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ANTIDIABETIC ACTIVITY OF MOMORDICA CHARANTIA EXTRACTED AGAINST IN ALLOXON INDUCED MICE

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ABSTRACT

The oral administrations of crude extract of 400mg/kg bwt of M.charantia and M.koenigii along with (1:1) proportion of M.charantian and M.koenigii for 21 days resulted a significant decrease in blood glucose levels, followed by a reduction in all the parameters tested for Liver and Renal function and Lipid profiles. Even a decreased LPO activity along with a decreased antioxidant enzyme activity of (SOD and Catalase) was reportes as against the diabetic rats. The observed effects is due to presences of Saponins, Flavonones, Alkoloids and Flavonoids present in the crude extracts of both the plants. Further, the observed reduction in all the tested parameters compared with a synthetic oral drug- Metformin (with 120 mg/kg) b.w shows which more or less closely related activity with the drug. The overall result reveals that the extracts of M. charantia and M.koenigii have a marked hypoglycemic, hyperlipidemic and Antioxidant activity.

Key words: Adiabatic Activities; antioxidant enzyme; hypoglycemic; hyperlipidemic

1. INTRODUCTION

Diabetes is a chronic metabolic disease characterized by an elevated blood glucose levels in the circulating blood that leads to morbidity and mortality. This symptomatic activity is due to distruction of pancreatic islet cell of langerhans β-cell with reduced insulin deficiency or ineffectiveness of secreted insulin. Diabetes is generally of two types, of which Type 1 diabetes mellitus or insulin dependent diabetes and Type 2 diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM) and Gestational diabetes mellitus (only during pregnancy) According to International diabetes federation, it is estimated around the world 537 million adults (20-79 years) are living with diabetes i.e., 1 in 10 are suffering and this number is expected to rise to 643 million by 2030 and 783 million by 2045 (IDF., 2021) and it is noted that diabetes is responsible for 6.7 million deaths in 2021 (1 person death in every 5 seconds). Over 3 in 4 (approximately 75-81%) adults with diabetes live in low and middle income countries. Around 541 million adults have Impaired Glucose Tolerance (IGT), which places them at high risk of type 2 diabetes. (IDF., 2021). 1 Globally 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized of diabetes has nearly doubled since 1980), rising from 4.7% to 8.5% in the adult population (Global report of WHO., 2021) and this number is expected to increase to 578 million (10.2%) in 2030 and 700 million (10.9%) in 2045. The prevalence of diabetes in women is estimated to be 9.0% and 9.6% in men in 2019. Worldwide nearly half billion people are suffering with diabetes. (Pauya Saeedi et al., 2019)

India is the largest and most populous developing country in the South Asian region. Type-2 diabetes has rapidly increased and has become a major public health problem in India. Indians are at increased risk of developing diabetes in comparison to other ethnic groups, as it has most diversified and aggressive natural history with higher degree of complications. (Hills et al., 2018). A total number of 77 million Indians are living/suffering with diabetes, with an estimated prevalence of 8.9% among adults. India is placed as 2nd largest country with diabetes population, where 1 in 6 adults is reported with diabetes (IDF., 2019) Indian council of Medical Research (ICMR) India reported the blood sugar level falls >140mg/dl.

The urban women show the highest blood glucose level 9.4% in both Mizoram and Tripura which is followed by Manipur with 8.8% and Sikkim with 7.8%. In case of men, highest blood glucose level can be seen in Nagaland 11.1% followed by Mizoram and Sikkim (10.7%). There is high prevalence of Type 2 diabetes among the overweight and obese group. In some states male show higher prevalence and in some other, female show higher. (Luxmi Devi, et al., 2018) In India 65.1 million people in the age group of 20-79 years are suffering with diabetes mellitus and this figure is predicted to increase to 79.4 million by 2030. According to National Urban Diabetes Survey, the prevalence of diabetes in Southern part of India reported 16.6% in Hyderabad, 13.5% in Chennai, 12% in Bengaluru, 11.7% in Kolkata, 11.6% in New Delhi and 9.3% in Mumbai (Ravi Babu, et al., 2019). Diabetes is mainly increasing because of rapid changes in lifestyle, food habits, urbanization, modernization and industrialization. Although the prevalence of risk factors is decreasing over time but smoking, alcohol was found to be positively associated with the chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels.

Long term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputations, Charcot joints, autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms. (Mitra et al, 2019) The most common adverse effect seen in diabetic patient was hypoglycemia. This condition is checked by using an antidiabetic compounds. Inspite of the beneficiary role, these compounds exert various side effects. Therefore it is necessary to look for new and if possible more effective drugs that have safer hypoglycemic activity. In present scenario it has become an important area of investigation. Plant materials that have been used as traditional medicine for the treatment of diabetes are considered as one of the best and good sources for a new drug or that can lead to make/develop a new drug. (Jyoti et al., 2017).

Usage of natural products for the treatment of various diseases, particularly of plant origin play an important role and are in practice from ages by some of the ethnic groups. Ease availability, low cost and least side effect make plant-based preparations the main key player of all available therapies, especially in rural areas. Moreover many plants provide rich source of bioactive chemicals, which are free from undesirable side effects and possess powerful pharmacological actions. (Bahare et al.,2019) According to the world health Organization (WHO), around 90% of the population in developing countries uses plants and its products as traditional medicine in primary health care across the world. WHO has listed out 21,000 plants, which were used for medicinal purposes, of which 2500 species are confined to India. The

plant derived compounds contain glycosides, polysaccharides, glucodines, steroids, tereoenoids, glycopeptides, alkaloids, flavonoids and peoptodoglycons, which have been proven to have effective activity against hyperglycemic condition (Marles and Farnsworth, et al., 1995), the plants like Aloe vera (Vogler and Ernst, et al., 1999); Gymnema Sylvestre (Bhaskaran et al., 2001); Eugenia Jambolana (Ravi et al., 2004),

2.Material & Methodology

Selecting the Plant

Murraya koenigii and Momordica charantia, were selected for the purpose of this study and the collected plant were identified up to species level and were authenticated with the help of a plant taxonomist from the Department of Botany, Osmania University. Hyderabad. The plant Murraya koenigii was known with the common name as curry leaves, which belongs to Family Rutaceae (Rutaceae). Momordica charantia is also known as bitter melon or bitter gourd, it belongs to cucurbitaceae Family.

