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ANTI-DIABETIC ACTIVITY OF BRASSICA OLERACEA VAR ITALICA AND MOMORDICA CHARANTIA LINN ON STREPTOZOTOCINE INDUCED DIABETES

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Abstract: Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus there is increasing demand by patients to use natural products with anti diabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine. Brassica oleracea and Momordica charantia juice has hypoglycaemic effects on blood glucose concentrations of diabetic rats and may be effective in cases of glucose tolerance impairment. However, it can be speculated that the antidiabetic activity of C Brassica oleracea and Momordica charantia juice may be due to non-specific mechanism and not due to the stimulation of insulin release from pancreatic beta cells since streptozotocin used in this study is well known to work by depleting the pancreatic beta cells thus reducing the release of insulin from these cells. Also, the synergistic effect of different bioactive chemicals may have a crucial contribution to the potential hypoglycaemic action of the plant species.

Keywords: Streptozotocin, Brassica oleracea, Momordica charantia, Column chromatography, HPTLC

Introduction: Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus there is increasing demand by patients to use natural products with anti diabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine

Plant profile : Selection of plant
Brassica oleracea
Botanical name- Brassica oleracea
Synoname - Brocolli, Cabbage,Cole wort, kail,Tender green, Gree gobi,Colza, Broccoflower Common names- Brocolli
Family- Brassicaceae Genus-Brassica
Species-Brassica oleracea
Broccoli is an edible green plant that is classified in the Italica cultivar group of the species Brassica oleracea. They are rich in vitamin C, dietary fiber and also contain glucoraphin, sulforaphane, selenium and

isothiocyanates. Broccoli is also an excellent source of indole-3- carbinol. These constituents present in broccoli are known to be very popular since they possess several anti-cancer properties and benefits. These ant i-carcinogenic compounds have a wide variety of uses and benefits for the treatment of various diseases and disorders. Broccoli is widely used in the treatment of several forms of cancer and also treats other neural disorders. The therapeutic potential of broccoli has been explained under its role in cancer, diabetes and other diseases. In the treatment of cancer, most of the constituents or the phytochemicals of broccoli such as brassinin, isothiocyanates, indole-3-carbinol etc. have been proved to be effectively beneficial. Even selenium plays a very important role in cancer prevention. The antioxidant activity of broccoli is induced by other phytochemicals such as glucosinolates, glucoraphin and sulforaphane. Sulforaphane in broccoli sprouts also has the potential to cure neural disorders such as Alzheimer's disease and Parkinson's disease. It is also used to bring about cure in asthma and diabetic patients. Flavonoids have the effect of reducing the risk of diabetes. Therefore sulforaphane is widely used to treat various diseases and disorders.

Chemical constituents: Glucosinolates, Kaempferal flavonoid, Isorhamnetin flavonoi, Quercetin flavonoidI, Alkaloid, Indol alkaloid

Momordica charantia juice

Botanical name- Momordica charantia
Synoname- Bittermelon, Bitter gourd, Balsam pear, Balsam Apple, Common names= Karela
Family- Meliaceae
Species- Momordica charantiaL – Balsampear

Use:Momordica charantia (Bitter melon), a climbing vine whose leaves and green fruits, although bitter, has been used to fight cancer, diabetes and many infectious diseases. It is also a powerful weapon against HIV/AIDS. According to Ayurveda, roots are useful in treatment of eye related diseases. The fruit is bitter, cooling, digestible, laxative, antipyretic, anthelmintic, appetizer, cures biliousness, blood diseases,anaemia, urinary discharges, asthma, ulcers, bronchitis etc. According to the Unani system of medicine fruit is very bitter, carminative, tonic, stomachic, aphrodisiac, anthelmintic, astringent to bowels and useful in treatment of syphilis, rheumatism, spleen troubles etc. Bitter melon is worldwide known for its effectiveness in treating diabetes. Bitter melon chemically contains a compound that is very much similar to insulin and sometimes also referred as p-insulin. Researchers have shown that when it is taken
continuously for some period has the ability to substitute the insulin in the body. It also contains steroidal saponins called charantin, peptides similar to that of peptides and certain alkaloids that effectively control sugar level in blood.
Chemical constituents

Triterpine, Alkaloid, Phenolic compound, Momordicin, Charntin, Vicine

Extraction

Extraction of Brassica oleracea

Extraction procedure

After identification of plant brassica oleracea then washed with running water to decontaminatefrom dust particle.
The plants were covered with cloth and dried in shade for 10 days at room temperature.
The plant were grinded through blender and converted into coare se of powder
The powder 200gm was extracted by continuous hot extraction using soxhlet apparatus at atemperature of 78 degree centigrate for 48 hrs using 95 % ethanol.

Extract are dry and preserved in a desiccator until used for further studies stored in air tight sterilizedcontainer in cool dry

Phytochemical screening

Phytochemical screening of extracts obtained by both the aforementioned mentioned methods was performed to profile various phytochemicals present in cabbage. The screening was done both qualitatively and quatitatively.

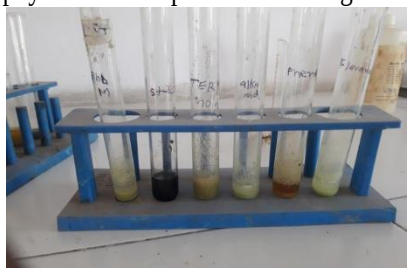
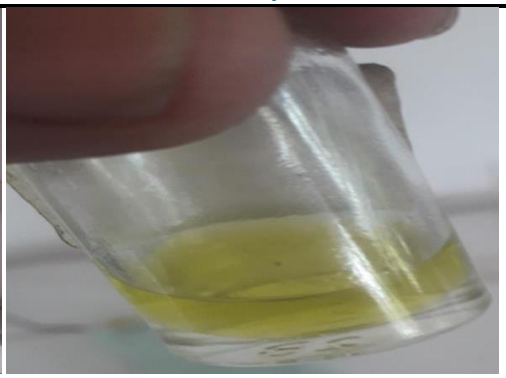


Figure: Phytochemical screening of Brassica oleracea and Momordica charantia

Test procedure
Test for tannins
2ml of aqueous extract
Add 2ml of distilled water
5 drop of Fecl3 solution added
Formation of green precipitate

Indication of presence of tannins
Test for flavanoid: 1ml of aqueous extract, 1ml of aqueous 10% lead acetate solution, Formation of yellow precipitate, Positive test for flavanoid
Test for phenol: 1ml of aqueous extract, Fecl3 were mixed, A deep bluish green solution, Indication of presence of phenols
Test for alkaloid: 1.27gm of 2gm of KI 100 ml water Readish brown colour indicate presence of alkaloid
Test for steroid: 0.5gm of extract, 2ml acetic anhydride, 2ml of H2SO4, Voilet or blue green colour
Terpenoid: 5ml of extract2ml of chloroform2ml of H2SO4Redish brown colour presence
Protein test: Ninhydrin dissolve in acetone, Treated with aqueous extract Purple colour
Column chromatography: Introduction to column chromatography
Column chromatography is a chromatography technique used to separate mixture of chemical substances into its individual compounds. Column chromatography is a widely used method for the purification or separation of chemical compound mixture in lab

Column Chromatography consists of two phases: one mobile phase and one contiguous stationery phase. The stationery phase is solid and the mobile phase is liquid. The compound mixture moves along with the mobile phase through stationery phase and separates depending on the different degree of adhesion (to the silica) of each component in the sample or the compound mixture.

