glucose lowering effect (Brian J. Welch and Ivana Zib, 2004), cholesterol lowering effect. The aim of present study was evaluate to putative beneficial antidiabetic effect of polyherbal formulation was evaluated in the alloxan induced diabetes in rats.

MATERIALS AND METHODS

Collection and authentication of the plants

The fruit of the plant *Momordica charantia*, seeds of the plant *Carica papaya*, seeds of *Trigonella foenum* and rhizomes of *Curcuma longa* were collected from the local market. They were authenticated by Dr. S.J. Hussain, Director, Al-Arif General Hospital & Unani Research Institute, Bandlaguda, Hyderabad and former Director in charge of Central Research Institute of Unani Medicine, Department of Ayush, Government of India.

Chemicals

Alloxan, Glibenclamide, Tween 80 (5%), Ethanol, Hexane, 5% glucose, Sodium citrate.

EXTRACTION OF THE PLANTS

Mormodica charantia

500 grams of *Momrdica charantia* was washed thoroughly and seeds were removed. Then fruit was cut into pieces and shade dried. The dried fruit was extracted with 90% ethanol using soxhletion method.

Carica papaya

The *Carica papaya* fruits were cut into pieces and the wet seeds were separated out. These were then gently but thoroughly rinsed in tap water twice and completely air dried at room temperature for 4 weeks. The dried seeds were pulverized into fine powder using a mixer grinder. 500 grams of the dried aqueous seed were extracted by maceration for 48 hours using 90% ethanol.

Trigonella foenum graecum

Trigonella foenum seeds were collected, air-dried and powdered. 1500 grams of the powdered seeds were extracted in Erlenmeyer flask with 90% ethanol for 48 hours by maceration process with occasional shaking and stirring.

Curcuma longa

500 grams of *Curcuma longa* rhizome was powdered and macerated in hot water (80^0 C) for 4 hours and the aqueous extract was evaporated. The product obtained was again extracted using soxhlet apparatus with 90% ethanol for 48 hours.

Preparation of Polyherbal formulation

The individual extracts of *Momordica charantia*, *Carica papaya*, *Trigonella foenum* and *Curcuma longa* which were prepared above were mixed in equal proportions using5% Tween 80 solution for pharmacological experiments.

Experimental designing

72 Healthy adult albino Wistar rats of either sex, 8-10 weeks old, weighing about 150-200 gm were used in the experiments. Animals were housed in polypropylene cages maintained under standard condition (12 hours light / dark cycle; 25 ± 3 °C, 45-65% humidity) and had free access to standard rat feed (Hindustan Lever Ltd., India) and water. All the animals were acclimatized to laboratory condition for a week before commencement of the experiment.

Acute Toxicity Study (OECD Guideline 423)

Animals were fasted prior to dosing, food but not water was withheld overnight. Following the period of fasting, the animals were weighed and test substance was administered. After the substance had been administered, food was withheld for further 3-4 hours. As a dose was administered in fractions over a period, it was necessary to provide the animals with food and water depending on the length of the period.(Ghosh MN, 1984; Turner R, 1965)

Three animals were used for each step. The dose level of the extract to be used as the starting dose was selected from one of the four fixed dose levels 500, 1000, 1500 and 2000mg/kg body weight (Lorke D, 1983). The starting dose levels such that which was most likely to produce mortality in some of the dosed animals. After administration of the test sample, the animals were observed continuously for first four hours for behavioral changes and at the end of 48 hour for mortality, if any.

Glucose Tolerance Test

Animals were fasted for 24 hours before experiment but were allowed free access to water. Fasted rats were divided into three groups of 6 animals each (WHO, 1999)

Group I - Control animals received 5% Tween 80 in distilled water at 5ml/kg b.w.p.o.

Group II - 200 mg/kg b.w. of polyherbal formulation (MCTC) p.o.

Group III - 400 mg/kg b.w. of polyherbal formulation (MCTC) p.o.

After 30 minutes of the treatment to the Groups I, II and III, 2gm/kg body weight glucose was given orally to the animals. Blood samples were collected from tail just prior to glucose administration and at 60, 120 and 180 minutes after glucose loading. The glucose levels were estimated for all the three groups by tail tipping method using Accu-check glucometer.

Effect of Formulation on Blood Glucose Levels in Alloxan Induced Diabetic Rats

30 male Wistar rats (150-200g) were made diabetic by a single i.p injection of Alloxan at a dose of 120 mg/kg i.p. after dissolving it in freshly prepared 0.1M citrate buffer (pH 4.5). The rats were maintained on 5 % glucose solution for next 24 hour to prevent hypoglycemia. Five days later blood samples were drawn from tail vein and glucose levels were determined to confirm the development of diabetes (>300mg/dl). The diabetic rats were divided into five groups, each containing six animals.

Group I- Normal control rats received 5% Tween 80 in distilled water p.o.at 5 ml/kg b.w.

Group II - Diabetic control rats received 5% Tween 80 in distilled water p.o.

