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Hepatoprotective And Hypoglycemic Activities of Morinda Tinctoria Leaves Extract on Alloxan Induced Diabetic Albino Rats.

KEYWORDS: Hepatic markers, Antioxidant, Morinda tinctoria, Hypoglycemic, HbA1

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ABSTRACT *The present study explores the hepatoprotective and hypoglcemic effect of Morinda tinctoria in alloxan induced diabetic rats. Alloxan (120mg/kg body weight of rats) induces chemical diabetes in a wide variety of animal species through damage of insulin secreting cell. The M. tinctoria leaf extract was prepared with ethanol and ethyl acetate solvents. Preliminary phytochemical screening of phenols, flavonoids, alkaloids, terpenoids and tannins were performed by standard protocols. Compounds responsible for these activities were identified by GC-MS with the inbuilt libraries. After 35 days of oral administration of M. tinctoria ethanol (250 mg/kg b.w) and M. tinctoria ethyl acetate (200 mg/kg b.w) extracts treatment, the physical and biochemical parameter such as body weight, blood glucose, lipid profile, hepatic markers and antioxidant parameters are assayed. Hypoglycemic activity may due to its stimulatory effect at cellular level on islets. Ethyl acetate extract of M. tinctoria produced more significant reduction in glucose, HbA1C, lipid profile, and enhanced hepatic marker enzymes and antioxidant activity in alloxan induced diabetic rats. Ethyl acetate extract yielded more promising results than the ethanol extract and even more or less same with the drug glibenclamide. The preliminary investigation on the hepatoprotective and hypoglycemic efficacy of ethyl acetate extract of M. tinctoria exhibit significant role due to its high alkaloid, terpenoid, coumarin and polyphenol contents. Further studies will be carried out to elucidate its active fraction and its mechanism of action.*

Introduction

Diabetes mellitus is a chronic disease which cannot be completely cured and may develop complications if not properly regulated. It's one of the most common non-communicable diseases globally. It has been associated with a syndrome of disturbance in the homeostasis of carbohydrate, fat and protein metabolism. Diabetes is primarily characterized by a hyperglycemia which results from lack of insulin or a weak response of tissues to this hormone. It is associated with long-term complications such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, retinopathy, amputations, renal failure and blindness. The prevalence of diabetes mellitus is growing rapidly worldwide and is reaching epidemic proportions. Diabetes is undoubtedly one of the most challenging health problems in 21st century (Gohel et al., 2013).

Diabetes mellitus has been classified into several subtypes based on its etiology (Rabi et al., 2006). Chiefly there are two types, type I diabetes (IDDM) and type II diabetes (NIDDM). Type I diabetes refers to endogenous insulin which is caused by a cellular-mediated autoimmune destruction of beta cells in pancreas, leads to increased blood and urine glucose levels. Type II diabetes is also known as Non-Insulin Dependent Diabetes Mellitus (NIDDM) or "adult onset diabetes" is developed due to lack of insulin and is the most widespread form of diabetes which accounts for 95% of cases. It has been proved that T2D is caused due to unhealthy life style such as smoking, alcohol, sedentary life and consumption of high calorie food (Misra Anoop et al., 2007). Current status of diabetes is estimated around 6% of the world's adult population now lives with diabetes (Meetoo et al., 2007). It is estimated that there are currently 285 million people with diabetes worldwide and this number is set to increase to 438 million by the year 2030.

Synthetic antidiabetic agents like sulfonylurea, biguanides, glucosidase inhibitors and thiazolindiones are being expensive and produce serious side effects. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes and some of them have been tested and their active ingredients isolated. The World Health Organization (WHO) has also recommended the evaluation of the plants effectiveness and conditions where we lack safe modern drugs. Hence phytotherapy has significant role to play in the developing countries compared to synthetic drugs because it is safe, less expensive and available as a gift of nature.