Extraction of Plant Extract:

Kingdom: Plantae (Plants) Subkingdom: Tracheobionta (Vascular plants) Super division: Spermatophyta (Seed plants) Division: Magnoliophyta (Flowering plants) Class: Magnoliopsida (Dicotyledons) Subclass: Rosidae Order- Sapindales Family: Rutaceae (Rue family) Genus: Murraya J. Koenig ex L. Species: Murraya koenigii (L.) Spreng. Curry leaf Murraya koenigii plant leaves (around 1200 gms) were thoroughly washed with distilled water and shade dried. The dried leaves are powdered with the help of an electric blender. About 150 gms of powder was subjected to Methanol extraction method by using soxhlet extractor. The solvent extract was concentrated by evaporation at room temperature and was used in the present study.

The rest of the powdered form was stored in an air tight container. Similarly, Momordica charantia fruits were collected, washed thoroughly and were sliced into small pieces (the seeds were removed manually). They were dried under shade at room temperature for 10 days and later it was grounded into powdered form with the help of an electric grinder and was stored in air tight container at 5°C for extraction. The active ingredients were obtained by Soxhlet extraction method using 100% methanol as a solvent. The solvent was evaporated in a rotary evaporator at 40-50°C with a percent yield about 85%. The wet residue was filtered through a whatman filter paper and the

3.REVIEW OF LITERATURE

Hyunjin Baek et al., (2018) studied the antihyperglycemic and anti hyperlipidemic effects of Momordica charnatia and Ligularia fischeri. MLM supplementation led to a more significant reduction of glucose levels in both STZ/ATH and db/db/ATH (authero genic diet) mice as well as lowered lipid profiles in mice. Samir Abd-elmonem Ahmed Ismail et al., (2017) reported the hypoglycemic effect of aqueous ethanolic extract of Momordica charantia L. fruit (MCE) and the interacting effects of co-administration of Momordica charantia on the antidiabetic activity of metformin and glibenclamide. This study showed that the aqueous ethanolic extract of Momordica charanita L fruit (50 mg kg body weight) was similar to the

glibenclamide (600µg kg body weight) exhibited significant anti-hyperglycemic activity in diabetic rats. Administration MCE induced significant protective effect and reflected in the reduction of serum levels of AST and ALT as well as on urea and creatinine in diabetic rats.

Mohammed Salem Moqbel, et al., (2017) carried out an investigation on renal and hepatic functions and biochemical parameters. Momrdica charantia (MC) was given to streptozotocine induced diabetic male Wister rats @ of 2, 5 and 10 % of the standard diet. An improved pancreatic, hepatic and renal function with an improvement in hematologic and biochemical parameters was noticed when compared to the groups and control group.

Selection and housing of experimental animal - Rat

Studies on in-vivo Rat System:

A series of experiments were carried out to study the efficacy of Murraya koenigii.(curry leaves) and Momordica charantia (Bitter gourd) in controlling the diabetes. The diabetic induced rats, were fed with crude extract of the leaves/fruit in different combinations along with the pelletized feed. 3.3.2. Selection of Experimental Animal model: For the present study, the male Albino Rats with an average weight of 170-200 gms were purchased from Jeeva Labs (CPCSEA No. 1757/PO/RcBiBt/S/14/CPCSEA). Rats were housed in a group of 6 in polypropylene cages and maintained at a controlled room temperature of 25 ± 2°C, relative humidity of 50 - 55% and 12 hours light and dark cycle, in animal house present in the department. Rats were acclimatized for 15 days and fed with the standard pelleted diet and water ad libitum. Precautions were taken to avoid any infections, by maintaining hygienic conditions through repetitive cleaning and sanitization of animal house, changing of beds/ husk of rat cages at regular intervals, cleaning and sanitization of cages, bottles and changing of water at regular intervals. Prior to commencement of the experiment, the acclimatized rats were subjected for fasting over a period of 12 hrs. The body weight of each rat was recorded before commencement of the experiment. The present study was carried out in accordance with internationally accepted principles for laboratory and was approved by IAEC (Institutional Animal Ethical Committee) of the department.

Experimental Design: All the experimental rats were fasted overnight and were tested for fasting blood glucose levels by drawing the blood from tail vein and was further analyzed for biochemical test like blood glucose; haemoglobin; serum glutamic oxaloacetic transaminases (SGOT); Serum glutamic pyruvic transaminase (SGPT); LPO; creatinine; albumin; urea; SOD; CAT; total proteins levels. Glucometer was used for analyses of FBS before and after treatment. To carry out the experimentation, about 36 male albino rats were segregated into 6 groups with 6 rats in each group. 40 The 1st group is (C group) - Control group Rats maintained as Control group were fed with the regular standard pelleted rat diet/feed and water provided ad libitum.

The 2nd group (AID) - Alloxan induced rat group Rats were fasted for 18 hours, before Diabetes induction. A single dose of alloxan monohydrate (i.e 150 mg/kg body weight) was injected intra-peritoneal for the induction of type-2 diabetes in experimental rats. After, a period of 72 hours of alloxan induction, the animals were fed with the standard pellet diet and water ad libitum. The animals were monitored and stabilized for a week/10 days and the animals showing the blood glucose levels higher than >250mg/dl

were selected for the study. The diabetic rats received standard pelleted rat diet and water ad libitum. Regular monitoring of FBS was done for the entire experimental period.

