

ATTENUATION OF BIOCHEMICAL PARAMETERS OF MORUS ALBA LEAVES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Morus alba . belongs to moraceae family ,traditionally is used for disorders of gastrointestinal, cardiovascular, biliary, diabetes, hepatic, urinary and respiratory origin and many more. Diabetes is associated with profound alterations in plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease. The presence of phytochemicals such as tannins, alkaloids, proteins, flavonoids, triterpenes and saponins in the ethanolic extract/fractions was revealed by the preliminary phytochemical screening, keeping in view of medicinal importance the present study is an effort to investigate the biochemical parameters of morus alba leaves in streptozotocin-induced diabetic rats for 21 days. The acute toxicity studies of oral doses of ethanolic leaves extract/fractions in rats revealed that it has a high safety profile, as the extract/fractions was well tolerated by the animals. The test drug was administered for 21 days at a four different dose level 100, 200 mg/kg for ethanolic extract and 100, 100mg/kg each of two successive fractions (chloroform and n-butanol) made in aqueous and given by orally. Body weight, urine sugar was analyzed before and after treatment of extract/fractions while serum glucose was analyzed every week and lipid and lipoprotein profile from serum was analyzed after 21 days. The ethanolic extract/fractions of *Morus alba* leaves significantly prevented loss of body weight and reduce urine sugar. The results indicated that the ethanolic extract/fractions produced significant ($p < 0.001$) in biochemical parameter.

Key words: *Morus alba* (MA), streptozotocin (STZ), total cholesterol, total glycerides

No: of Tables: 3

No: of References: 16

Introduction

Type II diabetes is the commonest form of diabetes constituting 90% of the diabetic population. The countries with the largest number of diabetic patients in the year 2025 will be India, China and United States. Therefore, it has become necessary to look for novel oral therapeutically effective treatment especially for usage in the developing as well as under-developed countries. India is a country with a vast reserve of natural resources and a rich history of traditional medicine. Ethno pharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity.

Morus Alba. belongs to Moraceae family is ,traditionally is used for disorders of gastrointestinal, cardiovascular, biliary, diabetes, hepatic, urinary and respiratory origin and many more. *Morus alba* species are used in folk medicine such as disorders of gastrointestinal, cardiovascular, biliary, diabetes, hepatic, urinary and respiratory origin. However, to the best of our knowledge and based on the citations of the use, the purpose of present study was to evaluate biochemical parameter of the ethanolic leaves extract/fractions of *Raphanus sativus* in streptozotocin induced diabetic rats.

Materials and Methods

Plant material

The fresh leaves of *Morus alba* was collected during January 2016, from the villages of Erode..The plant species was identified and authenticated by taxonomist Dr. S N Pandit.

Laboratory Animals

Healthy, adult Wistar rats of both sexes (180-220g) were selected for present study. Animals were maintained under standard

laboratory condition and the experimental protocol was approved from Institutional Animal Ethical Committee (IAEC).

Preparation of extract

The collected fresh plant materials were dried in shade for two days and then dried in a hot air oven at 25°C for three days and they were made in to coarse powder with the use of mixer grinder. The power of leaves of *Morus alba* obtained were weighed separately and transferred to a round bottomed flask and then went with soxhlet extraction using 95% ethanol for 24 hour. Then the extract of ethanol was concentrated and then the marc was stored for fractionation.

Preliminary Phytochemical studies

The preliminary phytochemical screening of extract/fractions was performed to identify the presence of alkaloids, proteins, triterpenoids, saponins, flavonoids and tannins.

Acute toxicity study

The study was carried out according to the OECD guidelines 423.

Female Wistar rats of weight (180-220g) were taken for the study and kept for overnight fasting. Next day, body weight was taken and standardized morus alba leaf extract and fractions were administered orally at a dose of 2000mg/kg in distill water. Then the animals were observed for mortality and morbidity at 0, ½, 1, 2, 4, 6, 8, 12 and 24 hr. Feed was given to the animals after 4 hr of the dosing and the body weight was checked at 6 hr after dosing. Morbidity like convulsions, tremors, grip strength and pupil dilatation were observed. The animals were observed twice daily for 14 days and body weight was taken. The same experiment will be repeated once again on 3 rats (preferably female) if there is no observable

clinical toxicity for the animals on the acute toxicity study.

Preparation and induction of type 2 diabetes in rats by Streptozotocin

The Streptozotocin was made at a final concentration of 50mg/kg body weight by dissolving in citrate buffer (pH 4.5) the solution was then kept refrigerated overnight to facilitate its dissolution. Non-Insulin dependent diabetes mellitus (NIDDM) was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of 50mg/kg streptozotocin. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72hr. The rats with permanent Non insulin dependent diabetes mellitus (NIDDM) (250-350 mg/dL) were used for the study.

Experimental design

The Wistar rats weighing 180-220 gm of either sex were used for the experimental study. The animals were divided into seven groups of six animals each.