Wet method

In wet method firstly a slurry of silica and solvent is prepared and then poured onto the column using a funnel. More solvent must be used until the silica is settled into it.

Materials AND equipments

Cylindrical chromatographic column, Separating funnel, Silica gel 60 (Mesh 230-400), Water (need for packing of column), Cotton

UV light detector (254 nm), TLC plate developing chamber, Pencil, Capillary tubes, Spatula

Organic solvents, Preparative TLC plates (20 cm x 20 cm)

Solvent mixer Isolation and purification of bioactive compounds from plant samples

A suitable size long cylindrical glass column (based on the amount of the sample) should be stand firm on a column-chromatography stand. Completely dried plant extract sample should be mixed with silica gel to make a fine powdered form for easy distribution of sample in already packed silica gel column.

Sample powdered mass should be placed on the top of the pre-packed silica column and sample should be covered with a layer of cotton. Then solvents of different polarities were passed through column at uniform rate under gravity to fractionate the sample extract. Each fraction was collected separately in a test tube and numbered consecutively for further analysis on thin layer chromatography. Thin layer chromatography (TLC) provides partial separation of both organic and inorganic materials using thin-layered chromatographic plates especially useful for checking the purity of fractions. Each fraction is applied on activated TLC plates with the help of capillary tube at a 1/2 inch apart from the lower edge of TLC plate, and plate is kept in a developing chamber containing suitable solvent system for specific time until the developing solvent reaches top of the upper edge of TLC plate.Plate is taken out from developing chamber, dried and solvent front is marked by lead pencil. Compound bands/spots visualized on TLC chromatoplate can be detected by visual detection,under UV light (254 nm), in iodine chamber and by using spray reagent (vanillin-sulfuric acid) for the presence of specific compounds. The visualized spots of the components in the chromatoplate are marked and the Rf value of each spot is calculated by the formula: Rf = distance travelled by the sample (cm)/distance travelled by the solvent (cm). TLC plate showing number of bands (compounds) for each fraction can be further purified using high performance liquid chromatography (HPLC). Based on the nature of the compounds, further spectral analyses such as infrared (IR), mass spectrometry, and nuclear magnetic resonance (NMR) can be performed to elucidate the chemical structure of target compounds.

HPTLC: Sophisticated form of thin layer chromatography It involves the same theoretical principle ofthin layer chromatography. Traditional Thin Layer Chromatography & its modern instrumental quantitative analysisversion HPTLC are very popular for many reasons such as visual chromatogram

In the present study, phytochemical profiling of *Brassica olerecea* extract and *Momordica charantia* was performed. Results clearly indicated the presence of various phytochemicals like tannin, flavonoid, alkaloid, anthocyanidin and phenols. It is reported that Brassica oleracea and Momordica charantia antibacterial, antifungal and anticancerous activity (5, 15). These activities may be attributed to various phytochemicals present in the extract. Tannin is reported to have antimicrobial activity and antibacterial activity. Alkaloids are TLC and HPTLC was performed to separate key component of cabbage extract i.e. alkaloid flavanoid which are reported to have anticancerous activity. We have separated hydrolysis products of alkaloid flavanoid This can be indicated from the known Rf values of the compounds (listed below). We have found the presence of

alkaloid and flavanoid .

In vivo study

Test system details

Species: Rat
Strain: Swiss albino
Sex: Male & Female
Source: Animal house Bn.University Udaipur
Age of animal: 15 week
Body weight: 100-200gm
Justification The albino rat are one of the recommended rodent species for oral studies as per sechedule Y guideline. Number of animal A total of 48 (24 male and 24 female) female will be nulliparous and Non pregnant, Test item Brassica oleracea extract Momordica charantia juice
Acclimatization: Animal will be selected and kept for acclimatization. The male animals will kept under acclimatization for minimum 5 days and female animals will kept under acclimatization for 6 days.
Randomization and grouping
Table 1 One day before the initiation of treatment 48 (24Male&24Female) rats will randomly divided into 8 groups of 6 animal each group (3Male&3Female).

	Groups (6Animal each groups)	DRUG	DOSE	Duration
	Vehicle control	CMC	0.5%CMC 5ml	28 Days
	Diabetic control	STZ	50mg/kg	28 Days
	Standard control	Glimepride	0.5mg/kg	28 Days
	HFI with low dose +low dose std	HFI+STD	5ml/kg+0.5mg/kg	28 Days
	HF1 with medium dose +low dose Std	HFI+STD	10ml/kg+0.5mg/kg	28 Days
	HF1 with high dose + low dose Std	HF1+STD	15ml/kg+0.5mg/kg	28 Days
	HF2	HF2	200mg/kg	28 Days
	HF1+HF2	HF1+HF2	10ml/kg+200 mg/kg	28 Days

Numbering and identification

The rats will mark on the picric acid solution prepare in water. The marking with in a cage as below;

Animal no.	Animal marking
1	Head
2	Body
3	Tail
4	Legs
5	Neck
6	No mark

The group no, cage no, sex of animal and animal no will be identified as indicated below using cage label and marking on the animal.
Animal husbandry
Housing: The rats will be housed in sterilized polypropylene cage with stainless steel top grill. Clean paddy husk or corn cob will use as a bedding material. Six rats will be hosed in one cage
Environmental condition: The animals will be kept 12 hours light and dark cycles. The room temperature will be maintained at 22±3°C and the relative humidity will be maintain between 30-70%.

Feed and feeding schedule: Feed will be provided ad libitum throughout the study period except over night fasting (16-20 hours) Prior to blood collection and will be offered immediately after completion of blood collection.

Water:Sterilized drinking water will be provided ad libitum in polypropylene bottles with a stainless steel sipper tube throughout the study to animals.

Oral glucose tolerance test: The Oral Glucose tolerance Test (OGTT) was performed on overnight fasting normal rats. Distilled water, respectively. Glucose (2 g/kg) was fed before study with distilled water, Blood glucose levels were measured at - 0hrs,1hrs,3hrs,8hrs The blood glucose was measured using blood glucose test strips and glucometer

Clinical signs: All animal will be observed daily once for any abnormal clinical signs and behavioral changes. Animal shown any pain or distress will be humanely killed. The cage side observation will include change in skin, fur, eyes and mucous membrane, occurrence of secretion or excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern stereotype like excessive grooming and repetitive circling or bizarre behavior like self – multilation, walking backward etc.