Morinda tinctoria belongs to the family of *Rubiaceae* that grows wild and is distributed throughout Southeast Asia. It is commercially known as *Nunaa* and is indigenous to tropical countries. *M. tinctoria* is considered as an important folklore medicine. The tribes of Australia have used the ripe fruits of *M. tinctoria* for the treatment of respiratory infections. In the traditional system of medicine, leaves, fruits and roots of *M. tinctoria* are used as astringent, deobstrent, emmengogueand to relieve pain in the gout (Thirupathy Kumaresan et al., 2009). Further, it has been reported to have a broad range of therapeutic and nutritional values. There is a greater demand for its fruit juice in treatment for arthritis, cancer, gastric ulcer and other heart diseases (Sivaraman et al., 2010). Anticonvulsant, analgesic, anti- inflammatory, antioxidant and cytoprotective effect of *M. tinctoria* leaves has been reported (Thirupathy kumaresan et al., 2009; Pattabiraman et al., 2011; Jeyabalan et al., 2009; Sivaraman et al., 2010).There was not much work has been carried out on hepatoprotective and hypoglycemic activities in vivo, the present study was designed to investigate the efficacy of leaves of *M. tinctoria* in alloxan induced diabetic rats.

2. Materials and Methods

2.1. Preparation of extracts

The leaves of *M. tinctoria* were collected from the banks of Cauvery river at Government Arts College(A), Kumbakon-

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am, 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 activities. The yields obtained for each fraction with respect to the initial dry material were ethanol 0.36% and ethylacetate 0.30%.

2.2. Phytochemicals screening

The phytochemical analysis of both ethanol and ethyl acetate extracts of *M. tinctoria* has been performed to find the presence of major secondary metabolites. The standard protocols (Trease and Evans 1989; Harborne 1998) were followed to analyze tannins, flavonoids, glycosides, terpenoids, alkaloids, coumarins and anthraquinones. Steroidal ring analysis was performed following method described by Sofowora (1982). Saponins were analyzed by the following protocol described Wall (1952).

The identification of phytochemicals were further confirmed by the analytical study carried out by GC-MS.

2.3. Experimental Animals

Adult male albino rats weighing about 175 to 300 gm were procured from Haja Nursery Garden, Mayiladudhurai. The animals were kept in polypropylene cages and allowed to get acclimatized to a standard laboratory and constant room temperature at 22° C - 24° C with 12 hour day and night cycle. Feed and drinking water were provided *ad libitum.*

2.4. Induction of diabetes

The animals were fasted over night and diabetes was induced by a single intraperitonial injection of freshly prepared solution of alloxan monohydrate (120 mg/Kg body weight dissolved in 0.9% saline). Two days after alloxan injection, blood glucose content was measured by using glucose test meter one touch horizon (China make) using a blood sample obtained from the tail vein. Those animals, whose blood glucose level above 200mg was considered as diabetic for the present study. Treatment with extract was started 48 hours after alloxan injection was considered as 0 hours of treatment. Retro orbital venous blood samples were collected in the fasting state at specific intervals during 0, 7, 14, 21, 28 and 35 days were tested for presence of glucose concentration.

2.5. Experimental Design

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

Group 1: Normal rats (Control)
Group 2: Diabetic Control (DC)
Group 3: Diabetic rats treated with 250mg/kg body weight of Ethanol fractions of *M. tinctoria* leaves (MtLEEt)
Group 4: Diabetic rats treated with 200 mg /kg body weight of Ethyl acetate fractions of *M. tinctoria* leaves(MtLEaEt)
Group 5: Diabetic rats administered orally with the reference drug Glibenclamide 5mg/kg body weight per day(DG)

2.6. Estimation of Biochemical Parameters

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 ethylene diamine tetra acetate depending on the respective biochemical parameter estimation. Serum glucose was estimated by the one touch horizon glucometer. The level of glycosylated haemoglobin (HbA1c) were estimated using the methods in the available literature (Sunil et al., 2011). The Total cholesterol (TC) (Allain et al., 1974), Triglycerides (TG), HDL, Serum Glutamate Pyruvate Transaminase (SGPT) (Reitman and Frankel, 1957) and Serum Glutamate Oxaloacetate Transaminase (SGOT) (Reitman and Frankel , 1957) in serum were estimated as per respective standard procedure using semi autoanalyzer (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 (pH–7.4) with a glass–teflon homogenizer. 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 (Das et al., 2000). CAT activity was assayed using the method of Sinha (1972). The GSH was assayed using the method of Moron et al., (1979). Gpx was assayed using the method of Rotruck et al., (1973) respectively.