Group III - Diabetic rats received polyherbal formulation (MCTC) 200mg/kg b.w., p.o.

Group IV - Diabetic rats received polyherbal formulation (MCTC) 400mg/kg b.w., p.o.

Group V - Diabetic received glibenclamide at the dose of 5mg/kg b.w., p.o.

The administrations of extracts were continued for 21 days, once daily. Blood samples were collected through the tail vein on days 1, 7, 14 and 21 after drug administration and the blood glucose levels were estimated using Accu-check glucometer.

Statistical Analysis

Results were expressed as Mean ± SEM. Statistical analysis were performed with Graph pad prism 5 software using one way analysis of variance (ANOVA) followed by Dunnett's t test. P values less than *p<0.05, p**<0.01, p***<0.001 was considered to be statistically significant, when compared with control and standard group as applicable (Diabetes and Metabolism 1989).

RESULTS

Extraction

The dried powdered parts of the respective plants were extracted using soxhlet and /or maceration method and percentage yield of the extracts are tabulated in Table 1.

Acute Toxicity Study

Acute toxicity study of polyherbal formulation was carried out in rats. It was observed that there was no gross evidence of any abnormalities up to 4 hrs and no mortality was observed in animals up to the end of 48 hours at the maximum tested dose level of 2000mg/kg b.w. in rats. This was considered as Maximum Tolerated Dose (MTD) and thus, $1/10^{th}$ of MTD i.e., 200mg/kg b.w. was taken as test dose and double the test dose i.e., 400 mg/kg b.w. was also selected for the experimental studies.

Oral Glucose Tolerance Test

The results obtained for oral glucose tolerance test with polyherbal formulation are given in Table 2 and illustrated in Graph 1. The results of the test indicated a significant increase in glucose tolerance at 60, 120 and 180 min. in extract treated group as compared to control treated group.

Effect of Formulation on different parameter in alloxan induced diabetes in rats

Effect of Formulation on Blood Glucose Levels

The results obtained with polyherbal formulation on blood glucose levels are given in Table 3 and illustrated in Graph 2. The results of the test indicated a significant decrease in blood glucose levels on day 7, 14 & 21 in extract treated group as compared to control treated group.

Effect of Formulation on Total Cholesterol and Total Triglyceride Levels

The results obtained with polyherbal formulation on total cholesterol and total triglyceride levels are given in Table 4 and illustrated in Graph 3 & 4. The results of the test indicated a significant reduction in elevated total cholesterol and total triglyceride levels in extract treated group as compared to Diabetic Control group.

Effect of Formulation on HDL, LDL and VLDL Levels

The results obtained with polyherbal formulation on HDL, LDL& VLDL levels are given in Table 5 and illustrated in Graph 5, 6 & 7. The results of the test indicated a significant decrease in total LDL & VLDL levels while it increased the HDL levels in extract treated group as compared to Diabetic Control group.

Table 1. Percentage Yield of Extracts

S. no.	Plant Extract	Part used	Extraction Method	Solvent Used	Percentage yield of extract (w/w)
1.	*Momordica charantia*	Fruit	Soxhlation	Ethanol	12.7%
2.	*Carica papaya*	Seeds	Maceration	Ethanol	6.3%
3.	*Trigonella foenum*	Seeds	Maceration	Ethanol	10.5%
4.	*Curcuma longa*	Rhizome	Maceration followed by Soxhlation	Ethanol	13.2%

Table 2. Oral Glucose Tolerance Test

Treatment Groups	Blood Glucose Levels (mg/dL) at			
	0 min	60 min	120 min	180 min
Normal control	93.67±3.87	156.54±9.21	189.08±8.99	247.72±10.22
Polyherbal formulation MCTC 200 mg/kg b.w.,p.o.	86.78±4.92	127.39±6.87**	97.64±5.81**	90.39±4.85**
Polyherbal formulation MCTC 400 mg/kg b.w.,p.o.	91.56±4.63	119.02±7.62**	95.37±5.83**	87.65±3.91**

Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Table 3. Effect of Polyherbal Formulation on Blood Glucose Levels

Treatment groups	Blood Glucose Levels (mg/dL)on			
	Day 1	Day 7	Day 14	Day 21
Normal	98.83±4.39	103.0±3.2	109.0±2.59	111.17±3.47
Diabetic Control	300.0±11.26	320.0±6.96	333.83±5.26	343.5±4.35
Polyherbal formulation MCTC 200 mg/kg b.w., p.o.	296.33±3.04	266.66±3.64*	201.34±3.59**	176.98±3.19**
Polyherbal formulation MCTC 400 mg/kg b.w.,p.o.	285.43±43	227.0±5.4*	172.43±4.59**	129.37±4.09**
Standard Glibenclamide 5mg/kg b.w., p.o	301±11.62	261.33±9.76*	174.5±8.03**	132.17±9.08**

Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Table 4. Effect of Polyherbal Formulation on Total Cholesterol and Total Triglyceride Levels

