World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC ACTIVITY OF MURRAYA KOENIGII BARK EXTRACT

***Rama Chandra Rout, Prof. (Dr.) Prasanna Kumar Panda and Dr. Gurudutta Pattanaik**

[1,2]University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar.
[3]School of Pharmacy & Life Sciences (SPLC), Centurion University of Technology and Management (CUTM), Jatni Bhubaneswar.

Corresponding Author: Rama Chandra Rout
University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar.

Article Received on 02/09/2020 **Article Revised on 23/09/2020** **Article Accepted on 13/10/2020**

ABSTRACT

In this research work albino male rats were randomly divided into four groups I.e. G-1,Gr-2, Gr-3 and Gr-4.Gr-1 and Gr-2 were served as vehicle control groups(Demineralised water) and Triton control (Triton WR-1339,200mg/kg IP).Gr-3 and Gr-4 were served with Atorvastatin(7.2 mg/kg) test sample 400 mg/kg/day as single dose for two days respectively. In the second study albino rats were divided into five groups, out of five groups 1st and 2nd groups were normal and diabetic control group respectively.Gr-3,Gr4,Gr-5 which are called as diabetic group were treated orally with Murraya Koengii bark extract (200mg/kg b.w,400 mg/kg b.w.,800 mg/kg b.w.) for 28 days. Sixth group was treated with Gliclazide(25mg/kg b.w). In the OGTT, it is found that the extract OD, extract BD and glyclazide treated group glucose level decreases non significantly at 60 mins and 120 minutes. It indicates that Murraya Koengii bark extract showed supportive action and also with STZ and NA induced rats significantly blood, glucose and also able to lowers the lipid by the same extract.

KEYWORDS: Diabetes mellitus,murraya koengii.streptozotacin,albino rats, OD once in a day, BD twice in a day.

INTRODUCTION

Diabetes is a chronic disease which is caused due to metabolic disorder of carbohydrate, lipid and lipoprotein. It is found that 4% population of world suffering from diabetes mellitus and it may increase 5% in 2025(Hyun SH and Chong SY et al. 2006).It is not curable. Except hyperglycemia it also causes varieties of complication like hyperlipidemia, hypertension and like atherosclerosis (Chalt A and brunzell JD et al 1996,Watkins PJ et al. 2003).Generally high levels of cholesterol in blood and high sugar level in blood increase the risk of cardiovascular diseases. Over the last two decades scientists have given emphasis on screening for high cholesterol and adopting interventions to reduce sugar level and cholesterol levels in order to reduce the risk of heart, kidney and lungs diseases(Jain V and others et al. 2012). The hepatic injury was characterize leakage of celluar enzymes into the blood stream and by centrilobular nicrosis(Muriel P and others et al. 2001,Poli G. et al.1993). High cost and side effects of hyperlipidemic and antidiabetic drug have forced people to turn for alternate treatment. Generally green leafy vegetables have less expensive and less side effects and easily available source of micronutrients. In the variety climate of India, many medicinal plants like spinach, coriander, Amarnath and curry leaves are readible available throughout the year. Murraya koenigii which is commonly known as curry leaves which represent more than 150 genera and 1600 species. Murraya koengii belongs to family rutaceae(Wong C. et al 2016). It is so popular for its Aroma and medicinal value it has also have anti oxidant and cholesterol reducing activities (Jain V and others et al. 2012). Murraya Koenigii grows throughout the Indian subcontinent which has wide culinary effect. It is one of the important component of formulation in the traditional ayurvedic system (Kesari AN and others et al. 2005).

The present day study able to evaluate the antihyperglycemic and antihyperlipidemic activities of murraya koenigii bark extract in experimental animals using Nicotinamid(NA) and Sterptozotacin(STZ) induced diabetic rats and triton induced hyperlipidemia.

