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Toxicity Study and Anti-Diabetic Activity of Indeginious Medicinal Herb Momordica Charantia

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Abstract:

Momordica charantia IS used as traditional medicines to treat diabetes mellitus. The current study is done on treatment of diabetes mellitus with various herbal plants of Maharashtra region. Initially acute toxicity studies were performed, and diabetes is induced in rats with alloxane and streptozocin in laboratory. Alloxan induced diabetes is like type I diabetes due to deficiency of insulin in the body because of no release of insulin and type II diabetes is studied on the basis of hyperglycemia or obesity of the patients. These are treated with different plant extracts and comparison is done with standard antidiabetic drug.

The two most important plants are selected from Maharshtra vidharbha region and this is also found all over the country and most widely utilized for controlling sugar and lipid profile. Here these plants and their toxicity were studies for antidiabetic purpose. Karela fruits and seeds are useful constituents for extraction and isolation of active constituents.

Keywords: momordica charantia, diabetes,, extraction and evaluation, toxicity, anti-diabetes evaluation..

1. Introduction

There are many plants that have been used in diabetes from very old days these are bulbs of allium cefa (piyas) and allium sativum (lahsun). The fruits of syzygium cumini (jamun) are also used for treatment of hyperglycemia. The fruits of momordica charantia (karela) are used in the treatment of diabetes mellitus. The plant ptercarpus marsupium is useful in the treatment of high sugar in urine.

Gymnemasylvestre is useful for the high sugar level in urine. The seeds of tetragonus cymbosis (guar) are utilized for patients of diabetes mellitus. These plants are easily available in Maharashtra and their water or alcoholic extracts are most powerful for treatment of hyperglycemia. Azadiractaindia is most precious plant and their leaves are useful for treatment of high sugar in urine.

Diabetes can be induced by different methods using streptozocine and alloxane. This can be generated with adrenaline injections in rats and other animals. The plants parts are collected, and water or

alcohol suitable solvent is used for extraction and administered to animals and three groups are made for measuring antidiabetic activities. These are standard, test and control groups.Toxicity studies are also performed on the animals for safetypurpose and drugs may be developed as ayurvedic formulation for better used in treatment and prevention of diabetes mellitus.

Sugar is a major complication at older age. Overeating and not controlling diet is major cause of diabetes. The pancreas release insulin but that insulin is not sufficient for digestion of more food so extra insulin is required. In old age cells are inactive and insulin is not secreted after requirement of our body. So, more sugar comes to the blood and reabsorbed by kidney. Pressure on the kidney is another complication and dangerous to the body. A regular load of sugar is the major cause of kidney failure. So regular checkup of body is beneficial and diet control is also required.

Hypoglycemia is major side effect of insulin therapy and oral hypoglycemia. Plants are easily cultivated and not harmful. The active constituents of these plants are carbohydrates, glycosides, alkaloids and falvanoids for treatment and prevention of sugar. Momoridica charantia contains momoridin and charanticene as antidiabetic glycosides.

2.0 Materials and Methodology

Plant profile: *Momordica Charantia*

Figure 1:

Common names – karela

Family – cucurbitaceae

Biological Source – It is fruit of *momordica charantia*, known as karela and bitter bottle gourd

Medicinal uses- It is mainly used in diabetes as hypoglycemic agent and antiobesity. These plants are also useful for reducing stress, anxiety, and obesity with frequent utilization. Taking fruit in diet is good habit that makes person health and mentally and physically strong.

Adults without diabetes as recommended by the Organization for Economics and Development's recommendations (AOT no. 425), acute toxicity experiments were conducted on male albino rats. For the following 48 hours, the rats' behavioral, neurological, and autonomic profiles, as well as any signs of fatality, were monitored continually.

Induction of diabetes

Alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg of body weight was administered intra peritoneally (IP) into the rats. Due to the potential for fatal hypoglycemia produced by the substantial mice had pancreatic insulin secretion administered a 15–20 ml of an intraperitoneal 20% glucose solution 6 hours after receiving alloxan. For an additional 24 hours, the rats were kept on bottles of 5% glucose solution to prevent hypoglycemia.

Chronic treatment model

A total of 60 rats (n = 6 groups of 5) were used in this study. The first group was the healthy controls, while the second was the untreated diabetics. Groups 3, 4, and 5 were administered 100 mg/kg, 200 mg/kg, and 500 mg/kg of ethanolic extracts of Cuminum cyminum and Trigonella foenum-graecum, and Chrysospermum mmi and Cicer arietinum L, respectively. Group 6 served as a control and received glibenclamide at 5 mg/kg daily for twenty-one days. In this

study, participants' blood sugar levels and weights were measured on days 1, 7, 14, as well as 21.

Six groups of diabetic rats (n = 5) were created,

- Group I- Tween 80 in purified water at 5% (5 ml/kg b.w., p.o.) was given to normal control rats.
- Group II - Rats with diabetes were given an intraperitoneal (p.o.) solution of Tween 80 (5% in water).
- Group III - Oral administration of a polyherbal formulation (FCAC) at 100 mg/kg body weight was used to treat diabetes in rats.
- Group IV- Polyherbal formulation (FCAC) 200mg/kg body weight, orally administered, was given to diabetic rats.
- Group V - Polyherbal formulation (FCAC) 500mg/kg body weight, orally, was administered to diabetic rats.
- Group VI - Glibenclamide (5mg/kg body weight, orally) was administered to diabetic rats.

For a total of 21 days, extracts were given once daily. The Accu-check glucometer was used to determine blood glucose levels from blood samples taken via the lateral tail vein on days 1, 7, and 21 following medication delivery.

Oral feeding needles were used for all medication administration.