Mortality and morbidity: All animal will observed once daily for any mortality and morbidity through out the dosing period.

Body weight: The body weight of each rat organ will be recorded before initiation of dosing and at weekly intervals throughout the study period. The last body weight will recorded one day prior to blood collection before all the group animal will be kept for over night fasting.

Food consumption: The food consumption of each rat will calculated by giving the feed on need basis and obtaining the left over at every week throughout the study period using below mentioned formula. Formula for food consumption calculation.

Food consumption by 6 animal /week/=total quantity of food offered during the week(gms) Feed left over on last day of week (gms)

Food consumed by each animal /week=feed consumed by 6 animal/6 Food consumed by each animal /day =food consumed by divided animal/7

Terminal studies: After completion of treatment period for 28 days animal will be fasted overnight after which blood will be collected on the following day under ether anaesthesia by retro orbital puncture

The 7.0-0.1 ml blood will be collected in pre- labels containers sodium citrate solution as anti coagulant . from which first the haematological investigation will be performed then after that blood will be centrifuged and the plasma will be used for biochemical analysis.

Haematological analysis

Table Below mention haematological parameter will be studied using automated haematology analyzer

Sr.no.	Parameter	Sr.no.	Parameter
1	Red blood corpuscles	8	Different White blood corpuscles
2	White blood corpuscles		Neutrophiles
3	Hemoglobin		Lymphocyte
4	Pack cell volume		Eosinophiles
5	Mean corpuscular volume		Basophiles
6	Mean corpuscular Hemoglobin		Monocytes
7	Platelets	9	Mean corpuscular Hemoglobin conc.

Biochemical analysis

Table Below mention biochemical parameter will be studied by using automated biochemistry analyzer

Sr.No.	Parameter	Sr.No.	Parameter
1	Glucose	7	AST:Aspartate amino transferase
2	Triglyceride	8	AST; Alanineamino transferase
3	Cholesterol	9	ALP:Alkaline phosphatase

4	Urea	10	Total protein
5	Creatinine	11	Albumin
6	Total bilirubin	12	Globulin

Necropsy and organ weight

On day 29th of study all animal from each group will be sacrificed using carbon dioxide gas. Animal which died during the experimental subjected to post mortem examination to determine the presence/absence of gross and histopathological lesion. Post mortem necropsy finding will be made by systemic approach (gross pathological changes in organ size, shape, and visible lesion) below mention organ will be weighed for all the animal belonging to all four groups

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Sr.No.	Organ	Sr.No	Organ
1	Brain	6	Small & large intestine
2	Kidney	7	Lungs
3	Liver	8	Stomach
4	Pancreas	9	Uterus
5	Heart	10	Testes

STATISTICAL ANALYSIS

Result will be expressed as mean ± SEM for each group. For comparison of two mean, student's t-test will be performed. In case of multiple comparisons of groups, the one way of analysis of variance (ANOVA) followed by Tukey's test using Graph Pad Prism 6 statistical software. The level of statistical significance will be set at $p < 0.05$.

The positive outcome of the study will lead to addition of agents in the treatment of various ailments

Result

Acute toxicity: Oral administration of fruit methanol extract of Brassica oleracea given to rats at graded doses (200, 400, 800, 1600, 2000, 2400, 2800 and 3200 mg/kg) did not cause any death or acute toxicity symptoms in the animals.

Test parameter Table Observation table of Phytochemical parameter

Test	*Brassica oleracea*	*Momordica charantia*
Tannins	Negative	Negative
Flavanoid	Positive	Negative
Phenol	Negative	Negative
Alkaloid	Positive	Positive
Steroid	Negative	Negative
Terpenoid	Negative	Negative
Protein	Negative	Negative

Table Observation table of column chromatography

Brassica oleracea	*Momordica charantia*	*Fraction*
Alkaloid	Alkaloid	Positive
Flavanoid	Flavanoid	B positive

Biochemical analysis

Table Below mention biochemical parameter will be studied by using automated biochemistry analyzer

Sr.No.	Parameter	Sr.No.	Parameter
1	Glucose	7	AST:Aspartate amino transferase
2	Triglyceride	8	AST; Alanineamino transferase
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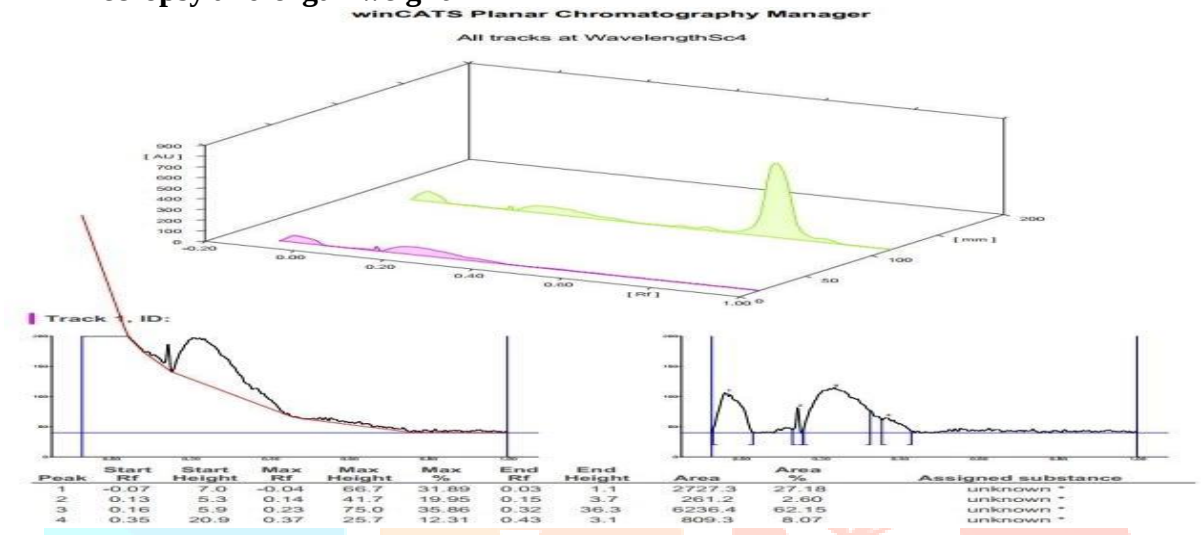
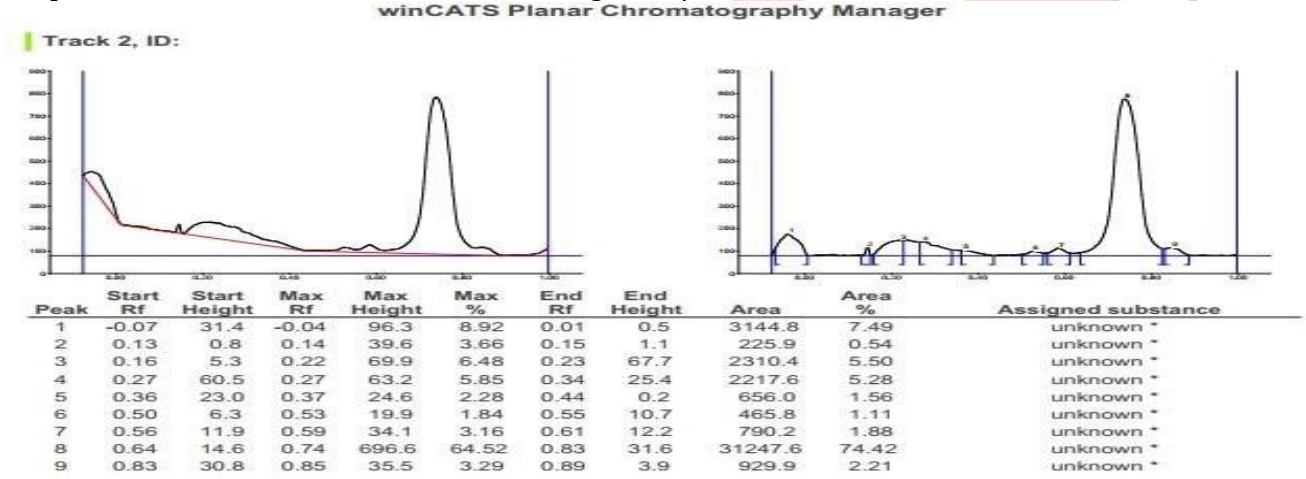
4	Urea	10	Total protein
5	Creatinine	11	Albumin
6	Total bilirubin	12	Globulin