Treatment groups	Total cholesterol levels(mg/dL)	Total triglyceride levels(mg/dL)
Normal	145.0±2.36	79.5±1.42
Diabetic Control	271.67±3.23	184.82±2.24
Polyherbal formulation MCTC 200 mg/kg b.w.,p.o.	197.5±3.25**	106.15±2.73**
Polyherbal formulation MCTC 400 mg/kg b.w.,p.o.	161.66±2.98***	97.5±2.09***
Standard Glibenclamide 5mg/kg b.w., p.o.	150.83±3.65***	83.16±2.71***

Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Table 5. Effect of Polyherbal Formulation on HDL, LDL & VLDL Levels

Treatment groups	HDL Levels	LDL Levels	VLDL Levels
Normal	58.03±1.11	71.07±1.21	15.9±0.76
Diabetic Control	21.37±1.38	213.34±2.13	36.96±1.12
Polyherbal formulation MCTC 200 mg/kg b.w.,p.o.	57.33±0.98**	118.94±1.79**	21.23±0.98**
Polyherbal formulation MCTC 400 mg/kg b.w.,p.o.	61.08±1.37**	81.08±1.99**	19.5±0.79**
Standard Glibenclamide 5mg/kg b.w., p.o.	74.83±1.75***	59.37±1.75***	16.63±1.02***

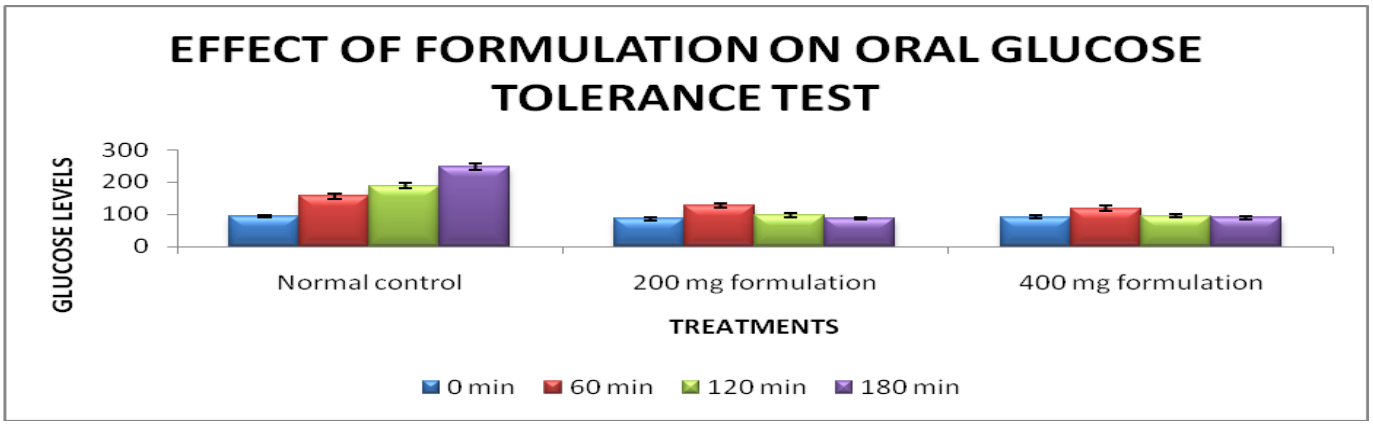
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Table 6. Effect of Polyherbal Formulation on SGPT and SGOT Levels

Treatment groups	SGPT levels	SGOT levels
Normal	27.83±1.32	27.17±1.32
Diabetic Control	66.00±2.11	57.17±2.01
Polyherbal formulation MCTC 200 mg/kg b.w.,p.o.	40.33±1.98**	35.15±1.98**
Polyherbal formulation MCTC 400 mg/kg b.w.,p.o.	35.33±1.67***	32.66±2.34***
Standard Glibenclamide 5mg/kg b.w., p.o.	30.83±2.03***	29.03±2.76***