Acute study

Studies on the acute toxicity of a polyherbal preparation in rats. No graphical indication of abnormalities was identified up to 4 hours, as well as no deaths were recorded up in creatures until at the highest tested dosage level of 2000 mg/kg body weight after 48 hours. Such was then utilized as the MTD, as well as a test dose of 1/10th of the MTD was selected (100 mg/kg b.w.); moreover, a test dose of 500 mg/kg b.w. was selected for the experimental trials.

Subacute study

The medications were given to the animals at regular intervals over the course of 28 days. On days 1, 7, and 21 GLs were estimated. After 21 days, patients were given a week off from taking their medication to recover. On day 28, we made our best guess at the GLs. Average GL SEM was used to summarize the data.

Statistical analysis

The standard deviation and mean were used to summarize the data. Statistical examination was carried out in Graph Pad Prism 5 employing a one-way analysis of variance (ANOVA) as well as t-test of Dunnett. Statistical significance was determined by a probability value of less than *p0.05, p0.01, or p0.001 when compared to the control as well as reference groups, respectively. (Diabetes & Metabolism, 1989).

3. Results and Discussion

Toxicity Studies

Acute Oral Toxicity Study (72 hours)

50 % ethanolic extract of selected plants *Momordica charantia* (PO), were evaluated for its acute oral toxicity in mice. Animals were divided into five groups of two mice each weighing about 20-25 g. acute oral toxicity was conducted in three sets of experiments.

In first experiment, 50% ethanolic extracts of *Momordica charantia* (PO) respectively was studied for their acute oral toxicity effects in mice. For this total thirty mice were taken & in each set of experiment, ten mice were divided into five groups of two mice each & groups were as follows: -

Group I ----- Normal Control. (2% gum acacia)
Group II ----- PO (500 mg / kg b.w)
Group III ---- PO (1000 mg / kg b.w)
Group IV ---- PO (1500 mg / kg b.w)
Group V ---- PO (2000 mg / kg b.w)

Mice were acclimatized for a period of 7 days before the start of treatment.

Ethanolic extract of PO in four dose levels was given orally in single doses to mice of Groups II, III, IV, V while Group I received only vehicle. (2% gum acacia). Extracts were administered in 2% gum acacia. animals were observed for mortality & general behaviour periodically, for 48 hr to 72 hr. behaviour of animals was observed daily for 1 hr in forenoon (10 to 11.am) animals were observed continuously for initial 4 hr. & intermittently for next six hr. & then again after 24, 48 & 72 hrs following administration of different doses of PO extract.

Following parameters were observed during acute oral toxicity study

- *Grooming* was considered in mice if animal cleared fur & skin of itself or another animal.
- *Hyperactivity* if there was any abnormal or excessive activity & animal was unable to relax
- *Sedation:* if animal was calm & composed without any stress
- *Having respiratory arrest*, if there was raising of head
- *Having convulsions* if there was tremor in tail or paddling of feet
- *Motor activity*, increased or decreased,
- *Mortality,* if any.

Subacute Toxicity study (14 days)

Ethanolic extract of *Momordica charantia* whole plant (PO) was administered orally once daily to mice. Before initiation of experiment, mice were acclimatized for period of seven days. To study subacute
Toxicity, animals of either sex (20-25 g body weight) were divided into five groups of
Six mice each. Treatment was given as per following protocol.
Group I- Normal Control (2% aqueous gum acacia)
Group II PO (200 mg/kg b.w)
Group III PO (400 mg/kg b.w)
Treatment was continued for 14 days. During this period, mice of control group received only 2% gum acacia. After 14 days, animals were fasted overnight & blood was collected by cardiac puncture. Blood samples were taken for haemoglobin & white blood corpuscles estimation. blood was allowed to clot for one hour & serum was separated by centrifuging & evaluated for different biochemical parameters results obtained were subjected to ANOVA followed by students t test, $p > 0.05$ was considered as non significant, $p< 0.05$ – significant, $p< 0.01$- highly significant & $p<0.001$ as very highly significant. After taking blood samples, animals were sacrificed. Liver kidney & spleen were excised from animals, preserved in 10% formalin & sent for histopathological studies. Following biochemical parameters were evaluated in subacute toxicity studies.

Antidiabetic Study

Study of 50% ethanolic extract of Momordica charantia whole plant (PO) against alloxan induced diabetes was done (10 days study)

In this study, ethanolic extract of PO (100 & 200 mg/kg b.w was evaluated for antidiabetic activity against alloxan induced diabetes mellitus in rats. Rats were divided into 10 groups consisting of 6 rats in each group. Initially 65 rats were taken to account for any mortality. Rats were acclimatized for period of 7 days before starting experiment. After overnight fasting, hyperglycaemia was induced by administering single dose of alloxan monohydrate supplied by s.d Fine-Chemical Ltd. Mumbai, India (120 mg/kg b.w) **(Vivek *et al.*, 2010)** prepared in sterile saline to all groups except group I which served as normal control. During this period, animals were given free access to water. After 5 days of alloxan administration, fasting blood glucose levels of rats were checked by glucostrips. Animals having blood glucose levels > 250 mg/dl were

separated & selected for further studies & then re-grouping of these hyperglycemic rats was done as per following protocol, for studying antidiabetic activity of different extracts.

Group I Normal Control (2% of gum acacia.)
Group II Diabetic Control (Alloxan monohydrate & 2% gum acacia)
Group III Alloxan monohydrate + Glibenclamide (10 mg/kg.).
Group IV Alloxan monohydrate + PO (100 mg/kg b.w)
Group V Alloxan monohydrate + PO (200mg/kg b.w).