Necropsy and organ weight



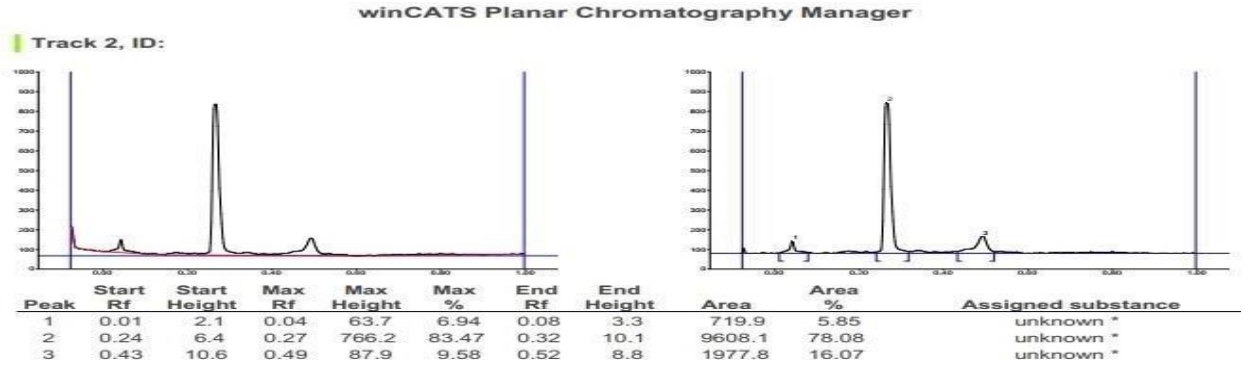
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.07	7.0	-0.04	66.7	31.89	0.03	1.1	2727.3	27.18	unknown *
2	0.13	5.3	0.14	41.7	19.95	0.15	3.7	261.2	2.60	unknown *
3	0.16	5.9	0.23	75.0	35.86	0.32	36.3	6236.4	62.15	unknown *
4	0.35	20.9	0.37	25.7	12.31	0.43	3.1	809.3	8.07	unknown *

Graph HPTLC chromatogram of Brassica oleracea extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.07	31.4	-0.04	96.3	8.92	0.01	0.5	3144.8	7.49	unknown *
2	0.13	0.8	0.14	39.6	3.66	0.15	1.1	225.9	0.54	unknown *
3	0.16	5.3	0.22	69.9	6.48	0.23	67.7	2310.4	5.50	unknown *
4	0.27	60.5	0.27	63.2	5.85	0.34	25.4	2217.6	5.28	unknown *
5	0.36	23.0	0.37	24.6	2.28	0.44	0.2	656.0	1.56	unknown *
6	0.50	6.3	0.53	19.9	1.84	0.55	10.7	465.8	1.11	unknown *
7	0.56	11.9	0.59	34.1	3.16	0.61	12.2	790.2	1.88	unknown *
8	0.64	14.6	0.74	696.6	64.52	0.83	31.6	31247.6	74.42	unknown *
9	0.83	30.8	0.85	35.5	3.29	0.89	3.9	929.9	2.21	unknown *

Graph HPTLC chromatogram of Brassica oleracea extract flavanoid standard

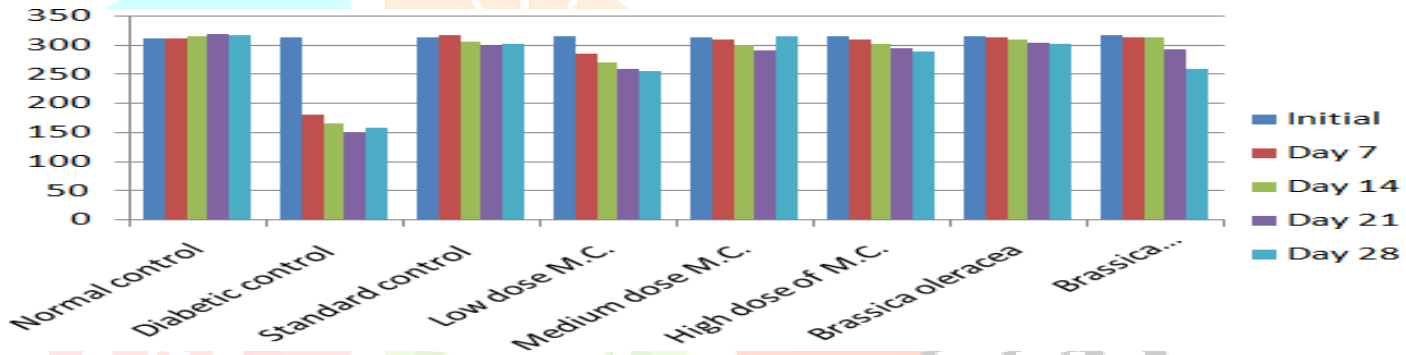


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	2.1	0.04	63.7	6.94	0.08	3.3	719.9	5.85	unknown *
2	0.24	6.4	0.27	766.2	83.47	0.32	10.1	9608.1	78.08	unknown *
3	0.43	10.6	0.49	87.9	9.58	0.52	8.8	1977.8	16.07	unknown *

