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Research Article

Anti-oxidant and anti-diabetic potential of *Morinda tinctoria* on STZ induced diabetic albino rats

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Abstract

The present study explores the anti-oxidant and anti-diabetic effects of *Morinda tinctoria* in *Streptozotocin* (STZ) induced diabetic rats. The diabetes was induced by single dose of STZ (45 mg / kg b.wt) in Citrate buffer (0.1 M), while the normal control group was given the vehicle (0.1 M citrate buffer) only. After 72 hours of induction, the diabetic animals (> 200 mg / dl) were identified and treated further for five weeks with ethanol and ethylacetate extracts in the ratio of 250 mg/ kg of b.wt whereas Glibenclamide in 5 mg/kg of b.wt. STZ - induced diabetic rats showed marked hyperglycemia, hyper cholesterolemia and hyper triglyceridemia. Body weight, plasma insulin, total haemoglobin were reduced abruptly while blood glucose and glycosylated haemoglobin levels were significantly increased in diabetic rats. Reduced activities of antioxidant enzymes such as SOD, CAT, GPx and GSH in liver and kidney tissues of diabetic rats shows considerable restoration after the administration of both extracts of *M.tinctoria* similar with glibenclamide treatment. In diabetic rats, SGOT (Serum Glutamate Oxaloacetate Transaminase) and SGPT (Serum Glutamate Pyruvate Tranasminase) levels were significantly elevated. These values were further reduced after the administration of both these fractions. The results confirm that because of its higher antioxidant property, it may sensitize the insulin receptors and reduce the diabetic complications in experimental diabetic rats.

Keywords: Anti-oxidant, Anti-diabetic effects, *Morinda tinctoria*, *Streptozotocin* (STZ) and Diabetic rats

1. Introduction

Diabetes mellitus is a group of complex metabolic disorders characterized by hyperglycemia, hyperlipidemia, polyphagia, polydypsia, ketosis, neuropathy, nephropathy and cardio vascular disorders with relative deficiency of Insulin or Insulin resistance due to increased oxidative stress. Harris SB, Macaulay AC (1998) and Wilson R. L., (1998). Despite advances in medicine, diabetes as a major health complication seems to be going at an alarming proportion world over particularly in India. By the end of 2030, 79.4 million Indians are expected to be affected by this metabolic disorder and this accounts for nearly one sixth of the world's diabetes (Wild, S., Roglie, G. Green, A., and King, H. Global 2004).

Oxidative stress in diabetes increases glycation of proteins, inactivation of enzymes, structural alterations of collagen basement and auto oxidation of glucose may result the generation of free radicals, which in turn catalyzes lipid peroxidation Baynes J.W, (1991); Jakus V. (2000). Such oxidative stress leads an imbalance between free radical generation. and radical

scavenging systems; thereby increased free radical production or reduced activity of antioxidant defenses or both (Bayness JW. 1991). The enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) which involved in the defense mechanisms against free radicals, whose activities contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals respectively (Soto C., Barron, Recoba, Ravaril. 2003).

Several antioxidants of plant origin have been screened against oxidative stress for their ability to scavenge free radicals and are useful as protective agents. Based on the WHO (World Health Organization) recommendations, a large number of plant origins used in traditional medicine for its excellence against hyperglycemic effects (WHO; 1980). Even though WHO recommends the evaluation of the plant products with available diseases effectively, the modern drugs prevalent in market (Day C. 1998). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes, but only a few have received scientific scrutiny (Pushparaj, P., C.H. Tan and B.K.H. Tan, 2000).