Treatment was started from same day except normal control & diabetic control groups for period of 10 days orally. During this period, animals in all groups had free access to standard diet & water. Blood glucose levels were estimated on 1st, 4th, 7th & 10th day of treatment. Besides this, during this study the body weight of rats were recorded on 1st, 4th 7th & 10th day of treatment. On the 11th day, blood samples were collected from overnight fasted rats by cardiac puncture. Animals were anaesthetized by mild ether anaesthesia before cardiac puncture. Blood was collected & allowed to stand for one hour, serum was separated by centrifuging & evaluated for different biochemical parameters.

Study of 50% ethanolic extract of Momordica charantia whole against Streptozotocin (STZ) induced diabetes was done(15 days study)

In this study, ethanolic extract of *Momordica charantia* whole plant PO (50 & 100 mg/kg b.w) was evaluated for antidiabetic activity against streptozotocin induced diabetes mellitus in rats. Rats were divided into nine groups consisting of six rats each. Initially sixty rats were taken to account for any mortality. Rats were acclimatized for period of 7 days before starting experiment. After overnight fasting, hyperglycaemia was induced by administering single dose of streptozotocin. (50 mg/kg bow) (Prasad *et al,* 2009)to all rats excepting group I which served as normal control. Streptozotocin was freshly dissolved in 0.1 M citrate buffer(pH=4.5) & injected intraperitoneally within 15 min of dissolution in vehicle volume of 0.4 mL with 1 mL of tuberculin syringe fitted with 24 gauge needle. Diabetes was confirmed by determination of fasting glucose concentration on third day post administration of streptozotocin. During this period, animals were given free access to water. After 3rd day of STZ administration, fasting blood glucose levels of rats were checked by glucostrips. Animals having blood glucose levels > 250 mg/dl were separated & selected for further studies & then re-grouping of these hyperglycemic rats was done as per following protocol, for studying anti-diabetic activity of different extracts.

Rats were given following treatment in this study.

Group I Normal Control (2% gum acacia).
Group II Diabetic Control. Received STZ (50 mg/kg b.w single dose i.p)
Group III STZ + Glibenclamide (3 mg/kg,)
Group IV STZ + PO (50 mg/kg b.w)
Group V STZ + PO (100mg/kg b.w)
Group VI STZ+ SGCF (50 mg/kg b.w)
Group VII STZ+ SGCF (100 mg/kg b.w)
Group VIII STZ + SGCS (50 mg/kg b.w)
Group IX STZ + SGCS (100 mg/kg b.w)

Treatment was started from same day except normal control & diabetic control groups for period of 15 days orally. During this period, animals in all groups had free access to standard diet & water. Blood glucose levels were estimated on 1st, 4th, 9th& 15th day of treatment. Besides this during this study body weight of rats were recorded on 1st, 4th 9th & 15th day of treatment. On day 16th, blood samples were collected from overnight fasted rats by cardiac puncture.

Animals were anaesthetized by mild ether an aesthesia before cardiac puncture. Blood was collected & allowed to stand for one hour, serum was separated by centrifuging & evaluated for different biochemical parameters. Animals were killed & liver, kidney & pancreas were taken out. Histopathology of these organs was also done.

Statistical Analysis

Data obtained from different studies & biochemical estimations is expressed as Mean ± SEM for each group. After this, statistical analysis was carried out using one way analysis of variance (ANOVA) followed by student's t-test. Values $p > 0.05$ were considered non-significant; $p < 0.05$ as significant, $p<0.01$ as highly significant & $p<0.001$ as very highly significant respectively.

Histopathological studies

Pancreas
Kidney
Liver

Organs were taken out, preserved in 10% formalin & sent for histopathological studies.

Physical Characteristics & Percentage Yeild of Different Extracts

a) Ethanolic extract of *Momordica charantia* (whole plant)

Weight of dried whole plant taken = 2750 gms Weight of extract obtained = 385 gms
% yield = Weight of extract obtained x 100
Weight of dried whole plant taken
% age yield of ethanolic extract = 14 %

Extract	Colour	Odour	% Extractive value
50% Ethanolic	Dark Brown	Characteristic	14%

PHARMACOLOGICAL STUDIES

50% ethanolic extract of whole plant of *Momordica charantia* (PO), 50% ethanolic extract evaluated for acute oral toxicity studies for 72 hours & sub-acute toxicity studies for 14 days antidiabetic activity, showed following results

Toxicity Studies

Acute Oral Toxicity Tests (72 hour study)

50 % ethanolic concentrate of chose plants Momordica charantia (PO) was assessed for their intense oral poisonous quality in swiss pale skinned person mice. In main investigation, half ethanolic concentrates of Momordica charantia (PO) was examined for its intense oral danger impacts in swiss pale skinned person mice.

a. ***Momordica charantia regulated*** at four measurement levels (500, 1000, 1500 & 2000 mg/kg b.w) uncovered accompanying impacts amid intense poisonous quality studies directed in mice for 72 hours. Control mice which had gotten 2% of gum acacia demonstrated ordinary conduct (Table 14*)*

- Prepping: After 48 & 72 hours, no preparing was seen at all four dosage levels.
- Hyperactivity: PO separated at regulated dosage levels had no impact on the action of mice which stayed typical.
- Sedation: After 48 hours, sedation was found in 50 % of creatures at measurements of 500 mg/kg b.w while 100 % of creatures in dosage scope of 1000,1500 & 2000 mg/kg b.w demonstrated sedation ,as creatures tried to avoid panicking & formed with no anxiety.
- Respiratory capture: After 48 hours, 50 % of creatures in measurements scope of 500 mg/kg b.w& 100% creatures in dosage scope of 1000, 1500 & 2000 mg/kg b.w demonstrated respiratory capture showed by bringing of head up in both creatures of these gatherings.
- Writhing : After 48 & 72 hours 50 % of creatures in measurements scope of 500 mg/kg b.w& 100% creatures in dosage scope of 1000,1500 & 2000 mg/kg b.w

indicated shakings as tremor in tail & paddling of feet.