The 3rd group (AIM) - Alloxan induced rats treated with metformine This group rats were first induced by diabetes by administration of Alloxan (procedure as mentioned in group 2) and these rats were given an oral synthetic drug metformine to control diabetes and the rats received standard pelleted diet and water ad libitum and regular monitoring of the FBS carried out till the completion of the experimental period. The 4th group (DMM) – Alloxan induced rats with M.Charantia fruit extract. The rats of this group were initially induced diabetes with alloxan (procedure as in group 2) after 10-15 days, the extract of M.chrantia fruit was given orally at the dose of 400 mg/kg body weight along with their regular diet and water ad libitum. Regular monitoring of the FBS was performed and the treatment lasted for 21 days.

Experimental Protocols:

Estimation of Hemoglobin concentration by Sahli's Method Principle:

Hemoglobin present in blood when in contact with HCl acid is converted to acid hematin complex, which gives brown colour. Further, the acid hematin solution is diluted with distill water until its colour matches exactly with that of the permanent standard of the comparator block present in the hemoglobinometer. The hemoglobin concentration is read directly from the calibration tube. Requirements: Sahli's hemoglobinometer; N/10 HCl and distilled water Procedure: Clean and dry hemoglobinometer tube and pipette were used. The hemoglobinometer tube or Sahli's tube was filled with N/10 HCl up to its lowest mark, with the help of a dropper. The hemoglobinometer pipette was filled with blood up to the mark 20 cu mm of the pipette. The tip of the pipette was wiped before transferring the blood from pipette to the hemoglobinometer tube containing the N/10 HCl, by immersing the tip of pipette and ensured no solution left in pipette. The solution present in the Sahli's tube was mixed thoroughly (for maximum conversion of hemoglobin to acid hematin).

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Group 6 (CMM) – Alloxan induced rats with a combination of crud extract of both M.charantia and M.koengeii The alloxan induced rats of this group were administered with a combination of both the extract i.e. M.charantia fruit (200mg/kg body weight) and M. koengeii leaf extract (200 mg/kg body weight) along with their regular pelleted diet and water. The monitoring of FBS was performed regularly for the period of 21 days of entire experimental period. Estimation of Hemoglobin concentration by Sahli's Method Principle: Hemoglobin present in blood when in contact with HCl acid is converted to acid hematin complex, which gives brown colour. Further, the acid hematin solution is diluted with distill water until its colour matches exactly with that of the permanent standard of the comparator block present in the hemoglobinometer. The hemoglobin concentration is read directly from the calibration tube.

Estimation Blood Glucose by Nelson and Somogyi method.

Principle: Blood proteins are precipitated by zinc hydroxide. The filtrate is heated with alkaline copper reagent and the reduced copper formed is treated with arsenomolybdate reagent resulting in the formation of violet colour which is read in the spectrophotometer. Procedure: 0.1 ml of blood was taken from the experimental animal into a clean dry centrifuge tube containing 3-5 ml of distilled water and mixed thoroughly. To this solution 2 ml of 0.3 N barium hydroxide solution was added. To the obtained brown mixture 0.2 ml zinc sulphate was added and centrifuged at 3000 rpm for 20 minutes. 1.0ml of the solution was taken from the above mixture into a clean test tube and 1.0 ml of alkaline copper reagent was added to it. The test tube was placed in a beaker containing glass marble and Kept for boiling in a water bath for 20 min. After 20 minutes the test tube were removed and cooled under running tap water. 1 ml of are arsenomolybdate reagent was added to the content and further it was dilute with 10 ml of distil water.

Estimation of Creatinine by Hawk and Oser method.

Principle: mg/dl Creatinine reacts with alkaline picrate to form a yellow-red colour known as the Jaffe reaction. Many substances present chiefly within the red cells contribute to a false high colour and therefore analysis in plasma or serum is preferred. Procedure: In a test tube, 2ml of serum was taken and 12.0-14.0 ml of water was added and mixed thoroughly. 1 ml of sodium tungstate solution and 1 ml of H2SO4 was added to the content in test tube. The solution were mixed well and allowed to stand for 10 min. the brown colour mixture was formed and the mixture was filtered into a dry container. 5 ml of the tungstic acid filtrate was transferred to a test tube and 2.5 ml of the diluted picrate reagent was added, mixed and allowed to stand at 25oC for 15 min exactly. The readings were noted on colorimeter at 520 nm within the next 5 minutes. (Note: For best reproducibility, the time interval between the addition of the alkaline picrate reagent and the reading in the colorimeter should be kept constant for all tubes). Simultaneously, a working standard solution of creatine containing 20 µg/ml was prepared. A series of various amount of the solution containing 5 to 20 µg creatinine per tube were pipetted out and the volume was adjusted to 2.5 ml in all the tubes. Water 7.5 ml and 5.0 ml of alkaline picrate reagent were added for the development of colour. The same procedure was followed for all samples. A reagent blank with each set of samples and standards was run by treating 5 ml water with 2.5 ml picric acid and the OD of the reagent blank is deducted from that of the samples and standard to obtain the true values. The creatinine values are expressed as mg/dl

Estimation of Urea by Richard Henry. J .

Principle: The urea of plasma is split to ammonia and CO2 by the action of urease and the ammonia is then determined photometrically by the phenol-hypochlorite reaction of Berthelot using sodium nitroprusside as a catalyst. The test is performed directly on the samples, there being no need to remove proteins because of the very high dilution used. The urease is buffered by EDTA which also performs the function of complexing metals that inhibit urease activity. For the present experiment, three test tubes were set up separately and used as blank, standard and unknown sample. The blank test tube consists of 20µL water and 0.3 ml of urease solution and 1 ml of phenol, 1ml of alkali-hypochlorite reagent, similarly the standard consisted of the 20µL standard solution and 0.3 ml of urease solution and 1 ml of phenol, 1ml of alkali hypochlorite reagent, whereas the 3rd test tube consisted of unknown sample 20µL and 0.3 ml of urease solution, 1 ml of phenol, 1ml of alkali-hypochlorite reagent. All the three test tubes were incubated at 50 - 60°C for 5 minutes and later 8 ml of water was added in all the test tubes. Absorbance values of standard and unknown against blank was recorded at spectrophotometrically at 630nm and the values are expressed in mg of urea/dl.