2.8. Statistical analysis

All the data expressed as mean+ SD of 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. Phytochemical constitutents

Phytochemical analysis of both ethanol and ethyl acetate extracts of leaves of *M. tinctoria* is shown in Table-1. It clearly indicates the abundance of alkaloids, coumarins, anthraquinones, steroids, tannins, phenolic compounds and terpenoids are more in ethyl acetate fraction than the ethanol fraction.

The results of GC-MS analysis in Table-2 clearly reveals the presence of many phytocompounds which contribute to the antioxidant, hepatoprotective and hypoglycemic activities. The main compounds such as 2-methyl-1-butanol, 6-methyl-octadecane, O-decyl hydroxylamine, 2-hydroxymethyl furan and hexa decanoicacid are identified and are suggested to be fragments of alkaloids, coumarins, anthraquinones, steroids, tannins, glycosides and terepenoids,

3.2. Effect of *M. tinctoria* leaves on body weight, blood glucose, total haemoglobin and glycosylated haemoglobin

In the present study, alloxan induced diabetic rats showed drastic reduction in body weight and elevation in blood glucose level compared to normal rats. Oral administration of ethyl acetate fraction of *M. tinctoria* extracts and gliben-

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clamide significantly increased the body weight on 35th day compared to diabetic control rats (Table-3). Administration of *M. tinctoria* extracts for a period of 35 days significantly ($P < 0.05$) decreased the level of blood glucose, glycosylated haemoglobin and increased the level of total haemoglobin in alloxan induced diabetic rats.

Table 1. Phytochemical screening of *M. tinctoria* leaf extracts

S.No	Phytoconstituents	Ethanol fraction	Ethylacetate fraction
1	Alkaloids	+	++
2	Saponins	-	-
3	Steroids	+	++
4	Phenolic compounds	+	++
5	Tannins	-	+
6	Flavonoids	-	-
7	Terpenoids	-	+
8	Coumarin	+	++
9	Glycosides	+	++
10	Anthroquinone	+	+

where '-' indicates Absent; '+' present in low concentration ; '++' Present in high concentration

Table 2. Phytocomponents identified in the ethylacetate leaf extract fraction of *M. tinctoria* by GC-MS

S.No	Retention time (min)	Area%	Molecular weight	Name of the compound	Nature of the compound
1	3.882	7.726	57	2-propen-1-amine	Alkaloid
2	6.951	4.85	88	2-methyl-1-butanol	Isoprene/ terpenoid
3	9.981	2.82	268	6 methyl octadecane	Alkaloid/ Steroid
4	11.135	5.567	173	O-decyl hydroxylamine	Alkaloid/ Steroid
5	13.363	7.04	98	2-hydroxy-methyl furan	Coumarin
6	15.992	9.18	98	6-oxa-bicyclohexan-3one	Terpenoid
7	18.692	5.36	150	2-methoxy-4vinyl phenol	Anthraquinone
8	20.976	8.87	284	Hexa decanoicacid	Terpenoid
9	23.079	5.78	247	4-Bromo3-chloro acetanilide	Phenol
10	23.237	3.71	222	Diethyl phthalate	Phenol

Table 3. Effect of *M. tinctoria* Leaves Extract for 35 days of treatment on body weight, blood glucose, total haemoglobin and glycosylated haemoglobin levels

Group	Body weight (g)		Blood glucose (mg/dl)		**Total haemoglobin (g/dl)** 35th day	**Glycosylated haemoglobin (Hb%)** 35th day
	Initial day 0	Final day 35	Initial day 0	Final day 35		
Normal	167.4±1.98	199.8±4.58	86.56±0.78	88.12±1.62	13.98±0.09	5.96±0.17
Diabetic control	181.8±1.59*	139.6±1.15*	242 ±1.45	265± 1.27*	7.2±0.07*	13.44±0.17*
MtLEEt	175.4±2.22*	192±1.39*	249 ±1.11*	150± 0.79*	9.86±0.14*	11 ±0.79*
MtLEaEt	187.8±1.43*	199.4±3.03*	234 ±0.79*	139 ±0.69*	11.14±0.13*	7.94 ±0.13*
DG	183.2±1.78*	192.4±1.98*	239 ±0.79*	130 ±0.49*	11.36±0.10*	8.28 ±0.17*

Each value is mean ± S.E of six rats in each group. Values are statistically significant at *p<0.05. Diabetic rats were compared with Control rats, while other treatment rats were compared with diabetic control rats.

