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Original Article

EVALUATION OF PHARMACOGNOSTICAL, PHYTOCHEMICAL AND ANTIDIABETIC ACTIVITY FRUITS OF *MOMORDICA CHARANTIA* LINN.

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ABSTRACT

Objective: *Momordica charantia* Linn. belonging to family cucurbitaceae, used in various ailments in Indian traditional system of medicine. Its fruit extract is used for the treatment of diabetes. Objective of present study was evaluation of pharmacognostical, physicochemical, phytochemical and antidiabetic activity of fruit extract of *Momordica charantia*.

Methods: The plant material was purchased from local market of District Hapur, U.P. (India). The plant drug was standardized as per W.H.O. guidelines. Albino Wister male rats of weighing between 150 to 200 gms were used for the study. Diabetes was induced by injecting alloxan (120 mg/kg, i.p.). Group I served as normal control, Group II served as diabetic control, Group III served as standard control and treated by Tolbutamide 100 mg/kg p.o. Group IV served as diabetic rats treated with hydro-alcoholic extract of fruit extract of *Momordica charantia* (MCE) at a dose of 300 mg/kg All the treatments were given for 21 days.

Results: Diabetic rats treated with fruit extract of *Momordica charantia* (MCE) at a dose of 300 mg/kg significantly ($P<0.01$) reduced fasting blood glucose and normalize the lipid profile, renal profile and hepatic profile. This reduced 44.24% blood glucose after 21 days treatment and normalizes various serum parameters. Histopathological changes of pancreas and liver of diabetic rats were improved by the treatment of MCE extract that confirmed its protective role in alloxan induced diabetes.

Conclusion: Our research project was preparation of polyherbal formulation by mixing four different crude drug extracts in an optimized ratio and MCE is one of the ingredients of this proposed formulation. For this purpose the plant material and extract were standardized and evaluated for antidiabetic activity. On the basis of results it can be concluded that 70% v/v hydroalcoholic extract of MCE possess antidiabetic activity and beneficial in improving complications associated with diabetes.

Keywords: Antidiabetic activity, *Momordica charantia* Linn., Fruit extract, Alloxan-induced diabetes.

INTRODUCTION

Momordica charantia Linn. belonging to family Cucurbitaceae. It is commonly known as bitter guard (in English) or karela (in Hindi). It is annual herb and commonly cultivated in coastal thickets, along creeks and streams and lowland forest. Plant contains Charantin (1:1 mixture of stigmastadienol β-D-glucoside and β-sitosterol β-D-glucoside), momordicosides A & B, vicine a non protein nitrogenous base. [1]

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins, and an increased risk of complications from vascular disease. According to reports of 2011 about 366 million people are diagnosed with diabetes globally and this may rise to 552 million by the year 2030. [2] Non-insulin dependent diabetes mellitus (NIDDM) is associated with morbidity & mortality, resulting from its microvascular, macrovascular and neuropathic complications. The abnormalities of the lipid metabolism in diabetes mellitus generally leads to elevation in the levels of serum lipids and lipoproteins that in turn play an important role in occurrence of premature and severe atherosclerosis, which affects patients and diabetes. [3]

The quality of medicinal plant depends on the geographical, climatic condition and time of collection. In present study, fruits of *Momordica charantia* Linn. were taken for the study and extracted by 70% v/v hydroalcoholic solution and evaluated for pharmacognostical, phytochemical and antidiabetic activity and various biochemical parameters.

MATERIALS AND METHODS

Plant material

The fruits of *M. charantia* Linn. were purchased from local market Hapur (U.P.) India. The specimen was given for authantification in Raw Material and Laboratory of National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (voucher no. NISCAIR/Consult/RHMD/-2010-11/1620/218).

The fruits of *Momordica charantia* was washed and dried in an electric hot air oven at a temperature 40° C.

Preparation of extract

Dried fruits are coarsely powdered and defatted with petroleum ether by soxhlet apparatus. Defatted drug than exhaustively extracted with 70% hydroalcoholic solution in soxhlet apparatus. The extract was concentrated under reduced pressure.

Standardization of plant drug and extract

The morphological and microscopical studies, ash value, extractive value in different solvents, heavy metal analysis of the extract and test for microbial contamination were also done for the purity of drug. The Phytochemical screening of the extract was carried out for the presence of Alkaloids, Proteins & Amino acids, Carbohydrates, Flavonoids, Phenolic group, Glycosides, Saponins, Tannins, Steroids, and Triterpenoids. [4-8]

Thin Layer Chromatography (TLC)

Solvent system Chloroform: Methanol (8.5 : 1.5) was developed for establishing the TLC patterns of hydroalcoholic extract of drugs. Various visualizing techniques were used for best TLC

fingerprinting, like UV 254, UV 366 nm, and R_f value (s) were determined.