GROUPING OF THE ANIMALS

- GROUP I – Untreated Control
- GROUP II – Diabetic control
- GROUP III – Positive control (glibenclamide 10mg/kg b.w i.p)
- GROUP IV – EtOH extract of *morus alba* (100mg/kg, orally)
- GROUP V – EtOH extract of *morus alba* (200mg/kg, orally)
- GROUP VI – ChF of *morus alba* (100mg/kg, orally)
- GROUP VII – BtF of *morus alba* (100mg/kg, orally)

The extract/fractions was administered for 21 days at a four different dose level 100, 200 mg/kg for ethanolic extract and 100, 100mg/kg each of two successive fractions

(chloroform and n-butanol) made in aqueous and given by orally. The blood was collected by sinus orbital under light diethyl ether anesthesia. The blood was centrifuged at 3000 rpm for 10 minutes. Body weight, urine sugar was analyzed before and after treatment of extract/fractions while serum glucose was analyzed every week and lipid and lipoprotein profile from serum was analyzed after 21 days.

Analytical methods

Determination of body weight and urine analysis

Body weight, urine sugar was analyzed before and after treatment of extract/fractions. Urine sugar was estimated by using Reagent strips from Diastix (2802B).

Estimation of serum glucose

Glucose estimation in serum was assayed by using Ecoline diagnostic kit.

Glucose content is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator. Measured the absorbance of the sample and standard against the reagent blank value at 500nm. Glucose level in serum was expressed as mg/dL.

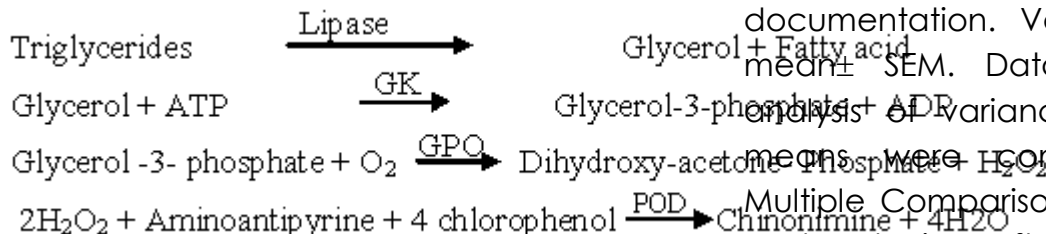
Estimation of Lipids and lipoprotein profile

Total Cholesterol (TC): Cholesterol in serum was estimated by using an Ecoline Diagnostic Kit. Cholesterol and its esters were released from lipoproteins by detergents. Cholesterol esterase hydrolysis the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 was formed. This was converted into a colored quinoneimine in a reaction with 4-aminoantipyrine and phenol catalyzed by peroxidase. The absorbance of the sample and of the standard was measured against the reagent

blank value at 546nm. Cholesterol level in serum was expressed as mg/dL.

Triglycerides (TG):

Triglyceride level in serum was estimated using an Ecoline Diagnostic Kit.



The absorbance of the sample and of the standard was measured against the reagent blank value at 546 nm. Triglyceride level in serum was expressed as mg/dL.

HDL (High density lipoprotein) Cholesterol:

The HDL cholesterol was separated from serum after precipitation of LDL and VLDL cholesterol by phosphotungstic acid precipitating reagent. The supernatant after centrifugation was estimated using Ecoline diagnostic kit. The absorbance of the sample and of the standard was measured against the reagent blank value at 546nm. HDL Cholesterol level in serum was expressed as mg/dL.

LDL (Low density lipoprotein) Cholesterol:

LDL Cholesterol was calculated by using the formula

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - [\text{HDL Cholesterol} + \text{Triglycerides}]$$

LDL Cholesterol level in serum was expressed as mg/dL.

VLDL (Very low density lipoprotein) Cholesterol:

VLDL Cholesterol was calculated by the formula

$$\text{VLDL Cholesterol} = (\text{Triglycerides})/5$$

VLDL Cholesterol level in serum was expressed as mg/dL.

Statistical analysis

Evaluation of statistical significance results by computer aided program and systemic documentation. Value were presented as mean ± SEM. Data were analyzed using analysis of Variance (ANOVA) and group means were compared with Bonferroni Multiple Comparisons Test using Instat graph pad and prism software.

Results

Effect on body weight and fluid intake

Gradual increase in body weight in untreated control while the diabetic control continue to loose the weight However treated diabetic group gained 16%, 11%, 16%, 15% as compared to diabetic control and body weight of diabetic treated towards normal range (P<0.001). On the other hand the administration of ethanolic extract/fractions. Ethanol (EtOH) (100mg/kg and 200mg/kg b.w.), Chloroform (ChF) and n-butanol fraction (BtF) (100mg/kg b.w.) of *Morus alba* decrease urine sugar level respectively. In diabetic control animal urine sugar remaining that +4 level but there was decrease of 2% to 3%, in urine sugar in case of treated diabetic rats. The change in body weight and urine sugar in all group of animals were given in table 5.1