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

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Induction of diabetes significantly altered the lipid profile levels on normal rats compared to diabetic control rats. Administration of ethyl acetate extract of *M. tinctoria* and glibenclamide significantly decreased ($p < 0.05$) TC, TG, LDL and VLDL level (Table - 4). 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. In case of diabetic control, there was a sharp fall in HDL level.

Table 4. Effect of M. *tinctoria* leaf extract for 35 days of treatment on total cholesterol, triglycerides, HDL, LDL and cholesterol of various groups of rats.

Group	Total Cholesterol mg/dl	Triglycerides mg/dl	HDL cholesterol mg/dl	LDL cholesterol mg/dl	VLDL cholesterol mg/dl
Normal	126.88±1.40	82.77 ± 0.80	35.69 ±0.70	35.48± 0.94	16.62 ±0.76
Diabetic control	241.79±0.73	173.9 ±1.16	17.29 ±0.51	65.14 ±1.23	30.68± 0.89
MtLEEt	187.89±1.59	169.05 ±0.93	22.95± 0.95	59.43 ±0.55	35.26 ±0.49
MtLEaEt	148.99± 1.09	120.58 ±0.60	28.12 ±0.79	44.38 ±0.52	22.55 ±0.58
DG	140.55± 0.69	116.07 ±0.81	26.99± 0.62	41.60 ±0.69	21.89± 0.35

Each value is mean ± S.E of six rats in each group. Values are statistically significant at *p<0.05. Diabetic rats were compared with Control rats, while other treatment rats were compared with diabetic control rats.

3.4. Antioxidant Status

A significant ($p < 0.05$) reduction of SOD, CAT, GSH and GPx level was observed in liver and kidney tissues of diabetic control rats compared to control rats. Administration of *M. tinctoria* extracts and glibenclamide significantly ($p < 0.05$) increased the activities of SOD, CAT, GSH and GPx levels compared to diabetic control rats in both liver and kidney and restored the condition as normal rats (Table - 5).

Table 5. Effect of *M. tinctoria* leaf extracts for 35 days of treatment on enzymatic antioxidants in various groups of rats.

Group	SOD (U/mg protein)		CAT (mM of H_2O_2 consumed/min/mg protein		GSH(µg of GSH/mg protein)		GPX(U/mg protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal	13.16± 1.09	12.48±0.57	65.33± 1.11	29.63±0.52	40.54± 0.89	30.28±0.50	6.94± 0.13	4.58±0.22
Diabetic control	7.42± 0.48	8.64±0.33	44.17± 0.53	19.63±0.29	22.06± 0.69	18.37±0.53	2.78± 0.20	3.06±0.16
MtLEEt	12.66± 0.76	9.58±0.45	51.49± 0.42	20..38±0.63	29.49± 0.58	21.47±0.55	4.6±0.15	3.14±0.33
MtLEaEt	14.2± 1.11	10.46±0.50	60.49± 0.81	23.74±1.17	33.58± 1.04	27.48±0.56	6.28± 0.56	4.14±0.10
DG	16.3± 0.60	10.04±0.44	54.63± 1.37	22.53±0.62	37.27± 0.54	25.54±0.52	5.96± 0.29	4.0±0.07

Each value is mean ± S.E of six rats in each group. Values are statistically significant at *p<0.05. Diabetic rats were compared with Control rats, while other treatment rats were compared with diabetic control rats.

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

Table - 6 shows the status of hepatic marker such as SGOT and SGPT on experimental rats. In diabetic rats SGOT and SGPT levels are raised at the end of 35th days when compared with normal rats. Administration of ethyl acetate (200 mg/kg body weight) extract of *M. tinctoria* reduces the SGOT and SGPT values as normal control. These results are similar with the treatment of glibenclamide. The present findings revealed that ethyl acetate extract of *M. tinctoria* have protective role on liver and its enzymes of alloxan induced diabetic rats.

Table 6. Effect of *M. tinctoria* leaves extract after 35 days of treatment on SGOT and SGPT.