Animals

Albino wistar male rats of weighing between 150 to 200 gms were procured from Indian Veterinary Research Institute Bareilly U.P. (IVRI).The animals were housed under standard conditions of temperature (25 ± 2^{0}C) and relative humidity (30-70%) with a 12:12 light-dark cycle [9] and acclimatized in the animal house facility of the department under ambient condition of Siddhartha Institute of pharmacy, Dehradun (CPCSEA Approval no. 1435/PO/a/11/CPCSEA). The animals were fed with standard diet (Amrut Rat Feed, India) and water *ad libitum*. The Institutional Animal Ethics Committee approved all the experimental protocols with approval no. SIP/IAEC/12/Polyherbal.

Acute Toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), received draft guidelines 420, received from committee for the purpose of control and supervision of experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. A total of six female albino rats were used for the study. The extract was administered in a single dose of 2000 mg/kg by gavage. All animals were observed individually after dosing during first 30 minutes, periodically during the first 24 hours and daily for 14 days. [10]

Induction of Diabetes:

The albino rats 150-200 gm either sex, were allowed to fast overnight prior to experimentation and rendered diabetic by injection a single dose of Alloxan 120 mg/kg body weight (Manufactured by Loba Chemie Company) administered as a 0.9% w/v in saline solution by I.P. route. It produces diabetes by selective necrosis of β - cells of islets of langerhans of pancreas. Since alloxan could evoke fatal hypoglycemia as a result of massive insulin release, rats received 20% of glucose solution for first 6 hr then simple tap water was given. The rats were then kept for next 24 hrs with free access of 5 % glucose solution to prevent hypoglycemia. [11] After 48 hrs of injection of Alloxan, Blood glucose level was measured for the evidence of diabetes by using commercially available kit "ACCU-CHEK ACTIVE" Glucometer from Roche Diagnostics GmbH, Germany. The rats which showed blood glucose level more than 200 mg/dl were considered as diabetic. The animals with sugar level more than 200mg/dl were selected. Animals were maintained for 72 hrs in diabetic condition for well establishment of diabetes.

Experimental design

Hydroalcoholic extract of the drug was suspended in 2% acacia solution and the dose of 300 mg/kg extract was given by oral route using a catheter [12]. Tolbutamide 100mg/kg was used as a standard drug. [13]

Animals were divided into six groups of six each.

Group-1: Healthy normal animals received only water served as Normal control (NC).

Group-2: Untreated alloxan induced diabetic animals served as a Diabetic control group (DC) also received water**.**

Group-3: The Reference Standard group (STD) was treated with Tolbutamide (100 mg/kg b.wt., p.o. /day).

Group-4: Diabetic animals treated with hydro alcoholic extract of fruits of *Momordica charantia* (MCE) (300 mg/kg b.w, p.o/day).

Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer (ACCU-CHEK ACTIVE" Glucometer from Roche Diagnostics GmbH, Germany). Blood samples were drown at weekly intervals till the end of study (i.e. 3 weeks). Blood glucose estimation was done on day 0, 7, 14 and 21 of the study. On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar [14] was estimated. Serum was separated and analyzed for serum cholesterol, [15] serum triglycerides, [16] serum HDL and LDL, [17] serum creatinine, [18] serum urea, [19] serum alkaline phosphatase (ALP), [20] bilirubin, serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase(SGPT) [21] were estimated.

Statistical analysis

All values of blood sugar and biochemical estimations were expressed as mean ± Standard error means (S.E.M.) and analysed for ANOVA and post test TURKEY-One Way Analysis of Variance. Differences between groups were considered at $P<0.01$ levels.

Histopathology of isolated liver and pancreas:

Small pieces of liver and pancreas tissues were collected in 10% formalin for proper fixation. Tissues were fixed in Bouin's fixative (without acetic acid) for histopathological studies. Sections of tissues (6 microns in thickness) were stained with haematoxylin and eosin (H & E) for histological examination. [22] The photomicrographs of histological studies are presented in fig. 2 and 3.