Graph:HPTLC chromatogram of M. charantia fruit juice alkaloid standard

Group	Initial	Day 7	Day 14	Day 21	Day 28
Normal control	311.69±2.08	313.27±2.18	315.97±2.52	320.51±3.8	318.41±2.8
Diabetic control	313.79±2.92	180.7.±2.32	165.9±2.48	152.33±1.37	158.21±1.26
Standard control	313.79±2.43	318.27±2.2.18	306.43±1.78	301.71±1.52	303.24±1.83
Low dose M.C.	316.94±2.43	287.16±2.68	271.24±2.78	259.69±1.12	255.7575±3.8
Medium dose M.C.	42±2.36	310.04±2.88	301.36±1.94	291.72±1.83	315.43±2.78
High dose of M.C.	316.94±2.43	310.12±2.21	303.81±1.64	296.64±1.22	290.64±1.22
Brassica oleracea	315.89±2.32	314.85±2.30	310.80±1.90	305.70±1.72	303.72±1.83
Brassicaoleracea+M. C.	317.94±2.43	313.90±2.42	313.90±2.42	294.64±1.12	259.69±1.12

Table Effect-of weight on rats Brassica oleracea and different dose of Momordica charantia juice streptozotocin-induced-diabetic-rats.



Graph Effect-of weight on rats Brassica oleracea and different dose of Momordica charantia juice streptozotocin-induced-diabetic-rats.

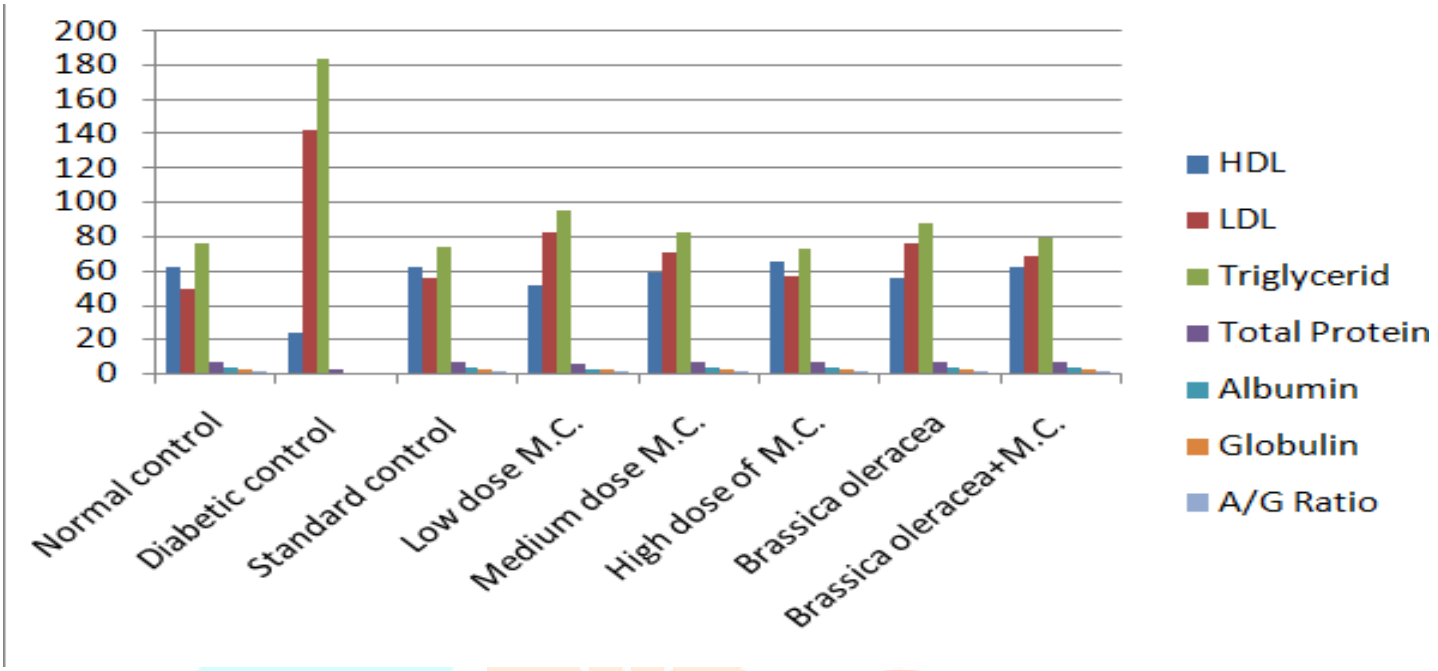
Normal control groups are not changes in weight 311.69±2.08 to 320.51±3.8 and diabetic control group body weight is decreased 152.33±1.37 to 158.21±1.26 and standard control group body weight is normal 313.79±2.43 to 301.71±1.52 and low dose of Momordica charantia given some animal body weight is decreased 259.69±1.12 to 287.16±2.68 high dose of Momordica charantia less body weight decreased 310.12±2.21 to 290.64±1.22 and Brassica oleracea treated group animal are body weight is normal 314.85±2.30 to 310.80±1.90 and Brassica oleracea with Momordica charantia treated group body weight decreased as compare to Brassica oleracea treated group. 313.90±2.42 to 259.69±1.12.

Determination of biochemical parameters after treatment with extracts

Group	HDL (mg/dl)	LDL(mg/dl)	Triglycerides (mg/dl)	TotalProtein(gm/dl)	Albumin(gm/dl)	Globuin(gm/dl)	A/G Ratio
Normal control	62.6±2.4	49.8±4.2	76.4±3.8	6.7±2.9	3.73±2.6	2.8±0.58	1.2±0.47
Diabetic control (STZ 50 mg /kg)	23.6±3.1*	142.3±2.6*	183.7±1.7*	2.5±4.5*	0.63±4.1*	0.15±0.61*	0.51±0.29*

Standard control Glimepride50 mg/kg	61.9±1.8[a]	55.7±3.7 [a]	73.8±2.6 [a]	6.2±3.1 [a]	3.83±2.7 [a]	2.2±0.18 [a]	1.3±0.43 [a]
Low dose *Momordica charantia* (5ml/kg)+Standard(0.5mg/kg)	51.7±2.7 [a]	82.6±2.3 [a]	95.0±3.4 [a]	5.1±2.8 [a]	3.12±2.1 [a]	2.1±0.39 [a]	1.6±0.24 [a]
Medium dose *Momordica charantia* (10ml/kg)+ Standard (0.5mg/kg)	59.6±3.6 [a]	71.03±2.9 [a]	82.0±2.7 [a]	6.8±3.6 [a]	3.56±3.4 [a]	2.7±0.72 [a]	1.2±0.32 [a]
High dose of *Momordica charantia* (15ml/kg)+ Standard (0.5mg/kg)	66.1±1.7 [a]	56.8±3.6 [a]	73.0±3.5 [a]	6.9±2.9 [a]	3.46±2.7 [a]	2.5±0.32 [a]	1.2±0.14 [a]
Brassica oleracea (200mg/kg)	56.3±3.4 [a]	76.2±2.1 [a]	87.3±2.9 [a]	6.1±3.2 [a]	3.71±3.9 [a]	2.4±0.53 [a]	1.3±0.56 [a]
Momordica charanita (10ml/kg) *Brassicaoleracea* (200mg/kg)	62.4±1.6 [a]	68.7±3.5 [a]	79.5±3.2 [a]	6.6±4.3 [a]	3.91±2.3 [a]	2.7±034 [a]	1.6±0.61 [a]