Groups	SGOT (AST) IU/dl		SGPT (ALT) IU/dl	
	Initial day 0	Final day 35	Initial day 0	Final day 35
Normal	19.41± 0.54	22.016±1.04	24.26± 0.60	24.98±1.36
Diabetic control	58.56 ±0.92	61.45±0.97	62.38 ±0.52	65.49±1.35
MtLEEt	29.65 ±0.76	32.42±1.26	48.15± 0.77	49.75±1.14

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MtLEaEt	28.24 ±0.85	31.75±1.09	26.91 ±0.69	29.96±1.33
DG	27.49 ±0.60	29.61±0.71	28.54 ±0.91	32.75±0.77

Each value is mean ± S.E of six rats in each group. Values are statistically significant at *p<0.05. Diabetic rats were compared with Control rats, while other treatment rats were compared with diabetic control rats.

4.0. Discussion

Management of diabetes without any side effects is still a challenge to the modern medicine. This leads to increasing the demand for searching new drugs from natural origin with antidiabetic and free from side effect or less side effects. In Ayurvedic or indigenous folk medicines, the hypoglycemic plants have been in use generally in their natural forms (fresh juice, paste or dry powder). These include both the inorganic and organic constituents of the concerned herbs. Further it is important to note that the inorganic part of a medicinal plant containing mainly mineral elements sometimes plays a contributory role in enhancing medicinal properties (including hypoglycemic activity) of that plant.

The major components identified in the *Nunaa* plant include octoanic acid, potassium, vitamin C, terpenoids, scopoletin, flavones, glycosides, lineoleic acid, anthraquinones, morindone, rubiadin, and alizarin (Levand et al.,1979; Fairne et al.,1996; Moorthy et al.,1970; Singh et al.,1976). The present phytochemical screening and GC-MS studies also reveals that leaves are rich in secondary metabolites such as phenolic compounds, followed by alkaloids, glycosides, steroids, coumarins and anthraquinones. Earlier investigations found natural polyphenol inhibit the activity of carbohydrate hydrolyzing enzymes thus in turn it reduces the release of glucose in serum (Tundis et al., 2010). Alkaloids have also been reported to have antidiabetic activity through the inhibition alpha glucosidase and decrease glucose transport through the intestinal epithelium (Patel et al., 2012).

In diabetic control rats, the loss of body weight is due to increased muscle wasting and loss of tissue proteins (Chatterjee et al., 2002). After 35 days of *Morinda tinctoria* plant extract administration, the results exhibited the gain of body weight in diabetic rats comparable with that of glibenclamide. The antihyperglycemic activity of the *Morinda tinctoria* may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration. In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including haemoglobin. Glycosylated haemoglobin is now considered as the most reliable marker of glycemic control in diabetes mellitus. The increase in oxygen free radicals in diabetes could be due to increase in blood glucose levels, which generate free radicals upon autoxidation. During diabetes the excess glucose present in blood reacts with haemoglobin. By knowing the degree of protein glycation, long-term blood sugar status can also assessed. Administration of *Morinda tinctoria* prevents elevation in glycosylated haemoglobin thereby increasing the level of total haemoglobin in diabetic induced rats. This could be due to the result of improved glycemic control through glycolysis by *Morinda tinctoria.*

The present study indicates significant increased levels of serum TC, TG, VLDL and LDL as well as marked reduction in serum HDL level in alloxan induced diabetic rats. Administration of both extracts of *Morinda tinctoria* decreases the levels of TC, LDL, VLDL and TG as well as increased levels of HDL in diabetic rats. Lipids play an important role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. Lowering of serum lipid level through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase (Al-Logmani et al., 2009). On the other hand glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipedemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolutic hormones on the fat depots. The rise in blood sugar is accompanied with the increase in total cholesterol. However, administration of *Morinda tinctoria* and glibenclamide exhibited hypocholesterolemic and hypoglycemic effect. Sharma (1993) have reported that administration of *E.Jambolana* seeds can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics.