MATERIALS AND METHODS

Animals

For animal tests, male adult albino rats(225-250g) bred in the animal house of the institute were used. A group consists of six animals were kept in a cage. They are kept in a controlled conditions temperature I.e, 25-26^{o}c, relative humidity 70-80%.They are kept in 12 hours light(8am to 8pm) and 12 hrs dark. It is used picric acid

for marking them for identification purpose. The animals were kept in polypropylene cages with stainless still grill top. All facilities like clean water, food like clean paddy husk, fed on pellet diet and U.V purified, will be provided diets given in pellet form contain protein 20.5%,total oil 4.28%,dietary fibers 3.65%,moisture 8% was supplied by Rayan's bio-technologies pvt. Ltd, Hyderabad, India. Animal experimentation's study design was in compliance with guidelines of animal ethical committee (IAECR Regd. no:2024/PO/Re /S/18 /CPCSEA).

Preparation of extract

Ethanolic extract of Murraya koengii bark was purchased from cure herbs,New Delhi.

Toxicity studies

Albino rats weighing(175-200g) of either sex are used for all experiments in six animal in a group. The ethanolic extracts of murraya koenigii barks is administered to different groups of dose (200-2000mg/ kg). It is observed that there is no lethality in any group. From the toxicity studies, it is taken one tenth of maximum dose of the extract and its double dose,4th times of the dose tested for acute toxicity was selected for evaluation of anti diabetic i.e,200mg/kg,400mg/kg and highest dose was selected for anti hyperlipidaemic study (400mg/kg b.w.).The experimental tests were performed after the experimental protocol had been approved by School of Pharmacy & Life Sciences (SPLC), Centurion University of Technology & Management (CUTM), Ramachandrapur, Jatni, Bhubaneswar.

Chemical requirements

Sodium chloride and Triton WR 1339 were purchased from HiMedia Labrotories Pvt. Ltd.,India.Atorvastatin from Ranbaxy laboratories Ltd. India, Sterptozotocin (STZ, Himedia laboratories pvt. Ltd),Nicotenamide (NA,Himedia laboratories pvt. Ltd, Mumbai), gliclazide (chemical products, Mumbai, India), EDTA (qualigens, Mumbai, India), citric acid (nice chemicals Pvt. Ltd, Cochin), sodium citrate 2-hydrate (Merk Specialities Pvt. Ltd, Mumbai), Glucose (nice chemicals Pvt. Ltd, Cochin).

Biochemical kits

All the biochemical kit was obtained from crest bio system, a division of Coral Clinical Systems, Goa. The kits like glucose, triglyceride, choresterol , etc were used in Auto analyzer(3000 Evolution, BSI, Italy).

Details about experiments

Induction of Hyperlipidaemia in triton model

All the groups except vehicle control 1 were induced hyperlipidaemia by a single intraperitoneal injection of triton (Triton WR-1339-200mg/kg,i.p.).

Induction of type-2 diabetes melitus

Nicotinamide(NA) of dose 120 mg/kg is induced by a single Intraperitoneal injection which is followed by 60 mg/kg intravenously. Nicotinamide is converted into solution with normal saline and STZ is dissolved with citrate buffer (PH-4.5).The animals which is used for experiment were remain fast for 12 hours before induction of drug. The animals were given 5% glucose overnight to overcome the drug induced hyperglycemia. Diabetes was confirmed by increase of glucose levels in the plasma of the rats, it is found after 3 days of the induction. Those rats whose plasma glucose level found more than 200 mg/dl were used in the experimental study.

Determination of plasma glucose and other Bio chemical parameter

Animals were not given food overnight and 0.5ml blood was withdrawn from sublingual vein by giving anesthesia and was collected in micro tubes which are filled with EDTA (20µl of 10% EDTA/ml) of blood.
Then it is centrifuged at 4000rpm at 4^{o}c for 20 minutes to obtain clear plasma, then the plasma is analyzed for glucose by auto analyzer(3000 evaluation ,BSI Italy)using biochemical kits.