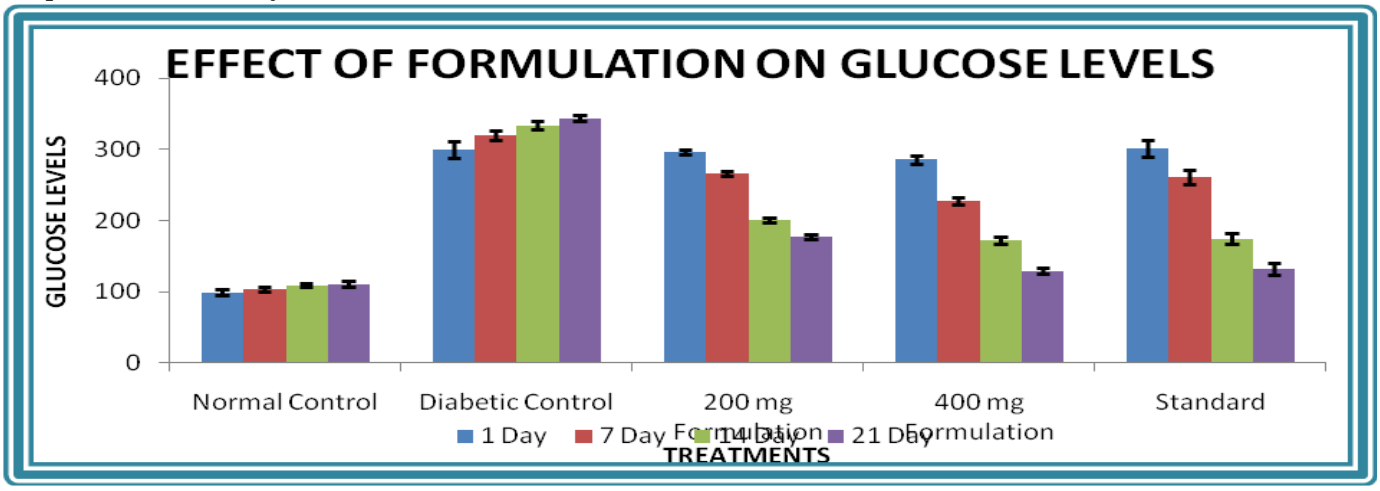
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 1. Oral Glucose Tolerance Test



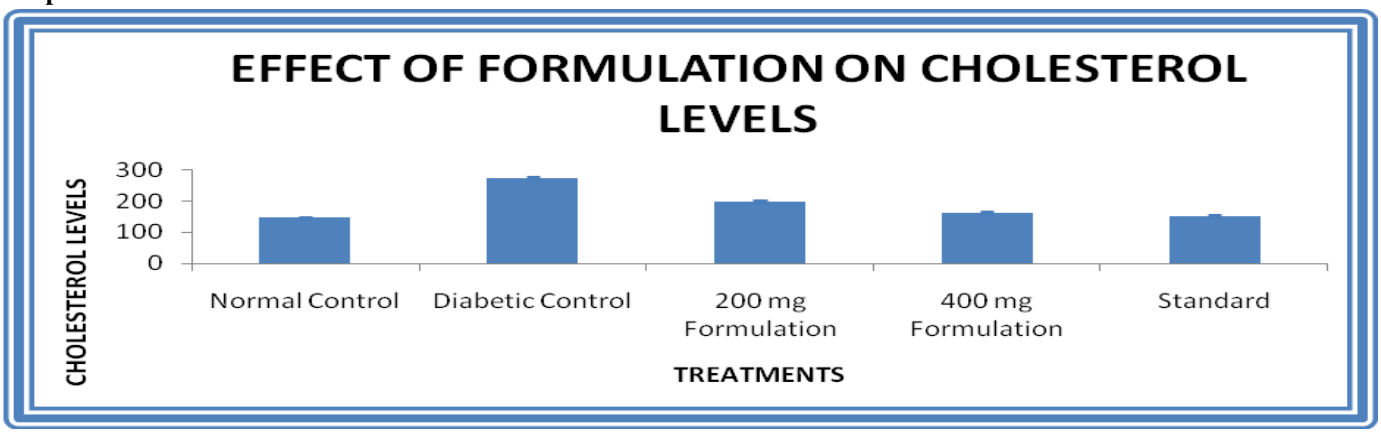
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 2. Effect Of Polyherbal Formulation On Blood Glucose Levels



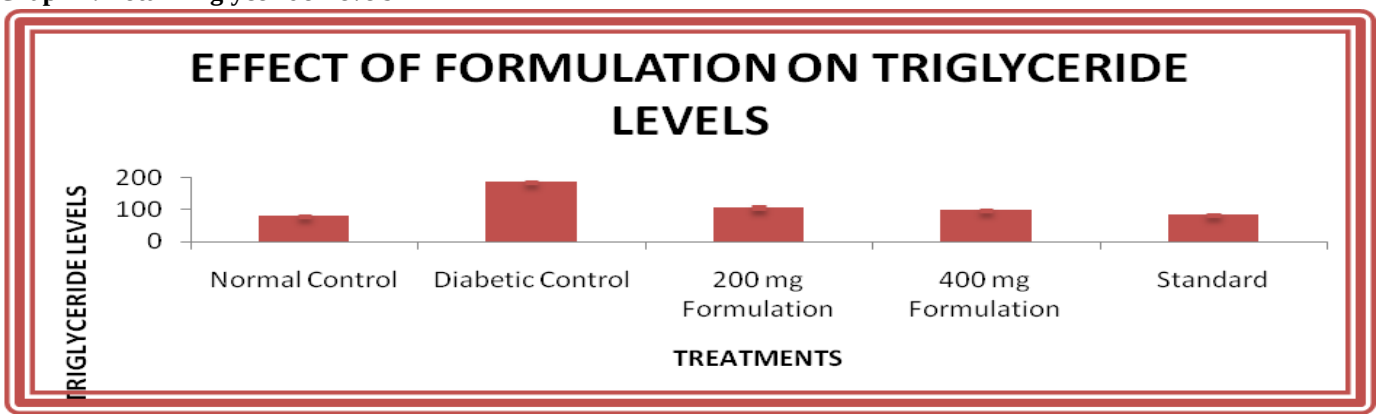
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 3. Total Cholesterol Levels