Serum Glutamic Oxaloacetic Transaminase (SGOT) Enzyme Assay

SGOT level in the blood was determined by the Philip Cabaud (1956) method Principle: The enzyme catalyst the following reaction: Aspartate + α Ketoglutar AsAT ate $\rightarrow$ Oxaloaceta te + Glutamate The determination of SGOT activity is based on the transamination of aspartic acid to α-ketoglutaric acid. One of the products of the reaction, namely, oxaloacetate is converted to pyruvate which is measured

colorimetrically. Since, pyridoxal-5'-phosphate of erythrocytes which is determines the vitamin B6 nutritional status.

Procedure: Preparation of erythrocyte haemolysate: Blood was collected in heparinized centrifuge tubes and centrifuged at 2000 rpm for 10 min. The Serum was removed and the erythrocytes were washed twice by adding 8-10 ml of 0.89% Nacl solution each time, mixed and centrifuged for 15 min at 2000 rpm. Finally, the volume is made up to the original volume of blood with 0.89% NaCl solution. Erythrocyte count is determined in a haemocytometer. A nineteen-fold volume of glass distilled water is added to the erythrocyte suspension (0.2 ml erythrocyte suspension of 3.8 ml glass distilled water) and kept for 30 min or 1 h. If samples are not used immediately, they are stored frozen at – 20°C. With all samples, in duplicate were analyzed. The serum sample was shaken well, cell membranes were allowed to settle and 0.5ml of sample was taken in centrifuge tubes according to the following procedure:

Blank (B) in duplicate - 0.5ml serum, Sample (S) in duplicate - 0.5 ml serum, Activated sample (A) in duplicate - 0.5 ml serum + 0.1 ml PLP The above tubes were incubated for 30 min at 37°C, preferably in a shaker water bath at low speed. All the samples were adjusted to 1 ml by adding 0.5ml and/or 0.4 ml of distilled water. The reaction in the blank tubes was stopped by adding 2 drops each of 100% TCA and aniline citrate solution. 0.5 ml aspartate - α – ketoglutarate reagent (Reagent d) was added to all tubes and mixed well. The tubes were incubated at 37°C for 30 min in a shaker water bath at low speed. The reaction is stopped by adding 2 drops each of 100% TCA and aniline-citrate solution. The reaction in S and A tubes of the same sample should be stopped together. All tubes are Shaken well and left at room temperature for 20 min. 2, 4-DNP solution 0.5 ml is added to all tubes and mixed. The tubes were left at room temperature for 5 min. Two ml toluene is added and the tubes shaken vigorously. All tubes were centrifuged for 5 min at 2000 rpm. 1 ml toluene layer is pipetted out and 3 ml alcoholic KOH is added and mixed thoroughly. Optical density is measured at 510 nm against a blank prepared by adding 3 ml alcoholic KOH to 1 ml toluene.

RESULTS AND DISCUSSION

Table:1 Blood glucose levels in alloxan induced diabeties Rat, crude extracted of Momordica charantia and Murraya Koengii

Sl No	Group	Fasting blood glucose level (mg/dl)			Parcentage (%) of change in glucose
		Initial glucose level	72 hr after alloxan treated	At 21 days	
1	Group-I (control)	82.16±2.46	Nil	84.20 ± 2.18	-2.04
2	Group-II (Alloxan Induced) AID	64.60 ± 1.18	346.20 ± 4.26	506.72 ± 8.38	-441.12
3	Group-III (Alloxan Induced + Metformin) AIM	88.40 ± 3.83	235.40 ± 3.16	62.00 ± 3.52	26.4

4	Group – IV (Diabetic Rat + M.Charnatia) DMM	70.20 ± 4.52	339.20 ± 4.62	68.20 ± 6.02	2
5.	Group – V (Diabetic Rat + M. Koenigii) DMK	76.10 ±4.68	330.60 ± 3.42	94.50 ± 2.53	-18.4
6.	Group – VI (Diabetic Rat + MC+ MK) CMM	70.26 ± 4.35	328.50 ± 4.95	60.60 ± 4.24	9.66