Grouping of animals in triton model

Animals were divided into five groups GR-1,Gr2, GR3, GR-4,GR-5. Gr-1,Gr-2 are under vehicle control (demineralised water) and Triton control(Triton WR-1339,200mg/kg), respectively. GR3 was treated with atorvastin 7.2mg/kg.Gr 4 was treated with test substance M.koengii bark extract 400mg/kg & 800mg/kg after induction of Triton,WR-1339 the dose of 200mg/kg. After that, blood samples of each animal was collected at 0,18,24,40,48 hours dose treatment. Serum will be separated for estimation of cholesterol and triglycerides.

Grouping of animals in STZ &NA Model for blood glucose estimation

Animals are divided into 5 groups. Each group contains 6 animals. Total animals of thirty rats of body weight range 150-200g were selected. Out of 30animals six animals were not treated with Nicotinamide and Streptozotacin and kept as non diabetic control and rest 24 animals were induced diabetes and designated as diabetic control group. From them whose group showed blood glucose level more than 200mg/dl were selected for further group.

GR-1- Non-diabetic contro l, given with normal saline daily.

Gr-2-Diabetic control (STZ+NA),given with normal saline.

Gr-3-Diabetic (STZ+NA),treated with Murraya koengii bark extract (200mg/kg b.w.) once a day.

Gr-4-Diabetic (STZ+NA),treated with M.koenigii bark extract (400mg/kg b.w) once a day.

Gr-5-Diabetic (STZ+NA),treated with M.koenigii bark extract (800mg/kg b.w) once a day.

Gr-6-Diabetic (STZ+NA)treated with 25mg/kg b.w of Gliclazide.

Oral Glucose Tolerance Test

This oral glucose tolerance test in rats who are not given food overnight, these rats were equally divided into 5 groups of six rats each. Group of normal control (Gr-2 & positive control) received only vehicle (1ml of 0.3 CMC,p.o).1 ml of the reference drug (Gliclazide 25mg/kg b.w) was received by the standard group (3).Gr-4 , Gr-5 & Gr-6 were administered with M.koengii extract(200 & 400 mg/kg,800mg/kg p.o) respectively. Then blood samples were collected from sublingual vein prior to dosing and then at 30,60 and 120 min after the glucose administration. This fasting blood sugar is analyzed by using auto analyzer using sugar testing kits.

Statistical Analysis

The data obtained from the studies subjected to one way analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dennett's test ,p values < 0.05 were considered to be significant. All the values expressed as mean+SEM.

RESULTS

The mean body weight of each group is represented in table 1 and figure 1.

It is observed that there is no significance difference in the mean body weight of vehicle control and Triton control group throughout the experiment period. The treated groups did not show any significant body weight change when compared to Triton control.

Table 1: Effect of different extracts on.

Groups for treatment	Body weight(g)		
	Day 0	Day 1	Day 2
I. Vehicle control (demineralisd water)10ml/kg	241.17±4.81	263.33±4.71	260.34±5.5
II. Triton Control (200mg/kg)	240.5±2.15	256.4±2.85	260.15±3.71
III. ATROVASTIN 7.2mg/kg	243.5±3.13	257.8±3.57	258.57±4.52
IV. Extract of M.Koengii bark (800/kg)	240.83±3.95	254.5±4.54	257.83±4.41

Body weight in triton-induced hyperlipidaemic rats

Values are expressed as mean ± SEM,N=6,except for groups 2 and 5 where N=5 here extract M is Murraya Koengii.

Effect of murraya koengii extract on body weight in triton induced hyperlipidemic rat.