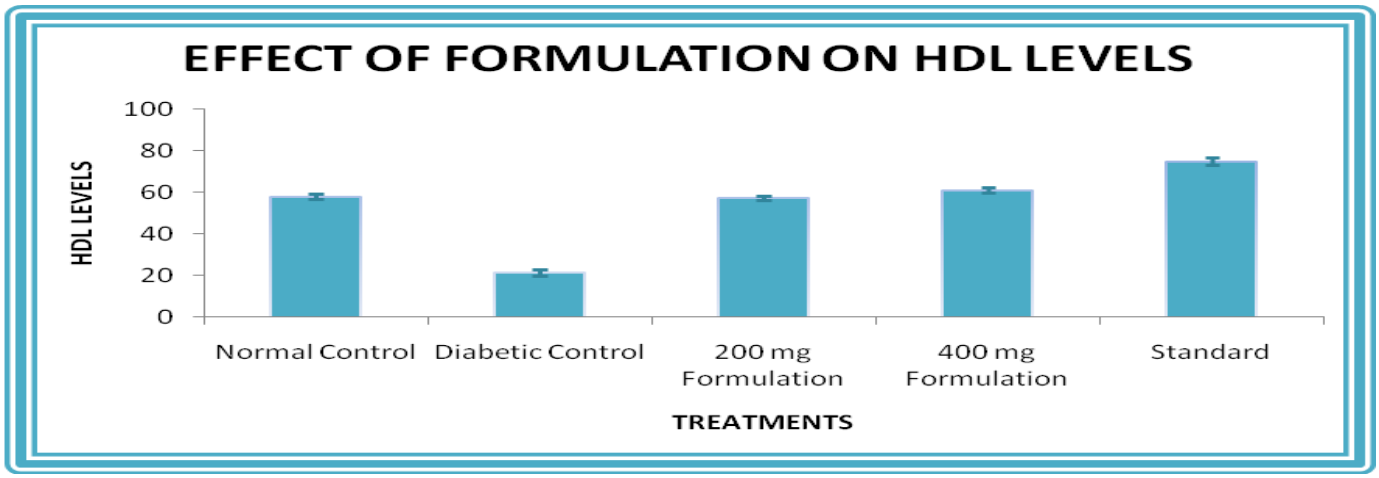
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 4. Total Triglyceride Levels



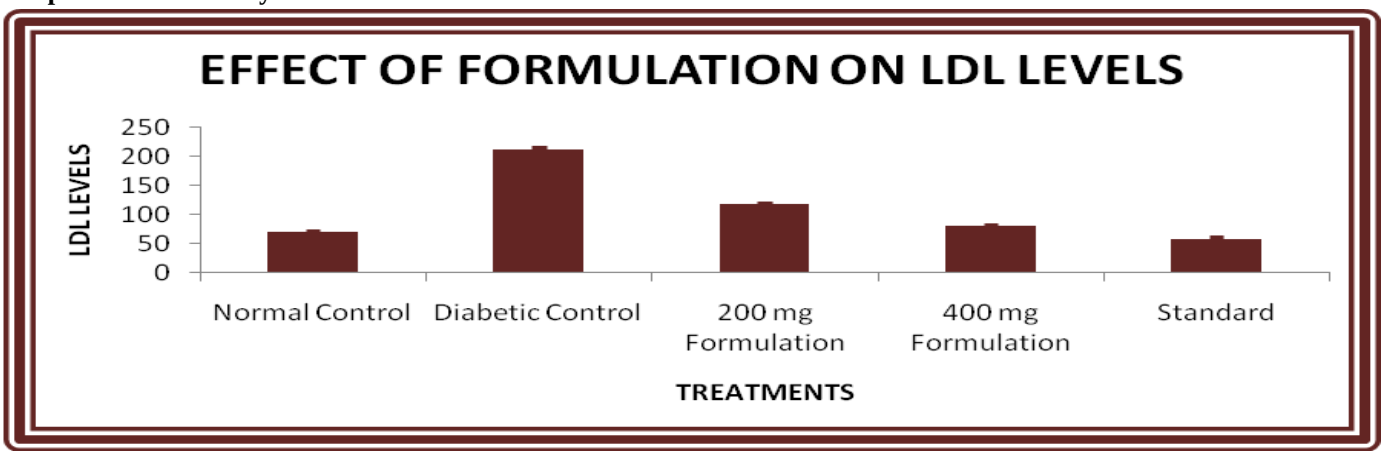
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 5. Effect Of Polyherbal Formulation On HDL Levels