Morinda tinctoria (M. tinctoria) belongs to the Rubiaceae family that grows wild and is distributed throughout Southeast Asia. It is commercially known as Nunaa and is indigenous to tropical countries. The tribes of Australia have used the ripe fruits of *M. tinctoria* for the treatment of respiratory infections (Wang My, Brett Jw 2002). There is a great demand for its fruit juice in treatment of arthritis, cancer, gastric ulcer and heart diseases (Farine Jp, Legal L. 1996). Octoamic acid, Vitamin C, terpenoids such as scopoletin, flavones, glycosides, linoleic acid, anthraquinones, morindone, rubiadin and alizarin are the major components identified in the Nunaa plant (Harbone JB. 1998). Since there were no documented reports available on the antioxidant and hypoglycaemic activity of *M. tinctoria* leaves either in experimental or clinical studies. The present investigation was envisaged to evaluate its potential in a STZ induced diabetic albino rats.

2.Materials and Methods

2.1. Preparation and fractionation of plant extracts

The leaves of *M. tinctoria* were collected from the banks of Cauvery river at Government Arts College, Kumbakonam, TamilNadu, India. The plant was identified and authenticated by Dr. N. Ramakrishnan, Head, Department of Botany, Government Arts College, Kumbakonam, TamilNadu, India. Fresh leaves from the plant were washed and dried in air at room temperature. The shade dried leaves (1 kg) were ground in to a powder form, macerated and soaked with 95% ethanol for three days at room temperature. The extracts were filtered and the filtrates are subjected to evaporation under reduced pressure to remove the excess solvents using a rotary evaporator. The crude filtrate was then extracted successively with ethyl acetate; then the extracts were condensed separately to obtain powder to use further for biological observation. The yields obtained for each fraction with respect to the initial dry material were ethanol 0.36% and ethylacetate 0.30%. The available standard procedures were followed to carry out preliminary phytochemical screening.

2.2. Experimental Animals

Male albino rats of wistar strain weighing about 150 – 200 g were obtained from the Venkateswara Enterprises, Bangalore. They were housed in standard cages and maintained on standard rat chow with water adlibitum. All the rats were acclimatized for two weeks before the initiation of the experimentation in order to adapt the rats with the environmental conditions. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.3. Induction of Diabetes

Diabetes was induced by freshly prepared solution of streptozotocin (STZ) (45 mg/kg b.wt in 0.1 M citrate buffer, pH 4.5) was injected intra peritoneally in a volume of 1ml/kg b.wt to over night fasted rats, (Pandit, R., Phadke, A. Jogtap, A.2010). STZ injected animals were given 5% glucose solution using oro gastric tube for 24 hr to overcome the drug induced hypoglycemia. Normal rats were injected with citrate buffer alone. Albino rats with a blood glucose level above 200 mg/dl on the third day (72 hr) were considered as diabetic and selected for further experimentation.

2.4. Experimental Protocol

The animals were randomly divided into five groups; each group consists of six animals and trail was made for 35 days.

Group 1: Normal rats served as the non – diabetic control group.
Group 2: Served as diabetic – untreated control
Group 3: Diabetic rats treated with 250mg/kg b.wt of ethanol fractions of *M.tinctoria* leaves.
Group 4: Diabetic rats treated with 250 mg /kg b.wt of ethyl acetate fractions of *M.tinctoria* leaves.
Group 5: Diabetic rats administered orally with the reference drug Glibenclamide 5mg/kg b.wt per day

2.5. Determination of oral glucose tolerance test (OGTT)

During the last week of treatment, glucose tolerances of all animals were assessed by the OGTT.(M.C.T. Fyfe, J.R. White, A. Taylor P. Wong – Kairin, 2007). The animals were orally gavaged with 2g/kg b.wt of glucose after the administration of drugs for the 12 hr fasted rats. Blood samples were collected from the tip of the tail at 0 (before glucose loading) - 120 minutes on every thirty minute interval after glucose loading (Aslan, M. Orhan, D.D., Orhan, N. Sezik. E., Yesiladda. E 2007). Blood glucose levels were tested using one-touch active glucose meter.