Table Values are expressed as mean ± SEM (Number of animals, n=6); *Significantly different from the normal control at P<0.05; [a]Significantly different from the diabetic control at P<0.0



Normal control groups are not changes in weight 311.69±2.08 to 320.51±3.8 and diabetic control group body weight is decreased 152.33±1.37 to 158.21±1.26 and standard control group body weight is normal 313.79±2.43 to 301.71±1.52 and low dose of Momordica charantia given some animal body weight is decreased 259.69±1.12 to 287.16±2.68 high dose of Momordica charantia less body weight decreased 310.12±2.21 to 290.64±1.22 and Brassica oleracea treated group animal are body weight is normal 314.85±2.30 to 310.80±1.90 and Brassica oleracea with Momordica charantia treated group body weight decreased as compare to Brassica oleracea treated group. 313.90±2.42 to 259.69±1.12.

Determination of biochemical parameters after treatment with extracts

Group	HDL (mg/dl)	LDL(mg/dl)	Triglycerides (mg/dl)	TotalProtein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A/G Ratio
Normal control	62.6±2.4	49.8±4.2	76.4±3.8	6.7±2.9	3.73±2.6	2.8±0.58	1.2±0.47
Diabetic control (STZ 50 mg /kg)	23.6±3.1*	142.3±2.6*	183.7±1.7*	2.5±4.5*	0.63±4.1*	0.15±0.61*	0.51±0.29*
Standard control (Glimepride 50mg/kg)	61.9±1.8[a]	55.7±3.7[a]	73.8±2.6[a]	6.2±3.1[a]	3.83±2.7[a]	2.2±0.18[a]	1.3±0.43[a]
Low dose of *Momordica charantia* (5ml/kg)+ Standard(0.5mg/kg)	51.7±2.7[a]	82.6±2.3[a]	95.0±3.4[a]	5.1±2.8[a]	3.12±2.1[a]	2.1±0.39[a]	1.6±0.24[a]

Medium dose of *Momordica charantia* (10ml/kg)+ Standard(0.5mg/kg)	59.6±3.6 [a]	71.03±2.9 [a]	82.0±2.7 [a]	6.8±3.6 [a]	3.56±3.4 [a]	2.7±0.72 [a]	1.2±0.32 [a]
High dose of *Momordica charantia* (15ml/kg)+ Standard(0.5mg/kg)	66.1±1.7 [a]	56.8±3.6 [a]	73.0±3.5 [a]	6.9±2.9 [a]	3.46±2.7 [a]	2.5±0.32 [a]	1.2±0.14 [a]
Brassica oleracea (200mg/kg)	56.3±3.4 [a]	76.2±2.1 [a]	87.3±2.9 [a]	6.1±3.2 [a]	3.71±3.9 [a]	2.4±0.53 [a]	1.3±0.56 [a]
Momordica charanita (10ml/kg) *Brassicaoleracea* (200mg/kg)	62.4±1.6 [a]	68.7±3.5 [a]	79.5±3.2 [a]	6.6±4.3 [a]	3.91±2.3 [a]	2.7±0.34 [a]	1.6±0.61 [a]

Table Values are expressed as mean ± SEM (Number of animals, n=6); *Significantly different from the normal control at P<0.05; [a]Significantly different from the diabetic control at P<0.05

Graph 6 Effect of various treatments on (A) haematological and (B) serum biochemical parameters of rats in various groups.

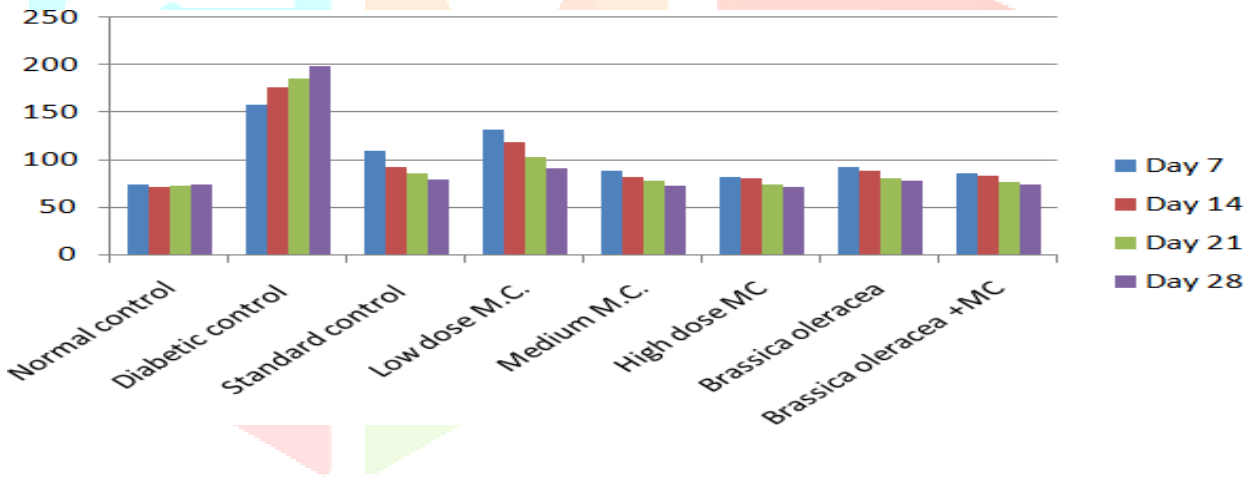
They are increase in LDL 49.8±4.2 to 142.3±2.6 and Triglyceride 76.4±3.8 to 183.7±1.7 level on diabetic control group as compare to normal control group and increase in triglyceride level of low dose of Momordica charantia 76.4±3.8 to 95.0±3.4 and decrease in compare to diabetic control group and HDL 62.6±2.4 to 23.6±3.1level is decrease in diabetic control group Total protein 6.7±2.9 to 2.5±4.5 decrease in diabetic control group..Albumin level is decrease in diabetic control group as compare to normal control

group 3.73±2.6 to 0.63±4.1Globulin level decrease in diabetic control group 2.8±0.58 to 0.15±0.61and A/G ratioof diabetic control group as compare to normal control group. 1.2±0.47 to 0.51±0.29 Effect of extracts on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 7	Day 14	Day 21	Day 28
Normal control	74.8±3.1	72.1±4.8	73.3±3.6	75.2±2.9
Diabetic control (STZ50 mg /kg)	159.2±4.8*	176.7±2.5*	186.4±2.7*	198.7±3.1*
Standard control (Glimepride 50mg/kg)	110.9±2.6[a]	92.6±.3.9 [a]	86.0±4.1 [a]	79.3±2.7 [a]
Low dose of *Momordica charantia*(5ml/kg)+ Standard (0.5mg/kg)	132.1±.3.9	119.4±.2.4 [a]	102.8±3.8 [a]	91.4±4.2 [a]