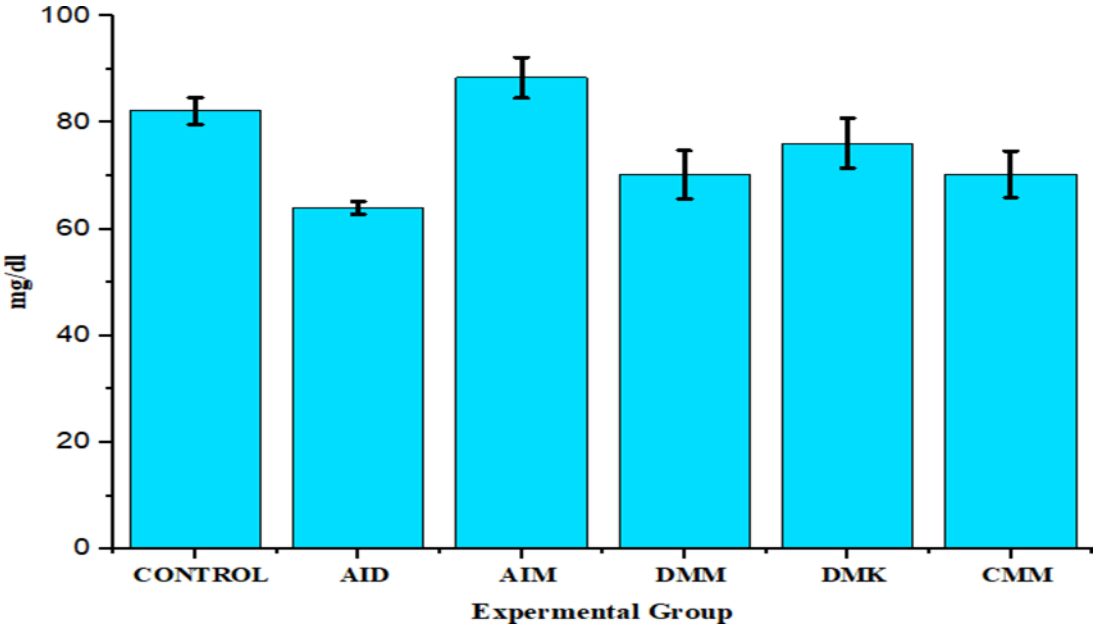


Fig- 1: Initial blood glucose levels in experimental group

Haemoglobin and Glucose Level:

The investigated data were summarized in Table – 2 and fig- 2. After 21 days of experimental period, there was not much significant variation in the levels of haemoglobin observed, except a moderate decreased Hb levels was noticed in Alloxan induced diabetic rat. The observed values were reported as mean ± SD mg/dl of Hb. The mean value of Hb reported as 14.75 ± 0.50; 14.78 ± 0.11; 14.75 ± 0.60 and 14.26 ± 0.74 in group – III, IV, V and VI against the mean value of 14.98 ± 0.29 in controls. Concomitant to this, a mean Hb value of 13.25 ± 0.51 in Group - II in comparison to 14.98 ± 0.29 in Group – I. A 506.72 ± 8.38 mg/dl glucose were reported in Group – II, after 21 days of experimental period against the value of 84.2 ± 2.18 mg/dl in control (Group I). The diabetic rat received therapy with a synthetic compound metformin (Group- III) showed a value of 62.0 ± 3.52 mg/dl as against to the value of 506.72 ± 8.38 in Group – II and also against a value of 84.2 ± 2.18 mg/dl in Group-I.

Table: 2 Effect of Momordica charantia and Murraya Koenigii, crude extract on Hb, Blood glucose levels in alloxan induced diabetic rat for a period of 21 days

Sl No	Parameter	Control n=6 Group - I	Alloxan Induced Daibetic Rat (AID) Group -II	Alloxan Induced Metformine (AIM) Group -III	Diabetic Rat + M.Charntia (DMM) Group -IV	Diabetic Rat + M. Koengii (DMK) Group-V	Crude extract of M.Charanti + M. Koengii (CMM) Group-VI
1	Hb (mg/dl)	14.98 ± 0.29	13.25 ± 0.51	14.75 ± 0.50	14.78 ± 0.11	14.75 ± 0.60	14.26 ± 0.74
2	Blood glucose mg/dL	84.40 ± 2.18	506.72 ± 8.38	62.00 ± 3.52	60.2 ± 6.02	94.50 ± 2.53	60.60 ± 4.24

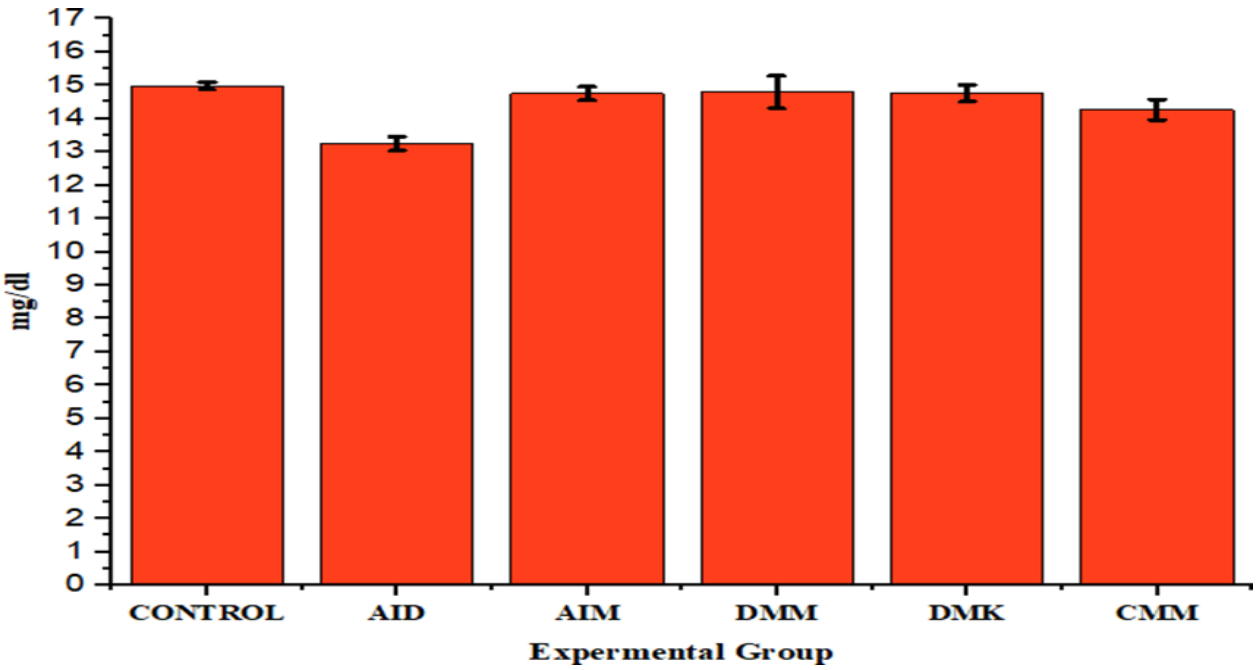


Fig-2 : Hb levels in Momordica charnatia and Murraya koenigii treated diabetic rat.