2.6. Determination of biochemical assays

At the end of the experimental period, the rats were anaesthetized and sacrificed. Blood samples were collected through retro – orbital plexus puncture and stored in with or without disodium ehtylenediamine tetra acetate depending on the respective biochemical parameter estimation. The levels of haemoglobin and glycosylated haemoglobin were estimated using the methods in the available literature (Sunil.C, Ignacimuthu.S, Agastin.P 2011). The Total Cholesterol (TC), Triglycerides (TG), HDL, Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and Total Protein (TP) in serum were estimated as per respective standard procedure using semi auto analyzer (Stat fax 3300,USA). The LDL and VLDL levels were calculated by the following equation.

$$VLDL = Triglycerides / 5$$

$$LDL = TC – (HDL + VLDL)$$

2.7. Antioxidant Assay

The liver and kidney tissues were dissected out and washed immediately with ice-cold saline to remove the excess blood. Tissues were cut into small pieces and homogenized in Tris – HCL buffer with a glass – Teflon homogenizer (pH – 7.4). The homogenate was centrifuged at 10,000 rpm for 10 min. and the supernatant was used for following antioxidant enzymes.

The activity of SOD was assayed using the method of Marklund and Marklund (Markland S.Markland G. 1974). CAT activity was assayed using the method of Aebi (AcbiH. Catalase, In: Bergmeyer. HV.Ed. 1974). The GSH and Gpx was assayed using the method of Sedlaok and Lindsay (Sedlak J, Lindsay R.H. 1968) and Lawrence and Burk (Lawrence RA, Burk RF, 1976) respectively.

2.8. Statistical analysis

All the data expressed as mean SEM for six animals in each group were evaluated by one-way analysis of variance (ANOVA), followed by least significant differences test. P-values less than 0.05 were considered as statistically significant.

3.Results

3.1. Effect of *M.tinctoria* leaves on body weight, blood glucose, glycosylated haemoglobin and Insulin levels

In the present study, STZ - induced diabetic rats showed significant ($P < 0.001$) reduction in body weight and elevation in blood glucose level compared to normal rats. Oral administration of *M. tinctoria* extracts and glibenclamide significantly increased the body weight from second to fifth weeks compared to diabetic control rats (Table-1). Administration of *M. tinctoria* extracts for a period of 35 days significantly ($P < 0.05$) decreased the level of plasma glucose, glycosylated haemoglobin and increased the levels of plasma insulin and total haemoglobin in STZ induced diabetic rats.

Table : 1 Effect of *M. tinctoria* leaves extract for 35 days of treatment on body weight, blood glucose, total haemoglobin, glycosylatedhaemoglobin and plasma insulin of various group of rats

Parameters	Body weight (g)		Blood glucose (mg/dl)		Total haemoglobin (g/dl)	Glycosylated haemoglobin (mg/g Hb%)	Plasma insulin (µu/ml
Group	Initial day 0	Final day 35	Initial day 0	Final day 35			
Control	172 ± 20.2	210 ± 17.1	89.0 ± 6.2	92.0 ± 4.3	14.3 ± 1.72	6.4 ± 1.13	16.21 ± 0.87
Diabetic control	186 ± 22.1	142 ± 7.3**	246 ± 8.2	269 ± 9.3	7.5 ± 0.87	13.6 ± 1.07	6.69 ± 1.35
Diabetic + MT & ET 250 mg/kg	181 ± 5.3	195 ± 6.7*	252 ± 6.1	152 ± 7.3**	10.1 ± 0.41**	10 ± 2.1*	11.32 ± 0.73**
Diabetic + MT & EA 250 mg/kg	191 ± 3.2	208 ± 2.7**	238 ± 7.6	141 ± 4.6**	11.2 ± 0.81**	8.2 ± 1.3**	12.46 ± 0.06**
Diabetic + glibenclamide 5mg/kg	189 ± 6.9	197 ± 3.5*	243 ± 6.9	134 ± 3.7*	11.8 ± 0.69**	8.3 ± 1.7**	13.67 ± 0.8**

Values are given as mean ± SD for group 6 animals each
Statistical significance using oneway ANOVA followed by Dunnett's test *P<0.05,**P<0.01 *vs* diabetic control

3.2. Oral glucose tolerance test

The diabetic control group exhibited noticeable elevation in fasting blood glucose level and showed marked impairment in glucose tolerance level when compared with normal control. The plasma glucose levels reached the maximum after 30 minutes of glucose load. After 60 and 90 minutes of treatment with *M. tinctoria* extracts, the blood glucose levels were reduced significantly ($P < 0.005$) in diabetic rats (Table-2) as same as glibenclamide treatment.