Mortality: 50 % of creatures passed on at dosage of 500 mg/kg b.w following 72 hours while 100 % of creatures in measurements scope of 1000, 1500 & 2000 mg/kg b.w kicked bucket following 24 hours.

Results of Sub-acute Toxicity Study (14 days)

Tab. 1Effect of 50% ethanolic extract of Momordica charantia(PO) on Serum Glucose Levels (mg/dl) in swiss albino mice (Subacute toxicity study 14days)

Serum Glucose Levels (mg/dl)					
Mice	**Group I Normal control (0.2 ml of 2% gum acacia)**	**Group II PO (200mg /kg)**	**Group III PO (400mg/kg)**	**Group IV SGCF (250mg/kg)**	**Group V SGCF (500mg/kg)**
1	88.12	65.15	43.66	55.50	23.45
2	97.98	51.46	51.34	46.24	19.56
3	98.02	47.35	57.23	51.12	25.34
4	95.67	68.67	42.87	46.06	31.78
5	93.45	68.46	44.87	47.00	31.23
6	96.89	64.45	43.33	46.80	27.67
Mean	95.02	60.92	47.22	48.79	26.51
Std. Deviation	3.788	9.175	5.821	3.783	4.7
SEM	1.54	3.74	3.37	1.54	1.91
P-Value		0.0114	0.0114	0.5918	P<0.0001
Statistical Compared Group		I & II	II & III	III & IV	IV & V

PO = 50% ethanolic extract of *Momordica charantia* whole plant
All extracts were administered orally in 2% gum acacia, daily for 14 days.
n=6 (Number of animals in each group)
** P< 0.01 highly significant,*p<0.05 Significant

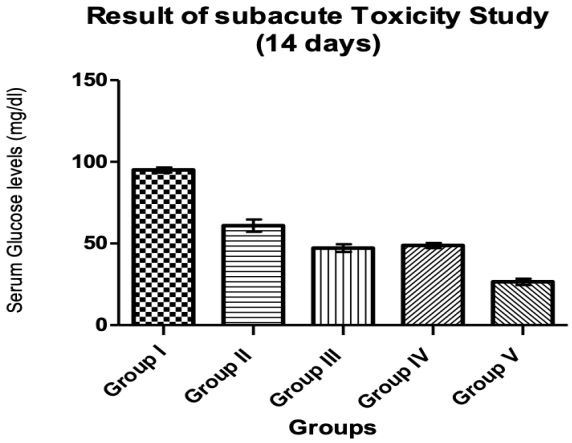

Figure 2: Effect of 50% ethanolic extract of PO whole plant on Serum Glucose Levels (mg/dl) in swiss albino mice.

B. Histopathology

Effect of 50 % Ethanolic extract of *Momordica charantia* (PO) whole plant on histopathology of different organs in subacute toxicity studies (14 days) are:

LIVER: Histopathological examination of liver slides of mice of Group I (Normal

control) showed portal triad area with no abnormality**.**

Momordica charantia (PO) whole plant when administered at two dose levels (200 & 400mg/kg) to mice of Group II & Group III respectively showed normal portal triad with no abnormality in liver.

SPLEEN: Spleen of mice of Group I (Normal control) showed normal spleen with red & white pulp areas in splenic parenchyma.

Momordica charantia (PO) whole plant when given at two dose levels (200 & 400mg/kg b.w) to mice of Group II & Group III respectively showed normal red & white pulp areas in splenic parenchyma

KIDNEY: Histopathological examination of kidney in mice of Group 1 (Normal control) showed glomerulus is of normal size & cellularity & tubules are within normal limits**.** ***Momordica charantia*** whole plant (PO) when given at two dose levels (200 & 400mg/kg b.w) to mice of Group II & Group III respectively showed that glomerulus is of normal size & cellularity & tubules are within normal limits**.**

Antidiabetic Studies

Antidiabetic studies using alloxan & streptozotocin as diabetic models revealed following results.

Alloxan

(10 days study) Effect of various dose of 50% Eth-Extract of *Momordica charantia* whole plant (PO), *Syzygium cumini* fruits (SGCF) &*Syzygium cumini* seeds (SGCS), against alloxan induced diabetes mellitus in rats was studied on following parameters

Statistical Analysis

Data obtained from different biochemical estimations is expressed as Mean ± SEM for each group. After this, statistical analysis was carried out using one way analysis of variance (ANOVA) followed by student's t-test. Values $p > 0.05$ were considered non-significant; $p < 0.05$ as significant, $p<0.01$ highly significant & $p<0.001$ very highly significant respectively.

Serum Glucose Levels (mg/dl)

There was very highly significant ($p<0.001$) rise in serum glucose levels in rats in diabetic group (Group II) (271.02±8.18) as compared to levels in normal control group (Group I) (84.71± 6.11 mg/dl). ***Momordica charantia*** extract (100 & 200 mg/kg b.w) showed decrease in serum glucose levels. At dose of 100mg/kg, administered to Group IV, decrease was highly significant ($p<0.01$) (142.82±2.76 mg/dl) while dose of 200mg/kg administered to Group V showed very highly significant decrease ($p<0.001$) (111.58±2.69 mg/dl) in serum glucose levels.