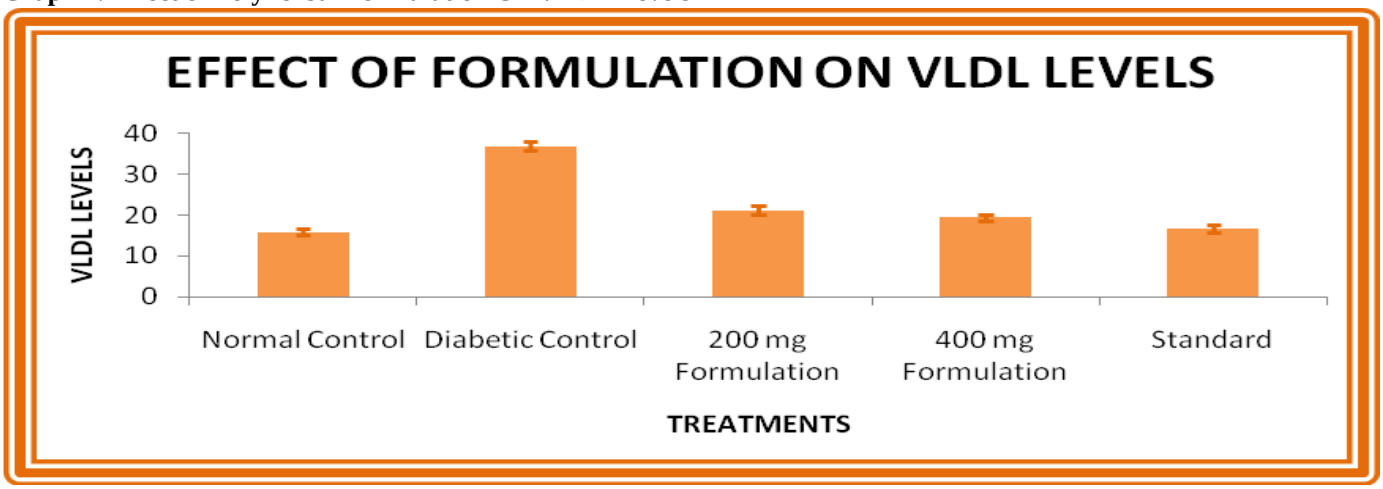
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 6. Effect Of Polyherbal Formulation On LDL levels



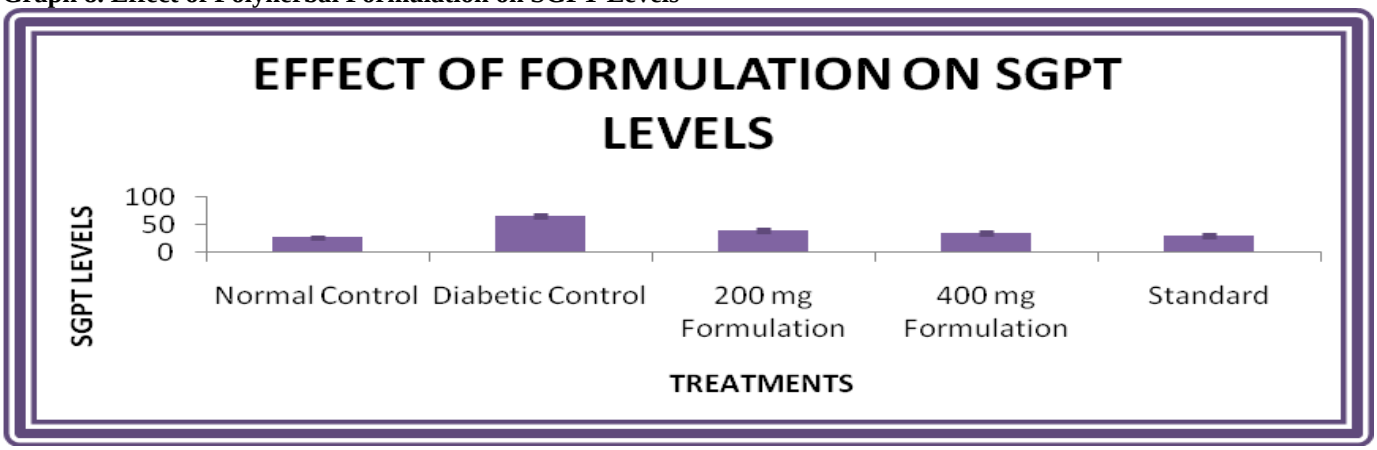
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 7. Effect of Polyherbal Formulation On VLDL Levels