Table: 2 Oral glucose tolerance test of *M. tinctoria*on normal and diabetic rats on 35th day

Group	Blood glucose level (mg/dl) at the end of			
	0 minutes	30 minutes	60 minutes	90 minutes
Control	95.78 ± 2.36	123.45 ± 3.12	102.67 ± 1.26	92.68 ± 2.84
Diabetic control	278.67 ± 6.17	315.64 ± 1.22	310.68 ± 2.78	271.46 ± 2.65
Diabetic + MT & ET 250 mg/kg	183.47 ± 5.24	202.41 ± 2.37*	156.38 ± 4.28**	142.65 ± 3.17**
Diabetic + MT & EA 250 mg/kg	146.53 ± 3.14	165.53 ± 6.13*	122.74 ± 1.64**	106.78 ± 1.69**
Diatic + glibenclamide 5mg/kg	152.73 ± 2.16	163.57 ± 2.67*	121.36 ± 2.67**	104.65 ± 2.35**

Values indicates mean ± SEM (n=6 rats);** P<0.01, *P<0.05 as compared to O minutes

3.3. Effect of *M.tinctoria* extracts on lipid profile

Induction of diabetes significantly altered the lipid profile levels on normal rats compared to diabetic control rats. Administration of both doses of *M.tinctoria* and glibenclmide significantly decreased ($p < 0.001$) TC, TG, LDL and VLDL level (Table - 3). Also, there was a significant ($p < 0.05$) increase of HDL cholesterol was observed in *M. tinctoria* treated rats after the span of 35 days. Incase of diabetic control, there was a fall in HDL level.

Table : 3 Effect of *M.tinctoria* leaf extract for 35 days of treatment on total cholesterol, triglycerides, HDL. Cholesterol, LDL and VLDL of various groupof rats

Group	Total cholesterol mg/dl	Triglycerides mg/dl	HDL cholesterol mg/dl	LDL cholesterol mg/dl	VLDL cholesterol mg/dl
Control	133.3 ± 5.21	86.56 ± 1.33	37.68 ± 1.34	39.4 ± 2.5	18.64
Diabetic control	249.56 ± 133^{a**}	178.70 ± 2.27^{a**}	19.28 ± 1.16^{a**}	68.7 ± 1.8^{a**}	34.93^{a**}
Diabetic + MT & ET 250 mg/kg	192.87 ± 7.37^{b*}	171.61 ± 8.67^{b*}	28.17 ± 1.24^{b*}	62.26 ± 1.7^{b*}	37.31^{b*}
Diabetic + MT & EA 250 mg/kg	156.36 ± 6.81^{b**}	124.78 ± 6.33^{b**}	31.17 ± 1.65^{b**}	46.34 ± 1.9^{b**}	24.95^{b**}
Diabetic + glibenclamide 5mg/kg	147.56 ± 2.24^{b**}	119.65 ± 7.81^{b**}	30.67 ± 1.31^{b**}	42.26 ± 1.3^{b**}	23.69^{b**}

Data's are expressed as mean ± SEM (n=6); a- Group II is compared with Group I; b - Group III,IV,V are compared with Group II ** P<0.01,*P<0.05

3.4. Antioxidant Status restoration:

A significant ($p < 0.001$) reduction of SOD, CAT, GSH and GPx level was observed in liver and kidney of diabetic control rats compared to control rats. Administration of *M. tinctoria* extracts and glibenclamide significantly ($p < 0.001$, $p < 0.01$) increased the activities of SOD, CAT, GSH and GPx levels compared to diabetic control rats in both liver and kidney (Table - 4).