Syzygium cumini fruits at dose of 50 mg/kg administered to rats of Group VI showed highly significant decrease ($p<0.01$) (157.04±3.09 mg/dl), while 100 mg/kg administered to Group VII showed very highly significant decrease ($p<0.001$) (128.64±1.61 mg/dl) & at 200 mg/kg b.w administered to rats of Group VIII also showed very highly significant decrease ($p<0.001$) in serum glucose levels in dose dependent manner (82.82±5.53 mg/dl) as compared to group II that had received only alloxan monohydrate (271.02±8.18mg/dl)($p<0.001$). **Thelevels of serum glucose levels in case of rats administered SGCF at dose of200mg/kg b.w were found to be similar to levels of normal control rats.**

seeds of ***Syzygium cumini*** at dose of 100 mg/kg administered to rats of Group IX showed highly significant decrease ($p<0.01$) of (155.54±2.54 mg/dl) while dose of 200 mg/kg b.w administered to rats of Group X also showed very highly significant decrease ($p<0.001$) (120.74±6.13 mg/dl) in serum glucose levels. Standard anti-diabetic drug, glibenclamide administered to rats of Group III also revealed very highly significant decrease ($p<0.001$) in serum glucose levels (114.84±3.20 mg/dl)

Serum Total Cholesterol levels (mg/ dl)

As compared to group I (85.25±8.51 mg/dl) levels in group II rats which had received only alloxan monohydrate, there was very highly significant increase ($p<0.001$) in total cholesterol levels (218.15±23.79 mg/dl). 50% ethanolic extract of ***Momordica charantia*** (100 mg/kg b.w) administered to rats of Group IV showed highly significant decrease ($p<0.01$) (196.80±25.95 mg/dl) while dose of 200 mg/kg b.w administered to rats of Group V revealed very highly significant decrease ($p<0.001$) in total cholesterol levels (101.85±19.17 mg/dl).

Serum Triglyceride Levels (mg/ dl)

Rats of Group I revealed serum triglyceride levels of (80.71±9.38 mg/dl). Rats of groups receiving 100 mg/kg b.w of extract administered to rats of Group IV & 200 mg/kg b.w administered to rats of Group V of 50% ethanolic extract of ***Momordica charantia*** showed dose dependent highly significant ($p<0.01$) reduction in serum triglycerides (140.58±18.62 mg/dl) & ($p<0.001$) (97.32±12.15mg/dl) indicating very highly significant reduction in lipid profile. Group II rats which received only alloxan monohydrate showed very highly significant increase in serum triglycerides. (194.56±14.99 mg/dl)($p<0.001$) dose of 50 , 100 & 200 mg/kg b.w of fruits of

Serum HDL Cholesterol Levels (mg/ dl)

There was non significant)($p.>0.05$) decrease in serum HDL Cholesterol levels in rats in diabetic group (Group II) (21.11±1.45 mg/ dl)as compared to normal control (Group I) (24.54±6.49 mg/dl) . ***Momordica charantia*** at dose of 100 mg/kg b.w

Serum LDL Cholesterol Levels (mg/ dl)

As compared to group I (52.03±3.21 mg/dl), levels in group II rats which had received only alloxan monohydrate, there was highly significant increase ($p<0.01$) in serum LDL cholesterol levels (89.59±13.82 mg/dl). When 50% ethanolic extract of ***Momordica charantia*** (100 mg/kg b.w) was administered administered to rats of Group IV it showed very highly significant increase ($p<0.001$) (143.14±19.38 mg/dl) but dose of 200 mg/kg b.w administered to rats of Group V showed significant decrease ($p<0.05$). In serum LDL cholesterol levels (60.06±12.46 mg/dl).

Serum Urea Levels (mg/ dl)

Rats of Group I showed level of serum urea level of (22.32±3.75 mg/dl). Group II rats which received only alloxan monohydrate showed non significant increase ($p>0.05$) in serum urea levels (23.06±0.69 mg/dl).

Rats of group IV receiving 100 mg/kg b.w of 50% ethanolic extract of ***Momordica charantia*** showed non-significant level ($p>0.05$) of (26.47±2.95 mg/dl) increase in serum urea levels. Rats of group V which received 200mg/kg b.w of ethanolic extract showed non-significant level ($p>0.05$) of (25.95±2.88 mg/dl).

Serum Creatinine Levels (mg/ dl)

There was non significant rise ($p>0.05$), in serum creatinine levels in rats in diabetic group (Group II) (1.14±0.12 mg/dl) as compared to normal control (GroupI) (0.85±0.07). ***Momordica charantia*** in dose levels of 100 & 200 mg/kg b.w showed non-significant decrease in serum creatinine levels. Dose of 100 mg/kg b.w administered to rats of Group IV showed non-significant level ($p>0.05$), of (1.65 ±0.24 mg/dl) & dose of 200 mg/kg b.w administered to rats of Group V also showed non significant level ($p>0.05$), of (1.38±0.14 mg/dl).

Serum Total Proteins Levels (g/ dl)

As compared to group I (4.95±0.61), levels in group II rats which had received only alloxan monohydrate, there was non significant change ($p>0.05$). In serum total protein levels (3.18±0.26 g/ dl). When 50% ethanolic extract of ***Momordica charantia*** (100 mg/kg b.w) was administered to rats of Group IV it showed non significant ($p>0.05$). Increase (4.66±0.77 g/ dl) while dose of 200 mg/kg b.w administered to rats

of Group V showed significant increase ($p<0.05$). In serum total proteins levels (5.20±0.66 g/ dl)

Average Body Weight (g)

Rats of Group I showed body weight of (222.05±4.74 g) in grams & rats of Group II showed very highly significant decrease ($p<0.001$). In average body weight (124.76± 2.35 g) in grams. Rats receiving 50% ethanoic extract of ***Momordica charantia*** showed dose dependent increase in average body weight indicating significant reduction in average body weight. Dose of 100 mg/kg b.w administered to rats of Group IV showed significant increase ($p<0.05$) of (145.50±8.35 g) & dose of 200 mg/kg b.w administered to rats of Group V showed highly significant increase ($p<0.01$).of (150.61 ±3.62 g).