Alloxan is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroys the beta cells of pancreas possibly by generating excess reactive oxygen species such as, H_2O_2, O_2^- and HO. The two major enzymatic antioxidants, SOD and CAT play an important role in eliminating reactive oxygen species formed during bioactivation of xenobiotics in hepatic tissues. SOD scavenges the superoxide anion to form hydrogen peroxide whereas CAT catalyzes the breakage of toxic H_2O_2 produced in the cell to O_2 and H_2O (Chance et al.,1992). The SOD and CAT are easily inactivated by lipid peroxide or reactive oxygen species. SOD and CAT are two major scavenging enzymes that remove the toxic-free radical in *vivo*. Reduced activities of SOD and CAT in liver, kidney and pancreas have been observed during diabetes and a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. In the present investigation administration of alloxan exhibited profound decrease the activity of SOD in pancreas and liver when compared with control rats. Oral administration of *Morinda tinctoria* and glibenclamide to alloxan treated rats showed SOD and CAT levels were significantly increased in pancreas. Similarly oral administration of *Hygrophila auriculata* (Ernst 1997) to streptozotocin treated rat showed SOD and CAT levels were increased.

Antioxidants may have a role in the prevention of diabetes. Antioxidant protect our body from free radicals. The persistent and chronic hyperglycemia generates free radicals and reactive oxygen species (ROS) which trigger an oxidative stress. The overproduction of free radicals and ROS results in enhanced lipid peroxidation; damages to DNA and protein degradation; and exhaustion of the antioxidative defense systems. Free radicals are continuously produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems that has increased free radical production or reduced activity of antioxidant defenses or both. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free-radical generation but also due to non-enzymatic protein glycosylation; auto-oxidation of

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glucose, impaired glutathione metabolism, alteration in antioxidant enzymes and formation of lipid peroxides. In addition to reduced glutathione (GSH), there are other defense mechanisms against free radicals, such as the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT), whose activities contribute to eliminate superoxide, hydrogen peroxide and hydroxyl radicals.

GSH is the most important intracellular thiol antioxidant and a major determinant of the intracellular redox status. Intracellular GSH levels and GSH redox status play a central role in regulating a wide variety of cell responses, including signal transduction, immune regulation, maintenance of protein structure, cell proliferation and apoptosis. Increased phospholipids levels in tissues were reported by Venkateswaran *et al.,* (2002) and Pari and Satheesh (2004) in alloxan diabetic rats. Administration of ethanol extract of *P. rosmarinifolia* whole plant and glibenclamide decreased the levels of phospholipids.

The glutathione antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. In the present investigation administration of alloxan exhibited profound decrease GSH levels in pancreas when compared with control rats. Oral administration of *Morinda tinctoria* and glibenclamide to alloxan treated rats showed the activity of GSH was significantly increased secretion in pancreas. The decrease in GSH concentration in the early stage of diabetes is probably due to reactive oxygen species (ROS) generation by mechanisms such as glucose auto oxidation, advanced glycation end-product (AGE) pathway, polyol pathway and activation of protein kinase C (PKC). These studies are also similar to the results of *Morinda tinctoria* fruit extract, which have higher antioxidant potential thereby it improves lipid metabolism (Pattabiraman et al., 2011).

Elevation of hepatic marker enzymes such as AST and ALT in serum of alloxan treated diabetic rats was observed which may have occurred by leakage of enzymes from the liver cytosol into the blood stream. The increased gluconeogenesis and ketogenesis was also observed in diabetes may be due to high level activities of these transaminases. The restoration of AST and ALT activities to their respective normal levels after administration of *Morinda tinctoria* extracts in this manner is further strengthens the antidiabetogenic effect of this extract. Moreover, AST and ALT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver. In accordance with these findings, alloxan treatments has a significant role in the alteration of liver functions since the activity of AST and ALT were significantly higher than those of normal value. On the other hand treatment of the diabetic rats with the ethyl acetate extract of *M. tinctoria* and glibenclamide caused reduction in the activities of AST and ALT enzymes compared to diabetic untreated rats. The maximum curative effect against alloxan induced diabetic aberrations was achieved with 200 mg/kg of ethyl acetate extract *Morinda tinctoria* . The present study is similar to several other plant extracts and zamzam water treatment which decreased the activity of AST and ALT (Ashraf Saif et al., 2014).

Conclusion

The present investigation shows that leaves of *M. tinctoria* posses high hypoglycemic potential. It also exerts protective effect against lipid peroxidation and enhances cellular antioxidant defense, thereby reducing diabetic complications.

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