RESULTS

Morphology of *Momordica charantia* fruits

Colour: immature fruit is green, pulp pithy, whitish yellow at maturity exposing numerous seeds, enclosed in whitish aril which becomes bright red on maturity. **Shape & size:** elongated, fusiform, longitudinally grooved, ridged and warty, 2.5 to 25 cm long, 2 to 7 cm in diameter. **Odour and Taste:** Bitter **Seeds:** seeds pale brown up to 1.5 cm long, flattened, elliptic with scalloped markings on the flat side and on the edge of seed.

Fig.1: Fruits of *Momordica charantia*

Microscopical characters

Microscopically, fruits shows epicarp, hypodermis, middle mesocarp, vascular bundles, prismatic calcium oxalate crystals, seeds, aril & inner mesocarp and the seed shows testa, endosperm, cotyledon, perisperm.

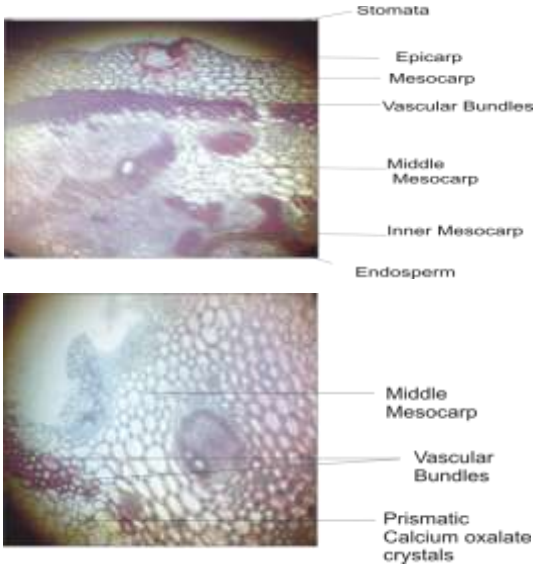

Fig.2: TS of *Momordica charantia* fruit

Powder microscopy

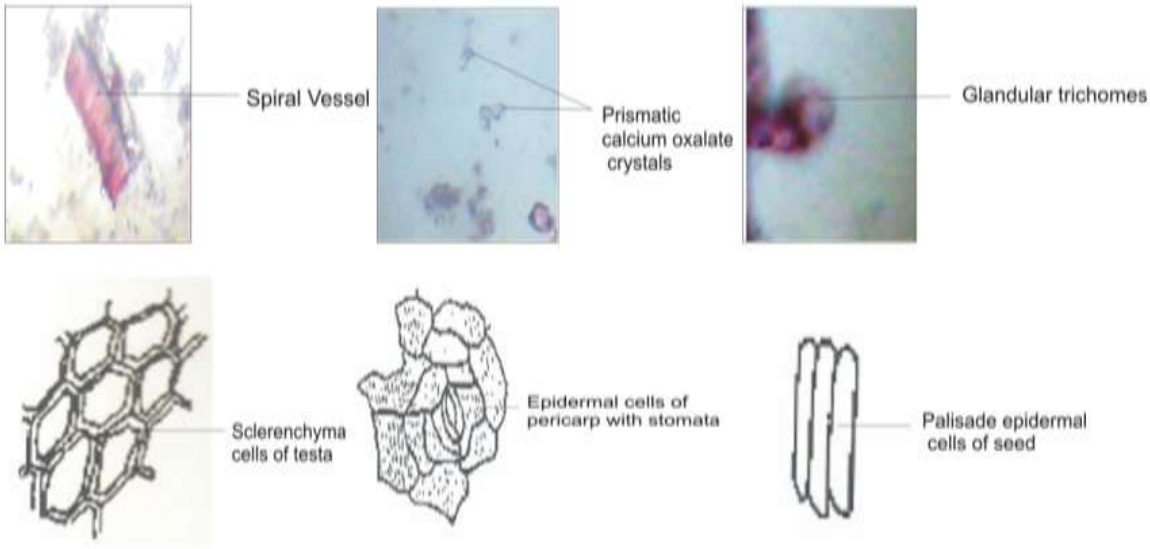

Fig.3: Powder microscopy of fruit of *Momordica charantia* Linn. Showing different structures (labeled accordingly)

Organoleptic characters

The colour of powder of fruit of *M. charantia* was brown, odour characterstic and taste was and bitter.