During course of these studies, blood glucose levels & average body weight were observed on day 1, day 4, day, 7 & day 10. **(Table 29; 38)**

Anti-diabetic Study using alloxan monohydrate in Rats (10 day study)

Tab. 2: Effect of various dose of 50% Eth-Extract of whole plant (PO), (SGCF) & (SGCS), on Serum Glucose Levels (mg/dl) against alloxan induced diabetes mellitus in rats (10 days study)

S.No.	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII	Group-VIII	Group-IX	Group-X
Rats	**Normal control (0.2ml of 2% Gum acacia)**	**Diabetic control (Alloxan monohydrate)**	**Alloxana + Std Anti diabetic drug Glibenclamide (10mg/kg.)**	**Alloxana + PO (100mg /kg)**	**Alloxana + PO (200mg / kg)**	**Alloxana + SGCF (50mg/kg)**	**Alloxana + SGCF (100mg /kg)**	**Alloxana + SGCF (200mg /kg)**	**Alloxana + SGCS (100mg /kg)**	**Alloxana + SGCS (200mg /kg)**
1	85.08	308.89	110.15	151.79	119.47	158.47	126.87	98.45	153.47	144.67
2	75.12	254.58	124.47	143.77	121.48	159.64	132.68	67.57	145.58	123.58
3	87.79	278.08	100.97	150.67	111.47	156.68	127.45	79.47	159.47	128.47
4	98.24	268.47	114.26	139.47	105.36	143.47	131.67	71.84	160.45	104.95
5	60.57	275.78	113.74	134.68	106.46	157.58	124.47	87.47	159.78	105.47
6	98.89	261.73	121.08	140.02	108.48	168.46	132.46	97.36	158.74	116.58
Mean	84.28	274.6	114.1	143.4	112.1	157.4	129.3	83.69	156.2	120.6
SD	14.61	18.94	8.300	6.727	6.827	8.038	3.455	12.94	5.801	15.10
SEM	5.965	7.732	3.388	2.746	2.787	3.282	1.410	5.282	2.368	6.165
P Value		**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**P<0.0001**	**0.0003**
Statistically compared groups		**I & II**	**II & III**	**III & IV**	**IV & V**	**V & VI**	**VI & VII**	**VII & VIII**	**VIII & IX**	**IX & X**

Alloxan monohydrate (120 mg/kg), was administered i.p, in sterile saline, single dose, 5 days before administration of different ethanolic extracts. Standard drug, Glibenclamide& three plants given as 50% ethanolic extracts in 2% gum acacia were administered orally for 10 days, in single dose daily five days after confirmation of hyperglycemia.

n=6 (No of animals in each group) except in group VII where n=5

Group II compared with Group I & all other groups compared with Group II

***p< 0.001 Very highly significant; ** P< 0.01; highly significant;

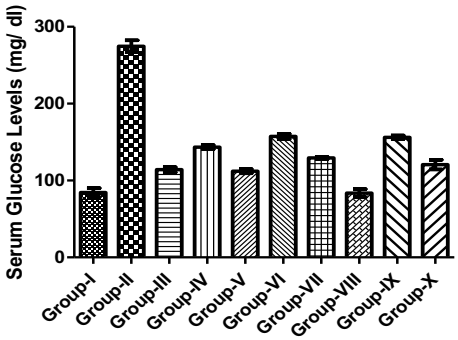

Figure 3:

Fig. 3. Effect of various dose of 50% Eth-Extract of PO whole plant, SGCF & SGCS, on Serum Glucose Levels (mg/ dl) against alloxan induced diabetes mellitus in rats.

Tab.3: Effect of 50% ethanolic extract of PO (whole plant) on Blood Glucose Levels (mg/dl) against alloxan induced diabetes mellitus in rats (10 days study)

Groups	**Treatment**	**Blood Glucose Levels (mg/dl)**			
		DAY 1	DAY 4	DAY 7	DAY 10
I	Normal control **0.2ml of 2% gum acacia**	85.07± 4.35	86.16±4.43	84.82±5.96	84.28±5.965
II	Diabetic control **Alloxan monohydrate**	261.47±8.37	264.28±8.29	268.03±8.48	274.6±7.732
III	**Alloxan monohydrate** + Std drug - Glibenclamide (10 mg/kg b.w)	200.37± 5.25	141.18± 2.43	124.52± 2.00	114.1±3.388
IV	**Alloxan monohydrate** + PO(100 mg/kg b.w)	203.38±4.04	147.87± 2.30	144.56± 2.56	143.4±2.746
V	**Alloxan monohydrate** + PO(200 mg/kg b.w)	201.24± 4.90	146.29± 2.06	130.44± 1.87	112.1±2.787
VI	**Alloxan monohydrate** + SGCF(50 mg/kg b.w)	201.17±4.85	159.48±2.68	158.76±2.81	157.4±3.282
VII	**Alloxan monohydrate** + SGCF(100 mg/kg b.w)	201.17±4.85	146.43±2.46	135.44±2.72	129.3±1.410
VIII	**Alloxan monohydrate** +SGCF(200 mg/kg b.w)	204.27±4.25	144.65±1.53	118.35±3.42	83.69±2.82
IX	**Alloxan monohydrate** +SGCS(100 mg/kg b.w)	204.27±4.25	156.99±2.48	156.21±2.85	156.2±2.368
X	**Alloxan monohydrate** + SGCS(200 mg/kg b.w)	202.95±4.95	144.67±1.56	128.22±5.55	120.6±6.165