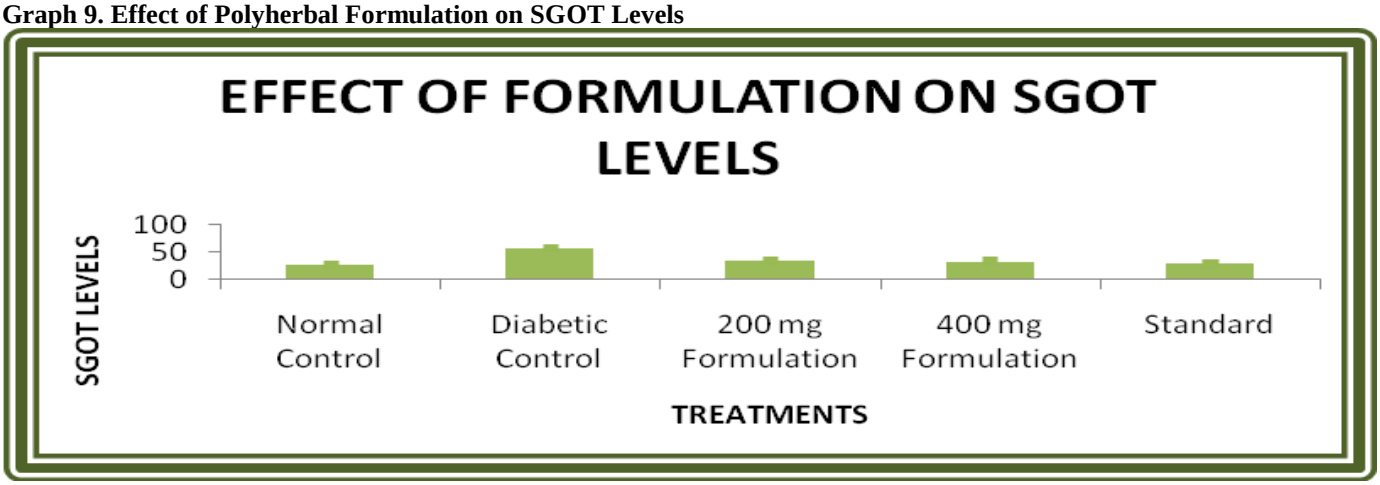
Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 8. Effect of Polyherbal Formulation on SGPT Levels



Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Graph 9. Effect of Polyherbal Formulation on SGOT Levels



Values are expressed as Mean±SEM (n=6); *P<0.05, **P<0.01, ***P<0.001 was considered significant with respect to control group using ANOVA followed by Dunnett's t-test.

Effect of Formulation on SGPT and SGOT Levels

The results obtained with polyherbal formulation on SGPT & SGOT levels are given in Table 6 and illustrated in Graph 8 & 9. The results of the test indicated SGPT & SGOT levels in extract treated group are 35.33±1.67 & 32.66±2.34 as compared to Diabetic Control group (66.0±2.11 & 57.17±2.01).

DISCUSSION

Diabetes mellitus is now recognized as one of the major killer diseases and a leading cause of death, claiming many lives world over. Oral hypoglycemic agents especially the sulphonylureas and biguanides have been commonly used in the disease management especially type II diabetes but are not without serious side effects. Consequently, attention has been focused on the use of plants and herbal remedies believed to be safer and devoid of serious side effects as alternatives in the treatment of diabetes.

The polyherbal MCTC is one of such herbal remedies prepared from the fruit of *Momordica charantia*, seeds of *Carica papaya*, seeds of *Trigonella foenum* and rhizome of *Curcuma longa* used to evaluate antidiabetic activity.

The extraction value of *Momordica charantia* fruit was 12.7% w/w, seeds of *Carica papaya* were 6.3% w/w, seeds of *Trigonella foenum* was 10.5% w/w and rhizome of *Curcuma longa* was 13.2% w/w. The Maximum Tolerated Dose (MTD) of the drug preparation was determined to be 2000 mg/kg b.w. for p.o.as there was no lethal effect at the dose. Thus, the test dose was decided as 200 mg/kg b.w.p.o. ($1/10^{th}$ of MTD) and 400 mg/kg b.w. p.o. was also decided for the experimental study.

The anti-hyperglycemic activity of the polyherbal formulation was screened using glucose tolerance test. The formulation tested for this activity exhibited significant antihyperglycemic activity at a dose level of 400 mg/kg b.w. (87.65±3.91) as compared to control (247.72±10.22) at 180 minutes. The results agree with the previous study on a polyherbal formulation i.e., Diabet containing six medicinal plants namely *Curcuma longa*, *Coscinium fenestratum*, *Strychnos potatorum*, *Tamarindus indica*, *Tribulus terrestris* and *Phyllanthus reticulants* also showed a significant antihyperglycemic effect at a dose level of 500 mg/kg b.w., p.o. at 180 minutes.