Similarly, were observed in M charantia and M koenigii crude extract treated diabetic rat (Group –IV, V and VI) showed a decreased blood glucose levels. A value of 60.2 ± 6.02; 94.5 ± 2.53; 60.6 ± 4.24 in Group – IV, V, VI was reported against the value of 506 ± 8.38 in Group – II, as well as a value of 84.4 ± 2.18 mg/dl in Group – I.

The effect of Momordica charantia (fruit) and Murraya koenigii (leaves) crude extract was investigated in the present study showed the hypoglycemic activity. The hypoglycemic effect of M.charantia fruit extract was found to be more effective in (Group – IV) when compared to that of M. koengii leaf extract treated rats (Group – V), whereas Group VI rats received a combination of M.charantia and M.koengii extract reported to have significant lower levels of blood glucose when compared to the Group IV, V, and blood glucose level values are more or less on par with the control values. In the present study the combination of plant extract (400 mg/ kg) exhibit a relatively favorable anti-diabetic activity when compared to the individuals received with the single plant extract. In the present investigation the efficacy of M. koenigii and M.charantia observed in Group – II and it was observed that M.koenigii extract had hypoglycemic properties and also improved the renal functions and its parameters (serum urea and creatinine).

The changes in rats were correlated to the diseased condition i.e. diabetes, as it could be the causative factor in influencing or hampering the physiological , metabolic functions and activities with the generation of free radicals, resulting in increased oxidative stress. Whereas M. koenigii extract was found to have the anti-diabetic properties which helped in controlling the blood glucose levels i.e. keeping diabetic disease in control without using synthetic drug. It was also observed that M.koenigii extract reduced the oxidative stress by exerting its antioxidant properties and the compounds present in the extract help in restricting the pancreatic alpha-amylase enzyme that exerts oxidative stress on the pancreatic cells. The mode of action M koenigii can be attributed to enhanced insulin secretion effect which helps in increased utilization and uptake of glucose by the cells. or M.Koenigii extract may have increased glycogenesis (or) it may have caused decreased glycogennolysis or gluconeogenesis and making it to be a potent hypoglycemic agent or a promising therapeutic medicine. Presence of carbazole alkaloid in M. koenigii extract may be the reason for the anti-hyperglycemic and anti-hyperlipidaemic activity or it could also be due to insulin secretion from the remnant or regenerated β-cells.

Liver enzymes.

SGPT and SGOT are the key enzymes which are produced by the liver and its cells. These enzymes act as an indicator's or biomarker for detection of inflammation or injury and liver damage in the living system. The SGPT and SGOT level was monitored in rat as a marker for hepatic injury induced by diabetes. The data on SGOT and SGPT were summarized in Table – 3 and Fig- 3.1. After 21 days of experimental period a drastic variation in transaminase activity (SGOT/SGPT) were expressed as mean ± SD mg/dl. An increase in enzyme level was observed in alloxan induced diabetic rat. whereas not much alteration in enzyme levels in (group III) rats (metformin) with that of the control and a slight insignificant increased levels was recorded in the other groups III, IV, V and VI against the control, mean values of SGOT reported as 62.3 ± 1.4; 64.4 ± 1.08; 67.7 ± 1.41 and 75.05 ± 1.68 in group III, IV, V and VI against the mean value of 61.47 ± 1.17 in controls. Concomitant to this, a mean SGOT value of 122.5 ± 0.11 in Group – II as compared to 61.47 ± 1.17 in control was observed in this study. The results were significant at P< 0.1. Group IV rats

administered with M charantia showed more or less similar levels of SGOT to that of control and decreased levels, when compared to other rats of Group V, VI and II. The analyzed data in the present study indicates that M charantia has anti- diabetic properties which protects the liver and reverse the diabetic induced damage caused and definitely influence on controlling the levels of SGOT.

Table: 3- Momordica charantia and Murraya koenigii, on Liver function in diabetic rat for 21 days

Sl.No	Parameters	Control Group - I	Alloxan Induced Daibetic Rat (AID) Group – II	Alloxan Induced Rat + Metformine (AIM) Group – III	Diabetic Rat + M.Charntia (DMM) Group – IV	Diabetic Rat + M. Koengii (DMK) Group – V	Crude extract of M.Charanti + M. Koengii (CMM) Group – VI
1	SGOT (mg/dl)	61.47 ± 1.17	122.50 ± 0.11	62.30 ± 1.4	64.40 ± 1.08	67.70 ±1.41	75.05 ± 1.68
2	SGPT (mg/dl)	53.90 ± 1.2	123.30 ± 1.24	54.9 ± 0.96	57.90 ± 2.07	64.92 ± 2.39	69.87 ± 1.99