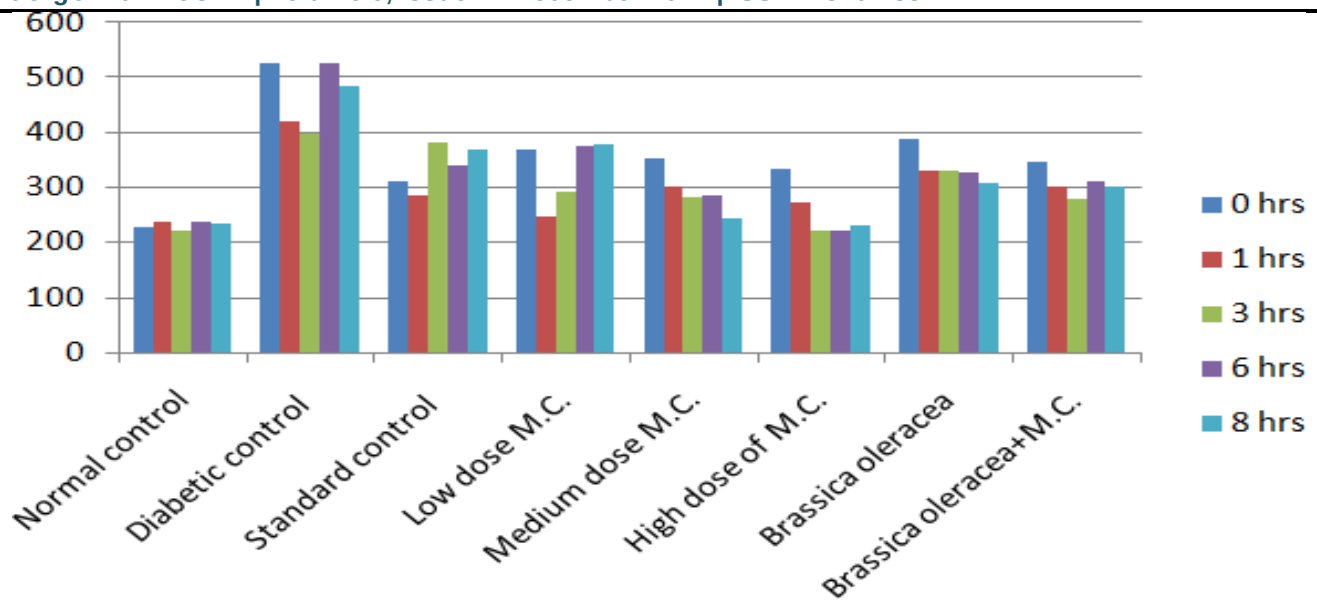
Medium dose of *Momordica charantia*(10ml/kg)+ Standard l(0.5mg/kg)	89.5±4.5 [a]	82.2±5.1 [a]	78.6±4.9 [a]	73.9±3.6 [a]
High dose of *Momordica charantia*(15ml/kg)+ Standard (0.5mg/kg)	82.7±.2.7 [a]	80.9±3.9 [a]	75.2±2.4 [a]	72.2±2.7 [a]
Brassica oleracea (200mg/kg)	92.6±3.4 [a]	89.4±2.5 [a]	81.7±3.8 [a]	78.6±4.9 [a]
Momordica charanita (10ml/kg) *Brassica*	86.2±2.3 [a]	83.7±4.6 [a]	77.2±2.7 [a]	74.5±3.4 [a]

different from the normal control at P<0.05; [a]Significantly different from the diabetic control at P<0.05



Graph Effect of extracts on fasting plasma glucose level in rats

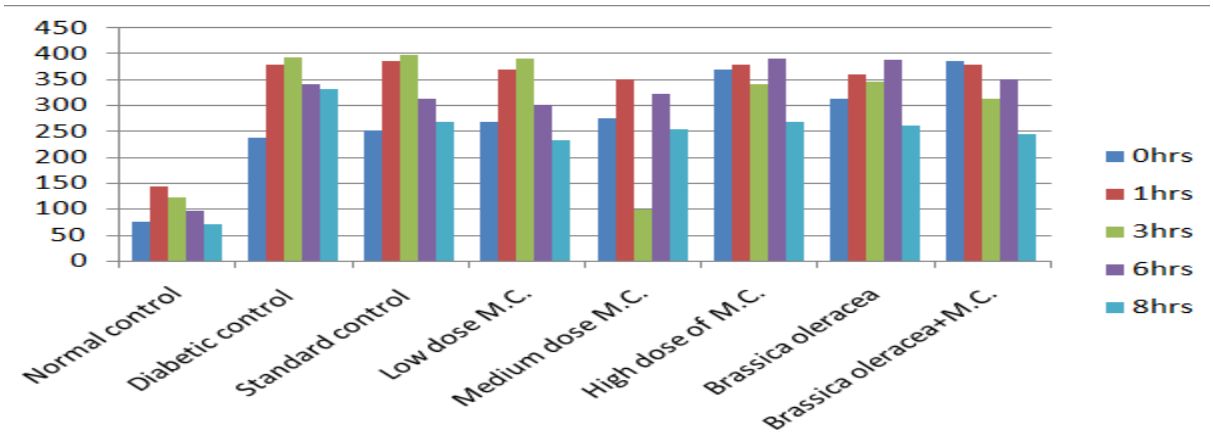
Normal control group blood glucose level 72.1±4.8 to 75.2±2.9 and diabetic control group blood glucose ratio is very high and increase in blood glucose level in every 7 days 72.1±4.8 to 198.7±3.1 standard control group blood glucose level decrease as compare to diabetic control group but some Ratio increase in compare to normal control group72.1±4.8 to 110.9±2.6.Low dose of Momordica charantis is less effect in medium dose 132.1±.3.9 to 89.5±4.5 and high dose of Momordica charantia decrease in blood glucose as compare to diabetic control group 198.7±3.1 to 82.7±.2.7.Brassica oleracea extract is lees effect on as compare to high dose and medium dose of Momordica charantia. 92.6±3.4 to 81.7±3.8 Both drug combination effect is decrease blood glucose as compare to Brassica oleracea extract 86.2±2.3 to 74.5±3.4.