The polyherbal formulation of drug was effective in decreasing the blood glucose levels in diabetic rats at both the low and high doses significantly. The results agree with the previous study on a polyherbal formulation Okudiabet containing three herbal plants namely *Stachytarpheta angustifolia*, *Alstonia congensis* and *Xylopia aethiopica* which showed antidiabetic activity dose-dependently. There was significant increase in total cholesterol, triglyceride, LDL, VLDL, SGPT and SGOT levels and a decrease in HDL levels in alloxan induced diabetic rats compared to normal rats. Administration of polyherbal formulation at doses 200 and 400 mg/kg b.w. and glibenclamide at a dose of 5 mg/kg b.w. reversed the elevated levels of total cholesterol, triglycerides, LDL, VLDL, SGPT and SGOT significantly and increased the HDL levels significantly. 400 mg/kg b.w. dose was found to be more effective than the lower dose. The results perfectly agree with the previous study on a polyherbal formulation Dianex containing *Gymnema sylvestre*, *Eugenia jambolana*, *Momordica charantia*, *Azadira chtaindica*, *Cassia auriculata*, *Aegle marmelose*, *Withania somnifera* and *Curcuma longa* and DRY/AY/5001 containing *Gymnema sylvestre*, *Syzygium cumini*, *Pterocarpus marsupium*, *Momordica charantia*, *Emblica officianalis*, *Terminalia chebula*, *Terminalia belirica* and *shudh Shilajit*.

MCTC, a combination of fruit of *Momordica charantia*, seeds of *Carica papaya*, seeds of *Trigonella foenum* and rhizome of *Curcuma longa* in the present investigation showed significant antihyperglycemic and hypolipidemic activity. So, it can be used as an agent for the treatment of diabetes mellitus. However, further studies are required to be done to explore the active principles and the exact mechanism of action. The results of the present study indicate that the antidiabetic effect of the MCTC may be due to increase in insulin secretion or decrease in insulin resistance or increased glucose absorption.

CONCLUSION

The WHO has recommended and encouraged the use of alternative therapy especially in countries where access to the conventional treatment of diabetes is not adequate. The ingredients present in polyherbal formulation i.e., *Momordica charantia*, *Carica papaya*, *Trigonella foenum* and *Curcuma longa* are widely used in various systems of medicine for a wide range of properties. In addition to antidiabetic activity, MCTC also exhibited hypolipidemic activity that may be beneficial for preventing diabetic complications. On the basis of these results, it could be concluded that MCTC, a combination of four herbal plants exerts a significant antidiabetic effect. This could be due to different types of active principles from various plants, which may have different mechanisms of action. Therefore, combination may be beneficial. However, it cannot be concluded that the combination of four plants may have synergistic or additive effect. Although, further studies are required to be conducted to investigate this hypothesis. The polyherbal formulation MCTC may be considered as safe supplementary therapy for a long term and effective management of diabetic patients. Thus, further studies can be done to identify the exact chemical constituents and mechanism of action which are responsible for the said activity.

In conclusion, the overall results of this study have clearly shown low potency (3X) or MT of *TO* to offer good protection against the deleterious renal side-effects of gentamicin. Although the exact nephroprotective

mechanism(s) of *TO* were not investigated in the present study, this could constitute an area of future studies. So, These strategies of treatment will be suitable/safe for clinical practice. The present findings coincide with those of earlier studies, which reported that, plants present in Dihar containing eight medicinal plants namely *Syzygium cumini*, *Momordica charantia*, *Emblica officinalis*, *Gymnema sylvestre*, *Enicostemma littorale*, *Azadirachta indica*, *Tinospora cordifolia* and *Curcuma longa* also produced similar effect.

REFERENCES

Anonymous1.http://www.stjohnsmercy.org/iiserrors/404.asp?404;http://www.stjohnsmercy.org/healthinfo/adult/liver/pancreas.asp.

Anonymous 2. http://www.who.int/topics/diabetes_mellitus/en/

Anonymous 3. http://www.mamashealth.com/organs/pancreas.asp.

Anonymous 4. http://www.healthinsite.gov.au/topics/Types_of_Diabetes

Anonymous 5. http://autoimmune.pathology.jhmi.edu/diseases.cfm?systemID=3&DiseaseID=23

Brian J. Welch and Ivana Zib. Case Study: Diabetic Ketoacidosis in Type 2 Diabetes: Look under the Sheets. *Clinical Diabetes*. 2004; 22(4): 198-200.

Ghosh MN. Toxicity Studies: In Fundamentals of Experimental pharmacology, Scientific book agency, Calcutta, 1984, pp.153-158.

Lorke D. A new approach to practical acute testing. *Arch. Toxicol.,* 1983; 53: 275-289.

Maske H, Weinges K. Untersuchungenüber das Verhalten der Meerschweinchengegenüberverschiedenendiabetogenen Noxen. Alloxan and Dithizon. *Naunyn-Schmiedeberg's Arch exper Path Pharmakol.* 1957; 230: 406–420.

Ramirez-Tortosa MC, Mesa MD, et al. Oral administration of turmeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental atherosclerosis. *Indian J PhysiolPharmacol.* 1999; 147 (2): 371-8.

Turner R. Acute toxicity: The determination of LD50. In Screening Methods in Pharmacology, Academic Press, New York, 1965, p 300.

World Health Organization and International Diabetes Federation. *Definition, diagnosis and classification of diabetes mellitus and its complications*. Geneva, Switzerland: World Health Organization, 1999.