Physicochemical parameters

Table 1: Physicochemical parameters

Sr. No	Physicochemical parameters	Value (% w/w)
1	Moisture content	3.21
2	Total ash	6.8
3	Acid insoluble ash	0.25
4	Water soluble ash	3.56
5	Alcohol soluble extractive	19.0
6	Water soluble extractive	36.9
7	Yield of extract	16.089

Phytochemical screening

It was found that alkaloids, Tannins, Phenolic group, Proteins & Amino acids, Saponins, Carbohydrates, Steroids, Triterpenoids and Flavonoids, were present and Glycosides were found to be absent in the extract.

Thin Layer Chromatography (TLC)

TLC of hydroalcoholic extract showed 3 spots. The Rf values of five compounds are 0.71, 0.8, 0.85, respectively.

Heavy metal analysis of the extracts:

The heavy metal analysis was performed as per guidelines of Indian Pharmacopoeia and found that hydroalcoholic extract pass the limit test of heavy metals.

Determination of microbial contamination

Microbial count included total viable aerobic count, total yeast and mould, *E. coli*, *S. typhi* counts have been determined. The results are given in Table 2.

Acute Toxicity studies

MCE treated rats showed no discernible behavioral changes given by oral route. No mortality was observed during observation period.

Table 2: Microbial analysis of Hydroalcoholic extracts

S.N.	Micro-organism	Limit	Results
1.	Total aerobic count	$1x10^4$	270 CFU/g
2.	Yeast/mould	$1x10^3$	Absent/g
3.	*E. coli*	Absent/g	Absent/g
4.	*S. typhi*	Absent/g	Absent/g
5.	*P. aeruginosa*	Absent/g	Absent/g
6.	*S. aureus*	Absent/g	Absent/g

Antidiabetic activity

Table 3: Effect of 3-week treatment with standard drug and MCE on blood glucose level after alloxan induced diabetic rats

S.N.	GROUP	0-DAY	7-DAY	14-DAY	21-DAY
1.	**NC**	79.33 ± 2.692	79.17 ± 1.740	79.33 ± 1.333	77.67 ± 0.988
2.	**DC**	318.2 ± 4.175	355.7 ± 6.097	367.8 ± 9.372	370.2 ± 9.928
3.	**STD**	358.3 ± 4.780*#	244.2 ± 21.60*#	199.0 ± 12.70*#	138.0 ± 13.43*#
4.	**D+MCE**	356.5 ± 7.046*#	271.8 ± 10.34*#	243.3 ± 10.56*#	203.8 ± 12.41*#

* P<0.01 (Turkey test) significant when treated with Normal control

# P<0.01 (Turkey test) significant when treated with Diabetic control

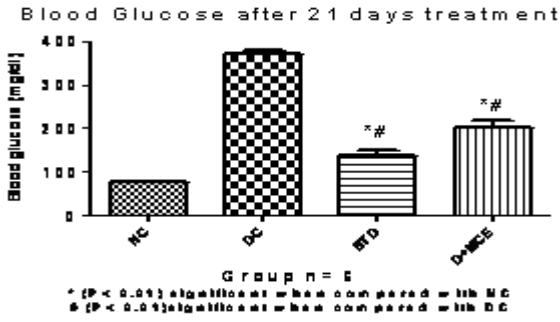

Fig.4: Graphical representation of effect of MCE on blood glucose.

Table 4: Effect of MCE on serum profile in alloxan induced diabetic albino rats after 21 days treatment

S.N.	Serum profile	Normal control	Diabetic control	Standard control	MCE treated
1.	Cholesterol	73.45±6.376	145.1±10.58	88.75±4.804#	97.75±7.718 #
2.	Triglycerides	40.75±3.262	92.33±4.645	51.98±3.975#	65.80±1.083*#
3.	HDL	26.72±0.8157	14.40±0.9980	21.98±0.9769*#	19.62±1.444*#
4.	LDL	29.50±3.896	94.80±6.225	41.91±3.373#	50.27±7.236#
5.	Urea	72.13±4.858	127.3±14.31	69.40±4.925#	76.65±7.466#
6.	Creatinine	0.525±0.02598	1.070±0.1060	0.6575±0.2287#	0.6600±0.1871#
7.	Albumin	3.854±0.03202	1.775±0.1377	3.193±0.04230#	2.850±0.1287*#
8.	SGPT	56.75±5.963	149.8±4.090	68±2.972#	79.06±3.298#
9.	SGOT	52.15±4.614	139±14.08	82.13±6.514#	90.93±2.454*#
10.	ALP	79.50±6.193	145.8±5.528	94.38±2.348#	103.4±2.599*#
11.	Bilirubin	0.2125±0.01315	0.4475±0.04820	0.2450±0.02363#	0.3375±0.02839*

* P<0.01 (Turkey test) significant when treated with Normal control, # P<0.01 (Turkey test) significant when treated with Diabetic control

HISTOPATHOLOGY

Photomicrographs (Fig.5) shows normal acini and normal cellular population in the islets of langerhans in pancreas of normal control and lesions in diabetic rats which maintained significantly after treatment by standard drug and MCE up to normal.