Graph Effect of extracts on fasting plasma glucose level in rats in different time duration Body organ weight

Group	Liver	L-Kidney	R-Kidney	Pancreas	Heart	Stomach
Normal control	4.58±0.09	0.87±0.008	0.86±0.01	0.0069±0.1	0.79±0.003	11.45±0.18
Diabetic control	4.96±0.01*	0.99±0.004*	0.87±0.02 [a]	0.071±0.10 [a]	0.77±0.005*	13.82±0.04*
Standard control	4.44±0.08 [a]	0.88±0.01 [a]	0.98±0.002 [a]	0.73±0.073 [a]	0.87±0.005 [a]	11.83±0.16 [a]
Low dose M.C.	4.32±0.06 [a]	0.82±0.01 [a]	0.85±0.05 [a]	0.74±0.10 [a]	0.82±0.03 [a]	11.62±0.08 [a]
Medium dose M.C.	4.41±0.07 [a]	0.81±0.004 [a]	0.86±0.01 [a]	0.08±0.10 [a]	0.71±0.01 [a]	11.24±0.20 [a]
High dose of M.C.	4.39±0.14 [a]	0.83±0.02 [a]	0.79±0.08 [a]	0.73±0.073 [a]	0.32±0.04 [a]	11.59±0.22 [a]
Brassica oleracea	4.53±0.08 [a]	0.78±0.06 [a]	0.89±0.08 [a]	0.0069±0.1 [a]	0.76±0.06 [a]	4.29±0.14 [a]
Brassica oleracea+M.C.	4.89±0.12 [a]	0.76±0.06 [a]	078±005 [a]	0.74±0.10 [a]	0.75±0.07 [a]	11.59±0.22[a]

Table Values are expressed as mean ± SEM (Number of animals, n=6); *Significantly different from the normal control at P<0.05; aSignificantly different from the diabetic control atP<0.05



Group	0 hrs	1hrs	3hrs	6hrs	8hrs
Normal control	75.9±4.95	144.45±1.851	123.59±1.5	96.27±1.58	71.42±2.86
Diabetic control	237.26±5.	378.46±8.67	394.25±7.93	342.57±10.45	331.88±8.45
Standard control	253.89±7.93	386.67±6.09	398.45±8.45	312.56±8.1	267.78±6.98
Low dose M.C.	268.23±9.56	369.49±9.11	391.67±8.1	301.69±9.2	232.82±8.54
Medium dose M.C.	276.44±8.63	351.62±8.23	98.8.93±9.27	321.85±7.59	254±9.34
High dose of M.C.	369.49±9.11	378.46±8.67	342.57±10.45	391.67±8.1	267.78±6.98
Brassica oleracea	312.56±8.1	360.34±7.68	345.45±9.25	388.55±7.1	260.75±8.35
Brassica oleracea+M.C.	386.67±6.09	378.46±8.67	312.56±8.1	351.62±8.23	245.82±7.54

Table Values are expressed as mean ± SD (Number of animals, n=6); significantly different at AP<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

Diabetes is a chronic metabolic condition recognized worldwide as an important cause of premature death and disability, especially in the developing world. According to the WHO, the number of adult people suffering from diabetes has almost quad rupled since 1980 mainly due to the increase number of people living with diabetes mellitus type 2 and related factors driving it including obesity and overweight. Additional 2.2 million of deaths are associated with the increase risk of cardiovascular diseases due to the high blood glucose concentrations. The cost of managing diabetes can be catastrophic in poor population. There is an urge to seek other possibilities of managing diabetes in order to reduce the high rate of mortality. In fact, about 80% of the population relies on herbal medicines especially in developing countries due to the low cost and availability of these medicines. The WHO has requested several governments to include herbal medicines with proven efficacy and safety in their healthcare programs

Phytochemical screening of the methanolic extract of fruit parts of Brassica olerace showed the presence of Flavanoid alkaloid as shown in Table 7a.1.Momordica charantia fruit juice are presence of alkaloid.

Column chromatography Wet method

In wet method firstly a slurry of silica and solvent methanol and petroleum ether is prepared and then poured onto the column using a funnel. More solvent must be used until the silica is settled into it.Column chromatography of Brassica oleracea fraction in alkaloid and flavanoid and Momordica charantia fraction on alkaloid presence Phytochemical fingerprinting

TLC and HPTLC analysis of the methanolic extract showed a number of bands when visualized under varying wavelengths, 254 nm, 366 nm and 530 nm (figure: 7a.1, graph: 7a.1, 7a.2, 7a.3). A similar study conducted by also showed the presence of several peaks and area under HPTLC analysis suggesting a number of different phytoconstituents present in the extract

Oral administration of fruit methanol extract of Brassica oleracea given to rats at graded doses (200, 400, 800, 1600, 2000, 2400, 2800 and 3200 mg/kg) did not cause any death or acute toxicity symptoms in the animals.Change in body weight

During the study, symbolic reduction in body weight disease control group body weight is decrease and Brassica oleracea and Momordica charantia treated group body weight is normal as compare to diabetic control group.

Changes in haematological and serum biochemical assay

They are increase in LDL and Triglyceride level on diabetic control group as compare to normal control group and increase in triglyceride level of low dose of Momordica charantia and decrease in compare to diabetic control group and HDL level is decrease in diabetic control group Total protein decrease in diabetic control group..Albumin level is decrease in diabetic control group as compare to normal control group Globulin level decrease in diabetic control group and A/G ratioof diabetic control group as compare to normal control group.

Effect of extracts on fasting plasma glucose level

Normal control group blood glucose level and diabetic control group blood glucose ratio is very high and increase in blood glucose level in every 7 days standard control group blood glucose level decrease as compare to diabetic control group but some Ratio increase in compare to normal control group.Low dose of Momordica charantis is less effect in medium dose 13 and high dose of Momordica charantia decrease in blood glucose as compare to diabetic control group.Brassica oleracea extract is lees effect on as compare to high dose and medium dose of Momordica charantia. Both drug combination effect is decrease blood glucose as compare to Oleracea brassica extract.

streptozotocin-induced diabetic rats significantly reduced the plasma glucose concentrations of the animals. Therefore it is possible to speculate that the presence of flavonoids, alkaloids may be contributing at least in part, to the antidiabetic activity of Brassica olerace and Momordica charantia observed in normoglycaemic rats and streptozotocin-induced diabetic rats in our study.

In the oral glucose tolerance test done in this project, administration of fruit methanol extract of Brassica olerace and Momordica charantia following oral glucose load on fasted normoglycaemic rats significantly reduced the increased blood glucose concentrations. Further supports the claim that the plant species has hypoglycaemic effect on normal fasted animals when compared to the results obtained from glemipride the hypoglycaemic reference drug, used.

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