Alloxan monohydrate (120 mg/kg) was administered i.p, in sterile saline, single dose, 5 days before administration of different ethanolic extracts. Standard drug, Glibenclamide& three plants given as 50% ethanolic extracts in 2% gum acacia were administered orally for 10 days, in single

dose daily five days after confirmation of hyperglycaemia.
n=6 (No of animals in each group) except in group VII where n=5;
DAY 10 compared with DAY 1; ***p< 0.001 very highly significant; p < 0.01 Highly Significant; p> 0.05 non-significant (NS)

Histopathology of Liver in Rats Diabetes Induced by Streptozotocin (STZ) (15- Days Study)

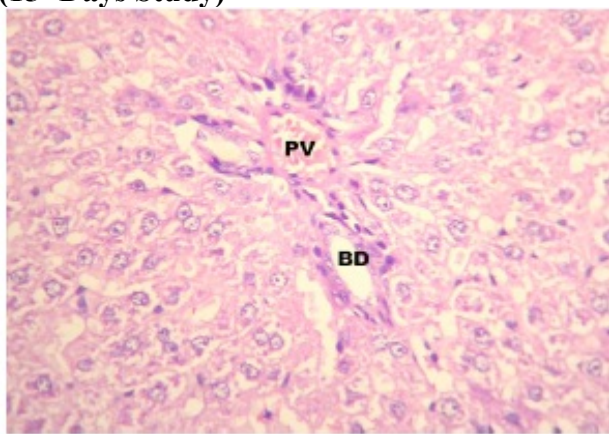

Fig. 4 Group –I Normal ControlLiver of rats showing portal triad area

4. SUMMARYAND CONCLUSION

4.1 Summary

Percentage Yeild

Percentage yield of 50% ethanolic extract of

- Whole plant of *Momordica charantia* was 14%.

Preliminary Phytochemical Screening

- *Momordica charantia* whole plant contains alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins & steroids.

Toxicity Studies

***a) Acute Oral Toxicity Studies* (72 Hours)**

LD50 of 50% ethanolic extract of

- *Momordica charantia* whole plant was found to be 500mg/kg b.w.

b) Subacute toxicity study (14 days)

50% ethanolic extract of *Momordica charantia* whole plant showed

- Hypoglycemic activity.
- Increase in urea levels.
- Serum creatinine levels showed non-significant decrease.
- Non significant increase in liver enzymes like bilirubin levels, total protein levels, albumin levels.
- Significant decrease in SGOT, SGPT, alkaline phosphatase levels.
- Non-significant increase in haemoglobin value.
- Significant decrease in W BC count.

Antidiabetic Studies

Alloxan (120 mg/kg b.w) & streptozotocin (50 mg/kg b.w) were found to induce diabetes in rats as evidenced by increased blood glucose levels.

a) Alloxan induced Diabetes mellitus (10 day study)

50 % ethanolic extract of *Momordica charantia* whole plant (PO)

- Revealed dose dependent **antidiabetic** potential in rats with doses of 100 & 200 mg/kg b.w. dose of **200 mg/kg b.w /day** was found to be having maximum activity,and effect was seen equal to levels of blood glucose with standard antidiabetic drug, glibenclamide.
- Exhibited significantly hypolipidemic effect in rats.
- **Non-significant increase** in kidney function tests, i.e., serum urea levels, serum creatinine levels & serum total protein levels.
- Significantly reversed decrease in **body weight** seen in diabetes.

b) Streptozotocin induced Diabetes mellitus (15 day study)

50 % ethanolic extract of *Momordica charantia* whole plant (PO)

- Revealed dose dependent **antidiabetic** potential in rats with doses of 50 & 100mg/kg b.w. dose of 100 mg/kg b.w /day was found to be more active.
- Exhibited significant **hypolipidemic** effect in rats.
- Exhibited significant **hepatoprotective** effect in rats.

- Significantly reversed decrease in **body weight** seen in diabetes.

Histopathological studies

- Momordica charantia whole plant when administered (50 & 100 mg/kg b.w) to rats showed few inflammatory cells in islets of pancreas at dose of 50 mg/kg b.w while at 100 mg/kg b.w showed no inflammatory cells, thereby indicating its

Antidiabetic potential.

- No abnormality in **livers**
- No abnormality in **kidneys.**

4.2 Conclusion

Diabetes mellitus is metabolic issue portrayed by resistance in development of insulin, lacking insulin transmission or both. It is finding opportunity to be champion amongst most extensively seen issue of world. Treatment for diabetes mellitus would be drug that not only controls glycemic level but also prevents development of atherosclerosis & other complications of diabetics. New drugs & new drug delivery systems for insulin have also been introduced. In this research two plants, Momordica charantia whole plant was evaluated for their toxicity studies & for antidiabetic activity as they have been reported to have hypoglycaemic activity in traditional system of medicine.