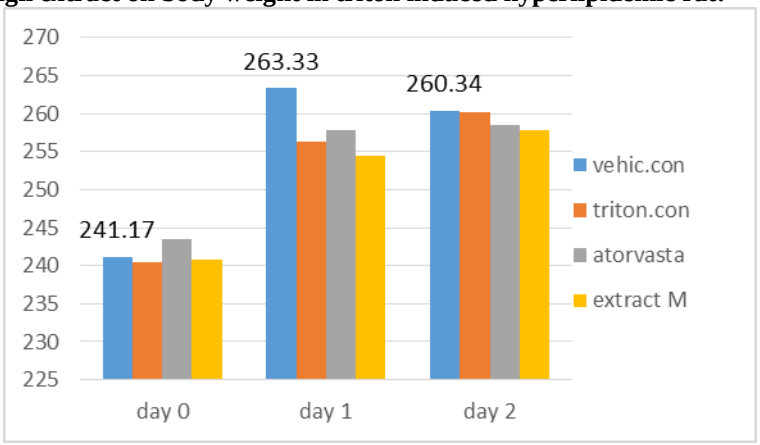

Fig. 1: Effect of murraya koengii extract on body weight.

Lipid Profile

The mean serum cholesterol level of each group is given in Table 2 and figure 2.in day 0'', there was no charge in serum total cholesterol level, but after induction of Triton, there was a significant increase of serum total cholesterol in hour18 and hour 24 when it is compared with Triton control group. Again there is decrease in serum total cholesterol level after administration of atorvastatin when compared with Triton control group. Again it is observed non significant decrease the serum total cholesterol level after administration of murraya koengii extract when compared to Triton control.

Table-2: Effect of different extracts on serum total cholesterol in trito-induced hyperlipidaemic rats.

Serum total cholestrol					
Treatment group	Hour 0	Hour 18	Hour 24	Hour 40	Hour 48
I. Vehicle control (demeralised water,10ml/kg)	61.8±3.21	37.8±4.81	39.51±2.24	49.50±2.26	46.89±1.91
II. Triton control (200mg/kg)	65.45±5.21	248.85±29.41	210.62±29.71	59.12±3.34	53.13±3.12
III. Astrovasration 7.2mg/kg	64.81±5.18	124.71±15.41	111.75±18.13	59.85±5.01	59.85±4.21
IV. Extract M 800mg/kg	64.9±2.71	189.8±12.81	143.65±12.81	58.82±3.89	53.98±2.31

Values are expressed as mean + SEM, except for group 2 where n=5,here extract M means murraya koengii.

P*≤0.05, vehicle control vs Triton control.
P** <0.05, triton control vs treated groups.

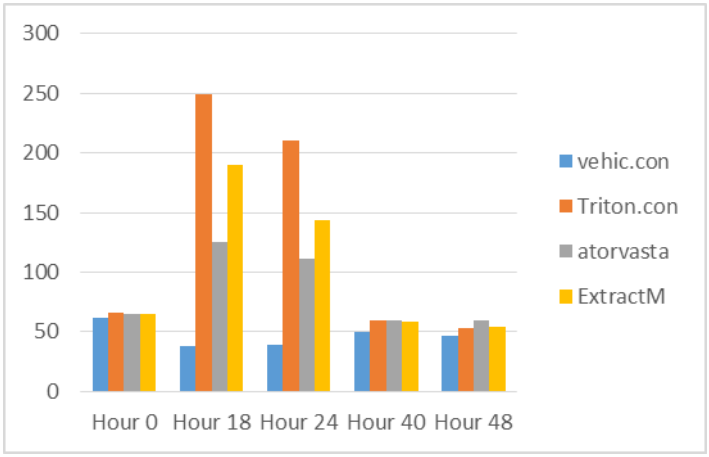

Fig. 2: Effect of murraya koenigii bark extract in serum total cholesterol.

Serum total triglycerides

Mean serum triglyceride level in each group is presented in table 3 and fig 3.It is observed that there is no significant change in serum glyceride level between all the groups at zero hour but after induction of Triton the vehicle control group showed a non significant increase in serum triglyceride level at 18 hours, 24 hours when compared with Triton control group. Atorvastatin also show no significant decrease in serum triglycerides level when compared with Triton control group, murraya koengii extract treated group shows no significant decrease in serum triglyceride level when compared to triton control group.