Table : 4 Effect of M.T leaf extracts for 35 days of treatment on enzymatic antioxidants in various groups of rats.

Group	SOD (U/mg protein)		CAT(µu of H_2O_2 consumed/min/mg protein)		GSH (µg of GSH/mg protein)		Gpx(µg of GSH utilized /min/ mg/ protein)	
	Liver	Kidney	Liver	Kidney	Liver	Liver	Liver	Kidney
Control	18.2 ± 0.04	13.72 ± 0.17	67.73 ± 0.6	32.84 ± 0.7	47.36 ± 3.7	32.68 ± 3.9	7.4 ± 1.6	5.2 ± 0.72
Diabetic control	9.4 ± 0.38	9.3 ± 0.64	46.32 ± 0.8	20.36 ± 0.36	24.28 ± 4.1	19.76 ± 2.7	3.8 ± 0.9	3.7 ± 0.63
Diabetic + MT & ET 250 mg/kg	13.9 ± 0.64*	10.12 ± 0.74*	54.61 ± 0.6*	23.71 ± 0.7*	31.67 ± 3.6*	23.68 ± 3.3*	5.2 ± 0.6*	4.1 ± 0.6*
Diabetic + MT & EA 250 mg/kg	15.6 ± 0.81'	11.68 ± 0.89**	62.17 ± 1.3**	28.17 ± 0.9**	36.71 ± 2.8**	28.17 ± 4.1**	6.8 ± 0.7**	4.6 ± 0.7**
Diabetic + glibenclamide 5mg/kg	16.2 ± 0.61	11.36 ± 1.14'	59.16 ± 1.8**	25.84 ± 0.5**	39.17 ± 4.3**	26.34 ± 3.7**	7.1 ± 0.3**	4.3 ± 0.2**

Values are given as mean ± SD for 6 rats in each group ' p <0.05 when compared with diabe tic rats.; ** P<0.01 Diabetic control compared with normal control;* P<0.05 Treatment groups were compared with diabetic control

3.5 Effect of *M. tinctoria* extracts on the hepatic markers

Table - 5 shows the activities of SGOT and SGPT on experimental rats. In diabetic rats SGOT and SGPT levels were raised to 2.7 and 2.27 times respectively in comparison to normal rats. Treatment with *M. tinctoria* extracts brought down SGOT and SGPT values on STZ induced diabetic rats to near normal.

A significant increase in serum urea and a significant decrease in plasma total protein level were observed in diabetic rats. After treatment with *M. tinctoria* ethanolic and ethyl acetate extract for 35 days, the protein levels (43% and 51%) were significantly increased compared to diabetic control rats, while the urea levels (34% and 35%) were significantly decreased (Table - 5).

Table : 5 Effect of *M. tinctoria* leaf extract for for 35 days of treatement on SGOT, SGPT and Total proteins.

Groups	SGOT (AST) IU/dl	SGPT (ALT) (IU /dl)	Total protein (g/dl)	Urea mg/dl
Control	23.64 ± 1.79	29.76 ± 2.67	8.12 ± 0.54	41.37 ± 3.76
Diabetic control	64.87 ± 2.05	67.63 ± 4.3	4.69 ± 0.75	72.58 ± 2.58
Diabetic + MT & ET 250 mg/kg	36.43 ± 1.74*	51.91 ± 2.18	6.71 ± 0.67	47.58 ± 3.86*
Diabetic + MT & EA 250 mg/kg	34.13 ± 2.58*	30.53 ± 2.61*	7.08 ± 0.34*	46.91 ± 4.37*
Diabetic + glibenclamide 5mg/kg	31.46 ± 5.05*	32.67 ± 1.98*	6.91 ± 0.68*	44.42 ± 4.29*

Values are mean activities of SGOT and SGPT ± SE (n=6)
*Statistically significant compared with diabetic control at $P<0.05$