Photomicrographs (Fig.6) shows normal hepatocytes and lesions in diabetic rats which maintained significantly after treatment by standard drug and MCE.

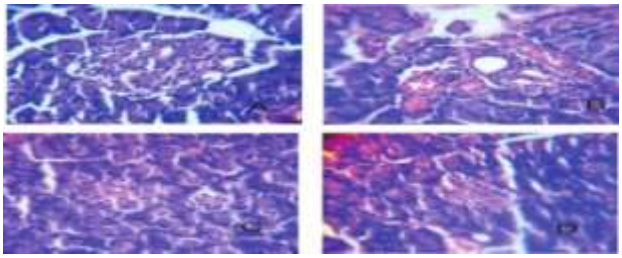

Fig. 5: Photomicrograph of rat pancreas stained by haematoxylin and eosin of normal control (A) diabetic control (B) standard (tolbutamide) treated (C) MCE treated (D)

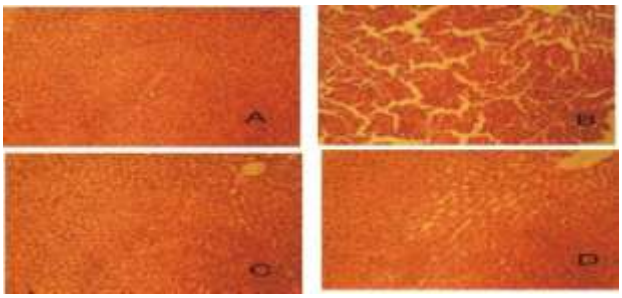

Fig. 6: Photomicrograph of rat liver stained by haematoxylin and eosin of normal control (A) diabetic control (B) standard (tolbutamide) treated (C) MCE treated (D)

DISCUSSION

Medicinal plants are the potential source of bioactive chemical agents and now are accepting throughout the world. [23] Herbal drugs should be standardized for making safe and effective medicines for human use. For this purpose studies on morphology, microscopy, ash value and extractive matter determinations, phytochemical screening, heavy metal analysis, microbial contamination are carried out. The next issue was evaluation of antidiabetic activity of same extract in alloxan induced diabetic rats. Alloxan induced diabetes has been commonly utilized as an animal model to study diabetes in experimental animals. Alloxan exerts its diabetogenic actions when administered intravenously, intraperitoneally or subcutaneously. The action of alloxan in the pancreas is preceded by its rapid uptake by the insulin-secreted beta cells. [24] Previous studies had reported that *M. charantia* enhances insulin secretion and increases the number of pancreatic B-cells in the islets of Langerhans. Some previous studies had also revealed that *M. charantia* fruit increases the glucose uptake in liver via promoting glucose-6- hosphate dehydrogenase and declining glucose-6- phosphatase activities. [25]

The present study indicates the effects of hydroalcoholic extract of *M. charantia* fruit on alloxan induced diabetic rats. The plant material and extract was standardized before starting the experiment (results are given). Administration of alloxan led to elevation of blood glucose levels, which was maintained over a period of 3 weeks. Three weeks of daily treatment of MCE at a dose of 300 mg/kg significantly reduce blood sugar levels. Various biochemical parameters in serum were also estimated that showed the beneficial effects of hydroalcoholic extracts of *M. charantia* fruits.

Histopathological findings of pancreas of the diabetic rats showed necrosis and atrophy changes in cell structures, but the pancreas treated with MCE and Tolbutamide showed minimal necrosis and MCE increased the number of pancreatic B-cells in the islets of Langerhans.

Histopathological findings of liver of the diabetic rats showed complete destruction of hepatocytes. These changes are restored near to normal in the group treated by the MCE extract.

CONCLUSION

Thus our study proves the beneficial effects of hydroalcoholic extract (70% v/v) of *M. charantia* fruit in the management of diabetes and its associated complications. Our findings supports the long term use of the extract at the dose of 300 mg/kg bw per day for better control of blood glucose and restorations of diabetes associated changes.