Table 3: Effect of different extrats on serum triglycerides in the trition hyperlipidaemic rats.

Serum triglyceride(mg/dl)					
Treatment group	**Hour 0**	**Hour 18**	**Hour 24**	**Hour 40**	**Hour 48**
I. Vehicle control (demineralised water, 10ml/kg)	50.14±4.95	58.61±10.16	86.86±16.6	90.35±13.13	80.91±6.35
II. Triton control (200mg/kg)	60.41±2.1	1130.35±45.8	647.71±74.3	86.89±4.21	62.91±4.41
III. Astrovasration 7.2mg/kg	40.68±4.95	661.72±150.31	355.34±105.01	75.16±7.12	66.5±5.91
IV. Extract of murraya koengii 800mg/kg	71.25±10.33	902.81±44.35	503.34±73.82	90.78±19.59	74.01±13.50

Values are expressed as mean ± SEM,n=6 except Gr-2 where n=5 Here extract m is murraya koengii

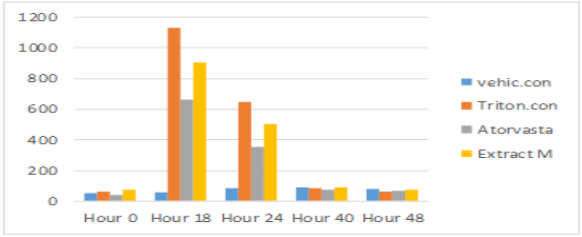

Fig. 3: Effect of Murraya Koenigii extract on serum triglyceride.

Blood Glucose Level (Stz&Na Model)

The rats were induced STZ and their blood glucose level were in the range of 240-293mg/dl. This range considered as higher or severe diabetes, when these diabetic rats were administered Gliclazide(50mg/dl)and murraya koengii extract(200mg/dl ,400mg/dl and 800mg/dl b.w). Then their blood glucose level decreased significantly I.e. from 195±10.81 to 143±3.41 in case of glyclazide and 240.5±7.5mg/dl to 208.83±1.7mg in case of Murraya koengii extract OD, 228.5±7.4 mg/dl to 200.5±4.31 mg/dl in case of murraya koengii extract BD and 227.5±7.7 mg/dl to 202.4±4.8 mg/dl in case of murraya koengii extract QD on 28th day respectively(Table 4 &fig 4).Therefore murraya koenigii extract (OD&BD) able top reduce the blood glucose levels in diabetic rats but values did not return to those of normal controls, so it is found that murraya koengii extract has antidiabetic effect (P<0.01)when it is compared with diabetic control. It is found that in the 28th day, there was a significantly decrease of blood glucose level in STZ diabetic animals.

Oral Glucose Tolerance Test

After administration of murraya koengii extract (200mg/kg and 400 mg/kg and 800mg/kg b.w) to the groups. it is found that there was no significant difference among the groups 0 min and 30 mins but significant difference in blood glucose level found in at 60 mins and 120 minutes among the groups(Table 5 & figure 5).therefore it is concluded that glyclazide (50mg/dl) and murraya koengii extract (200mg/kg and 400 mg/kg,800mg/kg b.w) groups the peak values of blood sugar significantly decreased from 86.5±6.51 to 72.16±4 in case of gliclazide between 60 min to 120 min,again from 121±4.81mg/dl to 112.83±4.2mg/dl in case of murraya koengii extract OD,again from 108±2.5 mg/dl to 80.6±4.2 mg/dl in case of murraya koengii BD extract and from 108.66±2.8 mg/dl to 80±4.8 mg/dl in case of murraya koengii QD extract on the 28th day, respectively indicates statistically significant(P<0.01) anti-diabetic activity when compared diabetic control. So it is concluded that murraya koengii bark ethanolic extract (OD&BD) able to reduce the blood sugar levels in diabetic rats non significantly but values did not return to those of normal control.