4.Discussion

The present study was undertaken to investigate the antidiabetic and antioxidative effect of ethanol and ethyl acetate fractions of *M. tinctoria* leaves on STZ induced diabetic rats. STZ, a diabetogenic compound, selectively destroys β - cells of pancreas resulting in a hyperglycemic state (Takasu, N.T. Komiya, T. Asawa 1991). The antihyperglycemic activity of the *M. tinctoria* leaf extract was associated with decrease in blood glucose, glycosylated haemoglobin and increase in Plasma insulin and total haemoglobin. The possible mechanism of hypoglycemic action may be through potentiation of pancreatic secretion of insulin or due to enhanced transport of blood glucose to the peripheral tissues. (Doda RF. 1996). In diabetic control rats, the loss of body weight is due to increased muscle wasting and loss of tissue proteins (Chatterjee M.N., Shinde. R 2002). After 35 days of *M. tinctoria* plant extract administration, the results exhibited the gain of body weight in diabetic rats comparable with that of glibenclamide. It is due to enhanced quantity of plasma insulin secretion. The decreased level of haemoglobin in diabetic rats is mainly due to the increased formation of HbA1C. During diabetes, the excess glucose present in the blood binds with haemoglobin to form HbA1C (Koening RL. Peterson CM. Jones RL 1976). The normalization of glycosylated haemoglobin was observed on *M. tinctoria* leaf extract treated rats. It indicates that there is decreased glycation of proteins which is similar to glibenclamide.

Normally, fasting plasma glucose (FPG) and Oral Glucose Tolerance Test (OGTT) are two most common tests for diagnosing diabetes (American Diabetes Association, Diabetes Care 33 2010). The OGTT is more sensitive than the FPG test for screening pre - diabetes.The OGT studies reveal a maximum fall of glucose after 90 minute by the administration of ethyl acetate extract and is almost similar to glibenclamide treatment. This indicated that the increased glucose tolerance was due to augmented glucose transport and utilization.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hyper cholesteromia. It is due to increased mobilization of free fatty acids from peripheral fat deposits (Al – Logmani et al., 2009). In the present study, it was noticed significant increased levels of serum TC, TG, VLDL and LDL as well as marked reduction in serum HDL level in diabetic rats. Administration of both extracts of *M. tinctoria* decreases the levels of TC, LDL, VLDL and TC as well as increased level of HDL in diabetic rats. The above action could be beneficial in preventing diabetic complications like coronary heart diseases and atherosclerosis.

SOD and CAT are the two major scavenging enzymes that remove toxic free radicals *in vivo*. Reduced activities of SOD and catalase in liver and kidney have been observed during diabetes; it leads to the accumulation of O_2 and H_2O_2 (Searle AJ, Wilson RL. 1981). Administration of both extracts of *M .tinctoria* and glibenclamide exhibit free radical scavenging activity, which could exert a beneficial action against pathological alterations caused by the presence of O^{2-}, H_2O_2 and OH.

GPx catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of GSH. GPx activity is also reduced in diabetic condition. This may be due to inactivation of the enzyme involved in disposal of oxygen species and also insufficient availability of GSH (Illing EKB, Gray CH, Lawrence RD. 1991). The present study also revealed that elevation in both GSH and GPx levels after the treatment with *M. tinctoria* fractions and glibenclamide.

Increased activities of SGOT and SGPT are used as the indices of liver damage (Ramesh B. and K.V. Pugalendi, 2006). The leakage of these enzymes from the cytosol to the blood stream, was noticed due to the increased activities. Upon treatment with *M. tinctoria* extracts and glibenclamide, the activities of these enzymes had reduced and thus inhibit the liver damage induced by STZ. Similar findings are consistent with the fruit extract of *Solanum xanthocarpum* on diabetic rats (Kar, D.M., L.Maharana, S. Pattnaik and G.K. Dash 2006).

Conclusion

The present investigation shows that leaves of *M. tinctoria* possesses an anti-diabetic effect in addition to anti-oxidant potential. It also exerts protective effect against lipid peroxidation and enhances cellular antioxidant defense, there by reducing diabetic complications.

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