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REFERENCES

1. Prajapati AN, Purohit SS, Sharma AK and Kumar T. A Hand book of Medicinal plants- A complete source book. Agrobiosis, India 2007, 346.
2. Ganugapati J and Swarna S. Molecular docking studies of antidiabetic activity of Cinnamon compounds. Asian Journal of Clinical and Pharmaceutical Research, 2014, 7 (2), 31-34.
3. Huse, DM, Oster G, Killen AR, Lacey MJ and Golditz GA. The economic costs of non-insulin dependent diabetes mellitus. Journal of the American Medical Association 1988, 262, 2708-2713.
4. Chitravadivu C, Manian S and Kalaichelvi K. Qualitative Analysis of Selected Medicinal Plants, Tamilnadu, India Middle-East Journal of Scientific Research 2009, 4 (3), 144-146.
5. Yadav RNS and Agarwala M. Phytochemical analysis of some medicinal plants. Journal of Phytology, 2011, 3(12), 10-14.

6. Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis, Springer 1973, 60-205.
7. Quality controls methods for medicinal plant materials. World Health Organization, Geneva AITBS publisher and distributors, Delhi 2002; 28-30, 38-40, 64-73.
8. Indian Pharmacopoeia 1996, Govt. of India, Ministry of Health and Family Welfare, Published by The Controller of Publication, Delhi, Vol. II (P-Z), A-110- 124.
9. Sujith K Ronald Darwin C, Dr.C.Roosewelt antidiabetic activityof methanolic extractofbuteafrondosaleaveswith Its possible mechanism of action. Asian J Pharm Clin Res, 2011, Vol 4,Issue 3, 93-98.
10. Revised Document OECD/OCDE 420. Guidelines on Acute Oral Toxicity. OECD Guidelines for the testing of chemicals, Dec-2001, 1-14.
11. Sangameswaran B, Singh, R, Balakrishnan BR, Jayakar B. Anti-hyperglycemic and anti-hyperlipidemic effects of Ipomoea reniformis Chois on alloxan induced diabetic rats. Journal of Research and Education in Indian Medicine 2007, 13(4): 27-30.
12. Sgrawat H, Sharma S, Chaturvedi M, Edein E, Shukla S. Evaluation of the phytochemicals and antidiabetic activity of Ficus bengalensis. Int J Diab Dev Ctries. 2007; 27(2):56-59.
13. Hemlatha S, AyyappanT, Shanmugam S, Nagavalli D, Shrivijaya Kirubha T, Evaluation of antidiabetic and anti diuretic activity of polyherbal formulation. Indian Journal of Traditional Knowledge, 2006, 5(4), 468-470.
14. Giordano BP, Thrash W, Hollenbough L, Dube WP, Hodges C, Swain A, Bunion DR, Klingensmith GJ. Performance of seven blood glucose testing systems at high altitude. Diabetic education 1989, 15, 444-448.
15. Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. Zeitschrift fur Klinische Chemie und Klinische Biochemi, 1974, 12, 226.
16. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clinical Chemistry, 1974, 20, 470-475.
17. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clinical Chemistry, 1972, 18, 499-502.
18. Bowers LD. Kinetic serum creatinine assays I. the role of various factors in determining specificity. Clinical Chemistry, 1980, 26, 551-554.
19. Wilson BW. Automatic estimation of urea using urease and alkaline phenol. Clinical Chemistry, 1966, 12, 360-368.
20. Sasaki M. A new ultra micro method for the determination of serum alkaline phosphatase. Use of Berthelot's reaction for the estimation of phenol released by enzymatic activity. Igaku To Seibutsugaku, 1966, 70, 208-214.
21. Patrícia S. Alves, Ermelinda A. Camilo, J. Pinto Correia, The SGOT/SGPT ratio in alcoholic liver disease. acta med port 1981, 3: 255-260.
22. Ramnik Sood, Concise book of Medical Laboratory Technology, Methods and Interpretations, Jaypee Brothers Medical Publishers(P) Ttd, New Delhi, 2009, 972-992.
23. Katewa SS, BL Chaudhary and A Jain. Folk herbal medicine from tribal areas of Rajasthan, India. J. Ethnopharmacol. 2004, 92: 41-46.
24. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001; 50(6):537-46.
25. M. Abdollahi A BZ, Zuki Y M Goh, Rezaeizadeh A. and MM. Noordin, The effects of *Momordica charantia* on the liver in streptozotocin-induced diabetes in neonatal rats. African Journal of Biotechnology 2010, Vol. 9(31), 5004-5012.