Table 4: Effect of Murraya Koengii bark extract on blood sugar level (STZ&NA MODEL).

	Non diabetic control	Diabetic control	Gliclazide	Murraya koengii (200mg/kg b.w)	Murraya Koengii Extract (400mg/kg b.w)	Murraya Koengii Extract(800mg/kg b.w.)
Week 0	88.56±6.5	256±4.25	195±10.81	240.5±7	228.8±7.4	227.5±7.7
Week 2	95.5±5.21	276.5±2.21	161±2.31	216.3±2.13	218.66±6.5	219±6.3
Week 4	95.41±5.41	296.5±6.51	143±3.41	208.83±1.7	200.5±4.31	202.4±4.8

P*≤0.05,vehicle control vs diabetic control

P*≤0.05, diabetic control vs treated groups

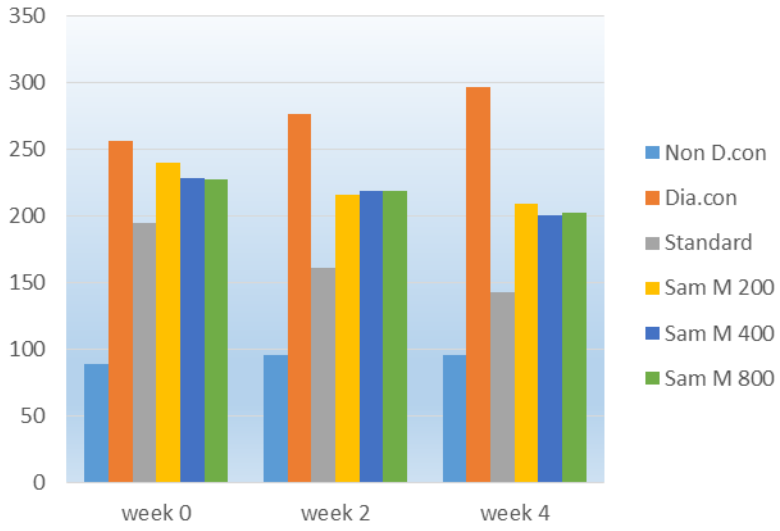

Fig. 4: Effect of murraya koengii extract blood sugar level (STZ&NA MODEL) in oral glucose tolerance test.

Table 5: Effect of murraya koengii bark extract on oral glucose tolerance test inSTZ and NA Model.

	Non diabetic control	Diabetic control	Standard	Murraya koengii (200mg/kg b.w)	Murraya Koengii Extract (400mg/kg b.w)	Murraya Koengii extract (800mg/kg b.w)
0 mins	74.83±7.10	72.5±7.21	70.21±3.81	78.8±3.11	72.1±3.2	71.2±3.7
30 mins	76.66±6.15	138.5±6.01	84.9±5.11	129±7.06	116±4.2	116.8±4.8
60 mins	75.4±4.43	128.3±6.5	86.5±6.51	121±4.81	108±2.5	108.66±2.8
120 mins	75.38±3.62	126.5±4.21	72.16±4.00	112.83±4.2	80.6±4.2	80±4.8

Values are expressed as mean ± SEM,n=6,P*≤0.05,vehicle control vs diabetic control, **P≤0.05 Diabetic control vs treated group