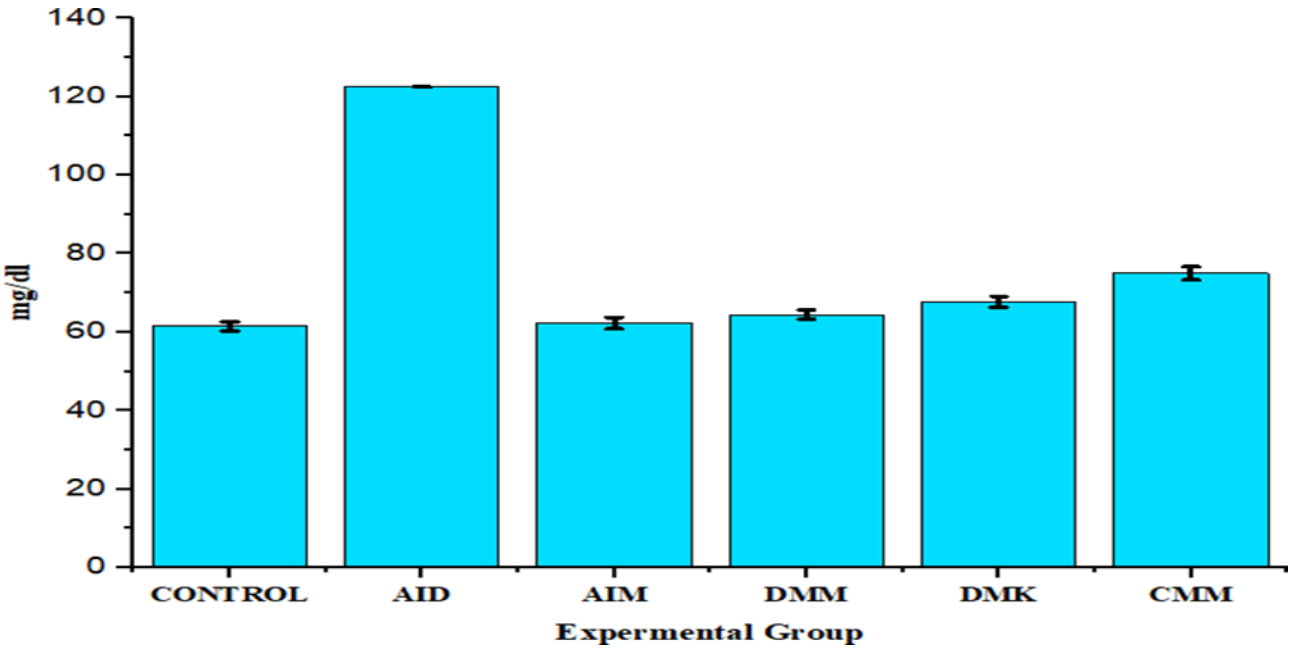


Fig-3 : SGOT activity in Momordica charnatia and Murraya koenigii treated diabetic rat

Oxidant and Antioxidant activity.

Lipid peroxidation – The lipid peroxidation results in generation of highly reactive products that exerts a marked effects on biological system, high concentration of LPO causes damages to DNA, Protein, it leads to cell toxicity, alterations in cell signaling pathways, which is harmful to health. MDA i.e. Malondialdehyde is a major product of lipid peroxidation which is measured via TBARS assay. An elevated levels of LPO was attributed to tissue damage in diabetes.

The LPO values were depicted in Table- 6. Fig - 6.1, It summarizes the serum MDA content and the observed values were reported as mean ± SD n mol L-1. The LPO levels were reported significantly high 0.36 ± 0.02 in alloxan induced diabetic rats compared against the control 0.13 ± 0.01 Group I. Group III was found to show similar level of LPO (0.13 ± 0.02) against (0.13 ± 0.01) of Group I. A significant decrease in LPO levels in Group IV, V and VI observed are 0.24 ± 0.23; 0.25 ± 0.03 and 0.24 ± 0.02 compared against the mean value of 0.13 ± 0.01 in controls and also against the mean LPO value of 0.36 ± 0.02 in alloxan induced diabetic rats of Group II. Group IV, V and VI diabetic rats receiving the crude extract either single or in combination showed no much variation in LPO activity when compared within the groups, but when compared to alloxan induced diabetic rats (Group II) it showed significantly low levels of LPO activity. All the observed values were statistically significant at $P<0.1$.

Table: 6 Ethanolic Extract of Momordica charantia and Murraya koenigii, on antioxidant

Sl.No	Parameters	Control Group - I	Alloxan Induced Daibetic Rat Group - II	Alloxan Induced Rat + Metformine Group - III	Diabetic Rat + M.Charntia Group-IV	Diabetic Rat + M. Koenigii Group – V	Crude extract of M.Charanti + M. Koenigii Group - VI
1 i	LPO Activity TBARS as MDA (nmol $L^{-}1$)	0.13 ± 0.01	0.36 ± 0.02	0.13 ± 0.02	0.24± 0.23	0.25 ± 0.03	0.24 ± 0.02
2 i.	Antioxidant enzymes SOD (Unita/mgHb)	80.6 ± 0.53	185.50 ± 0.29	87.50 ± 1.4	95.70 ± 3.9	104.05± 1.16	110.20 ± 1.8

ii.	CAT (Unitb/mgHb)	33.45 ± 3.3	9.87 ± 1.45	29.90 ± 1.6	29.60 ± 1.12	28.02 ± 2.29	27.20 ± 1.43
3	Total Protein (g/dl)	8.11 ± 0.43	9.91 ± 0.77	10.00 ± 0.70	10.80 ± 0.58	12.20 ±0.62	14.85 ± 0.53