Different model systems like alloxan, streptozotocin viruses, & insulin antibodies, hormones like dexamethasone, adrenaline & dithizone are available to screen anti-diabetic activity of given substance In this research chemicals like alloxan & streptozotocin were used to produce marked diabetic effects in animals. Alloxan diabetic model resembles type I diabetes (IDDM) without significant insulin resistance whereas streptozotocin induced diabetic animals inhibit reduced response to insulin in hepatic & peripheral tissues. Further rats treated with streptozotocin display many of features seen in human with uncontrolled diabetes mellitus. In this research, alloxan was given at dose of 120 mg/kg b.w, i.p, while streptozotocin was administered (i.p) at dose of 50 mg/kg, b.w, for inducing diabetes. Albino rats (Wistar strain) of both sexes, weighing 125-250g &swiss albino mice weighing 20-25g, were procured from IIIM Jammu & kept in clean polypropylene cages under uniform conditions of food, water, temperature & degree of nursing care. It was ensured that animals were in good health & free from any infectious diseases. Male & female animals were kept in separate cages so that there was no interference in evaluation of biochemical parameters during period of study. Temperature & humidity of room in which animals were housed were in range of 15-25oC & 70-75 % respectively. In this research, preliminary phytochemical screening, acute oral toxicity study (72 hours), subacute toxicity study (14 days), & antidiabetic studies using alloxan (10 days) & streptozotocin (15 days) for inducing diabetes, were carried out. Studies have reported that Momordica charantia whole plant (PO) contains alkaloids coumarins, tannins, flavonoids, glycosides, carbohydrates fixed oil, saponins proteins & amino acids & steroids & omega-3-fatty acids. Our studies have uncovered vicinity of alkaloids, saponins, glycosides, terpenes, phenolics, flavonoids, carbohydrates, proteins & steroids in Momordica charantia. Momordica charantia whole plant (PO) in streptozotocin induced diabetic model given at dose levels of 50 & 100 mg/kg b.w showed following effects in biochemical parameters. At dose level of 100 mg/kg b.w, serum glucose level($p<0.001$) showed significant decrease.

References:

1. Abate N. &Chandalia M. (2001), Ethnicity & type 2 diabetes: focus on Asian Indians, J diabetes Complications, (2001), 15, 320-7.

2. 2. Abou-Seif MA, & Youssef AA. (2004), Evaluation of some biochemical changes in diabetic patients, Clin Chim. Acta, 346, 161-70.
3. 3. ADA (American Diabetes Association) (1985), Responsible use of animals in Res. Diabetes, 34, 714.
4. 4. Agarwal SS. (2007), Herbal Drug Tech., University Press (India) Ltd. Hyderabad, 525.
5. 5. Allain CC., Poon LS., Chan CS., & Richmond W. (1974), CHOD-PAP method for determination of total cholesterol. Clin. Chem, 20, 470-475.
6. 6. Atta-ur-Rahman. & Khurshid Zaman. (1989), Medicinal plants with hypoglycemic activity. J Ethnopharmacol, 26 (2), 1.
7. 7. Augusti, K.T. (1973), Studies on effects of hypoglycaemic principle from Allium cepa Linn. Indian J Med Res, 61 (6), 1066.
8. 8. Awad N. (1994), Lipid content & antimicrobial activity of phenolic constituents of cultivated Momordica charantia L. Bull Fac Pharm, 32, 137-142.
9. 9. Baig NA., Herrine SK., & Rubin R. (2001), Liver disease & diabetes mellitus. Clin Lab Med, 21,193-207.
10. 10. Banerjee G. & Mukherjee A. (1996), Momordica charantia L: gem of aliens in India. J of Phytochem Res,9(2),111-115.
11. 11. Barbara BK. & Jeffrey SF. (2000), Obesity & insulin resistance. J Clin Invest, 106 (4), 473-481.
12. 12. Barnett AH. (2004), Exubera inhaled insulin. Rev.Int J Clin Prac, 58,394- 401.
13. Baynes JW. (1991), Role of oxidative stress in development of complications in diabetes. Diabetes, 40, 405-412
14. 13. Beaser R. & Hill J. (1995), Program for Managing your treatment: Joslin Guide to Diabetes. New York: Fireside Book: By Simon & Schuster.
15. 14. Bell R. (1983), Animal models of diabetes mellitus: physiology &pathology.J Surg Res, 433-460.
16. 15. Benjamin BD., Kelkar SM. & Pote MS. (1994), Catharanthus roseus cell cultures, Growth alkaloid synthesis & antidiabetic activity. Phytother Res, 8, 185-186.
17. Bowers GN. & McComb RB. (1966), continuous spectrophotometric method for measuring activity of serum alkaline phosphatase.Clin Chem 12(2), 70-89.
18. Bucolo G. & David H. (1973), Quantitative determination of serum triglycerides by use of enzymes. Clin Chem, 19, 476-82.
19. 16. Burkill HM. (1997), useful plants of West Tropical Africa. Edn 2.Vol 4.Families M-R. Royal Botanic Gardens Kew. ISBN. No.1-900347-13-X.
20. 17. Cernea S. & Raz I. (2006), Noninjectable methods of insulin administration. Drugs Today, 42, 405-424.
21. 18. Chandila HB. Talwalkar BS. &Rajha BS. (1988), Lipid profiles in Diabetes mellitus. J. Dia. Assoc. India, 21,155.
22. 19. Chandrasekar B., Bajpai MB. & Mukherjee SK. (1990), hypoglycemic activity of Swertia Chirayita(Roxb ex. Flem) Karst. Indian J. Exp. Biol Jul, 28 (7), 616- 618.
23. 20. Chattopadhyay S., Ramanathan M., Das J. & Bhattacharya SK. (1997), Animal models in diabetes mellitus. Indian J Exp Biol, 35, 1141-1145.
24. 21. Chen J. & Li WL. (2008), Progress in studies on Phytochemistry & Biological Activity of Folium Eriobotryae. Med & Aromatic Plant Sci & Biotech, 18- 23
25. Chitra V. &Leelamma S. (1999), Coriandrum sativum- mechanism of hypoglycaemic action. Food Chem, 67(3), 229-231.