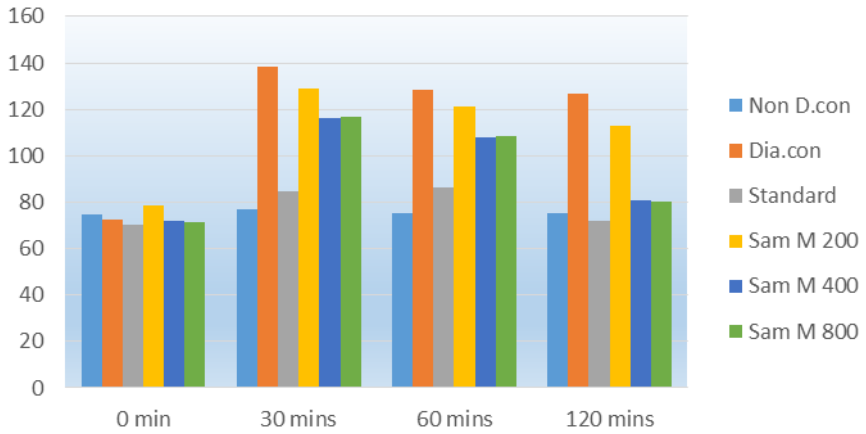

Fig. 5: Effect of murraya koengii bark extract on oral glucose tolerance test in STZ &NA model.

DISSCUSSION

Type 2 diabetes is induced by administration of NA&STZ which produced severe diabetic condition by which function of many organs is altered.In our study, after treating with murraya koengii bark extract, the behavioral abnormalities were significantly improved. Murrya koengii bark extract able to decrease blood sugar level .By action of STZ in β cells of pancreas affected adversely and there is a great alteration in blood insulin and glucose concentrations.STZ able to damages β cells of pancreas by generating excessive oxygen species and able to induce diabetic mellitus(Szkudelski T et al.2001). From the recent experiments,it is proved that the main cause for the STZ induced β cell death is alkylation of DNA. This alkylating activity of STZ is related to its nitrosourea moety,at the O_6 position of guanine. As STZ is a nitric oxide donor and this nitric oxide (NO) able to destruction of pancreatic islet cells. It was proposed that this molecule contributes to STZ induced DNA damage.It is also stated that STZ are main reason of toxicity. The synergistic action of both NO and reactive oxygen species causes DNA fragmentation and other abnormal changes caused by STZ. NO and reactive oxygen species can act separately or produce the highly toxic peroxynitrate. So, intracellular antioxidants or NO scavengers substantially attenuate STZ toxicity.STZ induced DNA damage able to activates polyADP ribosylation. This process results or leads to depletion of cellular NAD+, further decrease the ATP content and there is inhibition of insulin synthesis and secretion(Srinivasan K and Ramarao P et al. 2007).the rats who are administrated with STZ and stable non fasting hyperglycemia without any significant change in plasma insulin level. As NA is an antioxidant which exerts protective effect on cytotoxic action of STZ by scavenging free radicalls and leads to minor damge to pancrease β cells producing type 2 diabetes(Pavana P and others etal. 2007).

Blood Glucose Level

Due to excessive hepatic glycogenolysis and gluconeogenesis there is high sugar level in blood and decrease utilization of glucose by the tissues (Pavana P and others et al. 2007). It is found that diabetic rats showed high glucose level (200-300 mg/dl) throughout the experiment compared to non diabetic rats. When diabetic rats were treated with murraya Koengii bark extract and glyclazide,then there is decreased glucose level significantly as compared to diabetic control group. As the extract of murraya koengii bark helps the increase of plasma insulin level (Pavana P and others et al. 2007), so blood glucose level decreased significantly.

Biochemical parameters

Streptozotacin induced diabetic rats showed high cholesterol and triglyleride level. It is due to an increase in the mobilization of free fatty acids from the peripheral fat depots, as insulin inhibits the hormone-sensitive lipase. More fatty acids in the serum of diabetic rats are converted into phospholipids and cholesterol in the liver. Phospholipids, cholesterol and excess triglycerides are formed in the liver at the same time and discharged into

the blood in the form of lipoprotein. It is found from the animal experiment that murraya koengii extract and gliclazide treated rats showed no significant decrease in plasma cholesterol and triglyceride level as compared to diabetic control group.