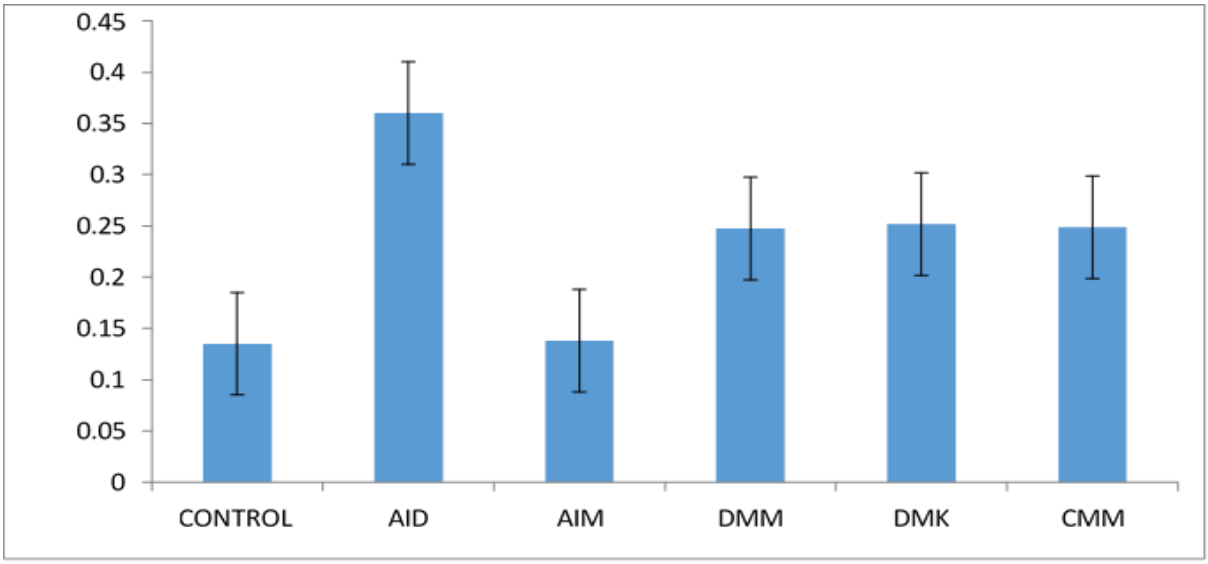


Fig-4 : LPO activity in Momordica charnatia and Murraya Koenigii treated diabetic Rats

CONCLUSION

Diabetes is one of the top ten causes of death globally, together with cardiovascular disease. The prevalence of diabetes is mainly increasing because of changes in lifestyle, food habits, urbanization modernization, industrialization and urbanization. Although the prevalence of risk factors is decreasing over time, Smoking Alcohol was found to be positively associated with diabetes. The increasing abdominal adiposity and the body mass index are the main curlprits in the increasing prevalence of diabetes all over the world. (Madhavi et al., 2017) To combat with this disease, several oral antidiabetic agents are in practice, but have various side effects. Therefore it is necessary to search for new and if possible more effective drugs that have safer hypoglycemic activity, that becomes an important area of investigation by several researchers. Plant materials that have been used as traditional medicine for the treatment of diabetes are considered as one of the good source for a new drug or a lead to make a new drug. (Jyoti Tara Mandhar Shrestha et al., 2017).

The observations of present study reveals that ethanolic extract of M.charantia fruit and M. koenigii leaf administration to Alloxan diabetic rat proven a promising results. The glucose levels were significantly higher in Alloxan received rat with respect to control due to the impact of the drug which will exert its action on pancreatic β-cells leads to poor insulin secretion. On contrary, extracts have shown an anti-hyperglycemic activity in reducing the glucose levels. In comparison M.charantia fruit extract showed a more anti-hyperglycemic activity in comparison with M.koenigii. when both the extracts in combination tested showed anti-hyperglycemic activity but in comparison the activity is moderately reduced against individual extract due to antagonistic activity. More so the investigated results on blood glucose levels with extracts are more or less matching with the synthetic compound-Metformin tested in this investigation.

REFERENCES

1. Ates M, Daniels J, Arslan Z, Farah IO, Rivera HF (2013) Comparative evaluation of impact of Zn and ZnO nanoparticles on brine shrimp (*Artemia salina*) larvae: effects of particle size and solubility on toxicity. Environ Sci Process Impacts 15:225–233. https://doi.org/10.1039/c2em30540b
2. Augustine R, Malik HN, Singhal DK, Mukherjee A, Malakar D, Kalarikkal N, Thomas S (2014a) Electrospun polycaprolactone/ZnO nanocomposite membranes as biomaterials with antibacterial and cell adhesion properties. J Polym Res 21(3):347. https://doi.org/10.1007/s10965-013-0347-6
3. Augustine R, Dominic EA, Reju I, Kaimal B, Kalarikkal N, Thomas S (2014b) Investigation of angiogenesis and its mechanism using zinc oxide nanoparticle-loaded electrospun tissue engineering scaffolds. RSC Adv 4(97):51528–51536. https://doi.org/10.1039/c4ra07361d
4. Bacchetta R, Tremolada P, Di Benedetto C, Santo N, Fascio U, Chirico G, Colombo A, Camatini M, Mantecca P (2012) Does carbon nanopowder threaten amphibian development? Carbon 50:4607–4618. https://doi.org/10.1016/j.carbon.2012.05.047
5. Baker TJ, Tyler CR, Galloway TS (2014) Impacts of metal and metal oxide nanoparticles on marine organisms. Environ Pollut 186:257–271. https://doi.org/10.1016/j.envpol.2013.11.014
6. Bishop PJ, Angulo A, Lewis JP, Moore RD, Rabb GB, Garcia MJ (2012) The amphibian extinction crisis—What will it take to put the action into the Amphibian Conservation Action Plan? SAPIENS 5(2). http://journals.openedition.org/sapiens/1406
7. Ema M, Kobayashi N, Naya M, Hanai S, Nakanishi J (2010) Reproductive and developmental toxicity studies of manufactured nanomaterials. Reprod Toxicol 30:343–352. https://doi.org/10.1016/j.reprotox.2010.06.002
8. Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetology 16:183–190. https://doi.org/10.1201/b15378-9
9. Halder AK, Melo A, Cordeiro MND (2020) A unified in silico model based on perturbation theory for assessing the genotoxicity of metal oxide nanoparticles. Chemosphere 244:125489. https://doi.org/10.1016/j.chemosphere.2019.125489

10. Husen A (2019) Natural product-based fabrication of zinc-oxide nanoparticles and their applications. In: Husen A, Iqbal M (eds) Nanomaterials and Plant Potential. Springer, Cham, pp 155–176. https://doi.org/10.1007/978-3-030-04076-4
11. Ikram S, Rais M, Gill S, Ahmed W, Saeed M, Akram A, Khan IA, Akhter MA (2022) Morphological and ecological correlates of anuran tadpoles in scrub and subtropical pine forests. Russ J Herpetol 29:317–323. https://doi.org/10.30906/1026-2296-2022-29-6-317-323