OGTT[8]

In the OGTT or glucose loaded hyperglycemic model, the murraya koengii bark ethanolic extract tested for antihyperglycemic activity showed exhibited significant antihyperglycemic activity at the dose level of 400 mg/kg b.w and 800 mg/kg b.w. The more or excess amount of glucose in the blood induces the insulin secretion. This secreted insulin controls the blood glucose level by stimulating peripheral glucose consumption. However, from the glucose control study it was sure that the secreted insulin requires 2-3 hours to bring back the glucose administration murraya koengii bark extract OD and murraya koenigii bark extract BD and murraya koenigii bark extract QD and glyclazide to diabetic treated groups. The glucose levels significantly decreased at 60 mins and at 120 mins and reached the normal level compared to diabetic group giving the indication regarding the favourable action of extract and glyiclazide.

The murraya koenigii bark extract consists of important constituents like carbazole alkaloids, coumarin galactoside, carbazole carboxylic acid, glycolipid, Phospholipids etc. and several of these constituents are responsible for having antidiabetic and antihyperlipidaemic activity. Glycolipids are potent antioxidants are known to modulate the activities of various enzymes due to their interaction with various biomolecules. Alkaloids and Phospholipids have antidiabetic effect and also antihyperlipidaemic activity. So, it is concluded that murraya koengii bark extract proved its effectiveness against metabolic disorders lies in its constituent. Further isolation of this extract will prove its exact mechanism of action.

ACKNOWLEDGEMENTS

The authors are thankful to IAEC(Registration No:2024/PO/Re/S/18/CPCSEA) to conduct the research work and School of Pharmacy & Life Sciences(SPLC),Centurion University of Technology & Management (CUTM), Ramachandrapur, Jatni, Bhubaneswar for providing the facilities.

REFERENCES

1. Chalt A, brunzell JD, diabetes lipid and atherosclerosis. Ini Taylor SI,olfsky JM eds, diabetes mellitus, Lipincott Raven's publisher, 1996; 467-469.
2. Hyun SH, Chong SY, Antidiabetic effect of cinnamon extract on blood glucose in db/d mice J ethnoPharmacol, 2006; 104: 119-123.
3. Jain V, momin M,Laddha K. Murraya Koengii,An updated review. Int J Ayur Herbal Med, 2012; 4: 607:627.
4. Kesari AN, Gupta RK, watal G. Murraya Koengii (curry leaves); A traditional plant. Res J Ethonopharmacol, 2005; 97-247-51.
5. Muriel P,Alba N,Perez-Alvarez VM,Shibayama M,Tsutsumi VK,Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp Biochem Physiol C Toxicol Pharmacol, 2001; 130: 219-26.
6. Poli G. Liver damage due to free radicals.Br.Med.Bull., 1993; 49: 604-20.
7. Pavana P, Sethupathy S,Manoharan S, Antihyperglycemic and antilipidperoxidative effects of Tephrosia Purpurea seed extract in Streptozotocin induced diabetic rats, Indian Journal of Clinical Biochemistry, 2007; 22: 73-77.
8. Ramesh B, Pugalendi KV,Antihyperglycemic effect of umbellifeyrone in streptozotocin-diabetic rats, J Med food, 2008; 40: 256-260.
9. Szkudelski T, The Mechanism of Alloxan and Streptozotocin Action in B cells of the Rat Pancreas, Physiol. Res, 2001; 50: 537-446.
10. Srinivasan K,Ramarao P, Animal models in Type 2 diabetes research: An overview, Indian Journal of Medicinal Research, 2007; 125: 451-472.
11. Watkins PJ, ABC of diabetes, 5th edn, BMJ publishing group Ltd, tavistock square, UK, 2003.
12. Wong C. herbal plant sterol helps to crab high cholesterol, the doctors book of natural health remedies https://www.google.com (Accessed on October), 2016.