Effect of morus alba on Serum glucose

The initial blood glucose levels of the diabetic rats selected for the study where in the range of 240-300mg/dL. In the untreated control (diabetic) rats the blood glucose level increase to 379 mg/dL on the 7th day the glucose levels on the 14th and 21th day of the animals which survived where 410 mg/dL respectively. In the morus alba treated rats the blood glucose level suddenly decreased (P<0.001, P<0.01) thus the *morus alba* treatment restore the serum glucose levels

almost nearer to normal value and comparable to that of positive control ($P < 0.001$). The changes in Serum glucose estimation in all groups of animal were given in table no. **5.2**

Serum lipid profile and lipoprotein profile

Effect of extract/fraction of MA on the control and experimental animals. STZ diabetic rats group were found to have significantly increased HDL, LDL, VLDL, TG, TC, levels as compared to control group ($P < 0.001$, $P < 0.01$) HDL cholesterol was also reduced significantly in diabetic rats after treatment of ethanolic extract/fraction of RS. Positive control was significantly preventing the increasing the serum TC, TG, HDL, LDL, VLDL, as compared to diabetic group. Diabetic treated group was significantly increased in HDL cholesterol level as compared to diabetic group. ($P < 0.001$, $P < 0.01$). Thus the MA treatment restores all these changes near to normal value. The change in serum lipid and lipoprotein profile were tabulated in table no **5.3**.

5.1 Effect of administration of feeding the Ethanolic extract/fractions of *MORUS ALBA* leaves on body weight and urine sugar analysis in normal and diabetic rats for 21 days.

S. No	GROUP	Body Weight (g)		Urine sugar	
		Before treatment	After treatment	Before treatment	After treatment
1.	Untreated control	194±1.88	220.5±1.839	Nil	Nil
2.	Diabetic control	202.66±2.33	168.5±2.513 ^{###}	+4	+4
3.	Diabetic+Glibenclamide (10mg/kg)	206.66±1.745	222.33±1.96 ^{***}	+4	+1
4.	Diabetic+EtOH(100mg/kg)	209±1.932	220.16±1.078 ^{***}	+4	+2
5.	Diabetic+EtOH(200mg/kg)	197±2.176	213.6±1.476 ^{***}	+4	+1
6.	Diabetic+ChF(100mg/kg)	205.83±2.182	220.83±2.182 ^{***}	+4	+1
7.	Diabetic+BtF(100mg/kg)	202.16±2.056	217.16±2.056 ^{***}	+4	+1

All values are expressed as mean ± S.E.M (n=6). ***P<0.001 as compared to diabetic control. ^{###}P<0.001 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison tests.

Table5.2: Effect of administration of feeding the Ethanolic extract/fractions of MA leaves on serum glucose estimation in normal and diabetic rats

S.No	GROUP	Serum glucose (mg/dL)			
		0 day	7 th day	14 th day	21 th day
1.	Untreated control	84.83±5.41	85.33±5.87	84.66±5.77	84.83±5.09
2.	Diabetic control	298.16±17.20	367.33±4.7 ^{##}	413.83±16.61 ^{###}	410±2.045 ^{###}
3.	Diabetic+Glibenclamide (10mg/kg)	280.33±2.44	205.33±1.145 ^{**}	165±1.29 ^{***}	114.83±1.302 ^{***}
4.	Diabetic+EtOH (100mg/kg)	277±7.69	223±4.195 ^{**}	164.66±1.406 ^{***}	116.5±1.232 ^{***}
5.	Diabetic+EtOH (200mg/kg)	282.66±4.49	223.33±1.94 ^{**}	164.16±1.406 ^{***}	115±0.966 ^{***}
6.	Diabetic+ChF (100mg/kg)	290.83±5.46	221.5±3.51 ^{**}	167±1.065 ^{***}	115.83±0.945 ^{***}
7.	Diabetic+BtF (100mg/kg)	285±5.33	219±2.63 ^{**}	166.83±1.77 ^{***}	115.33±0.881 ^{***}

All value are expressed as mean ± SEM (n=6). ***P<0.001, **P<0.01 as compared to diabetic control. ##P<0.01, ###P<0.001 as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison tests.

Table 5.3: Effect of ethanolic extract/fractions on serum lipid and lipoprotein profile in streptozotocin induced rats

NO	GROUP	TC(mg/dL)	TG(mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL(mg/dL)
1.	Untreated control	86.5±0.76	54.83±1.138	54.80±0.693	18.38±0.40	8.793±0.40
2.	Diabetic control	156.68±0.72 ^{###}	183.5±11.59 ^{###}	21.33±0.44 ^{###}	37.73±6.75 ^{###}	37.10±2.85 ^{##}
3.	Diabetic+Glibenclamide (10mg/kg)	69.33±2.81 ^{***}	116.5±5.21 ^{***}	32.63±2.30 ^{***}	16.27±1.39 ^{**}	23.96±0.19 ^{**}
4.	Diabetic + EtOH(100mg/kg)	126.88±0.25 ^{**}	64.16±1.53 ^{***}	35.45±3.66 ^{***}	52.38±1.51 ^{**}	13.82±0.22 ^{**}
5.	Diabetic + EtOH (200mg/kg)	126.95±0.22 ^{**}	69.66±4.63 ^{***}	38.4±2.725 ^{***}	53.07±0.79 ^{**}	13.98±0.78 ^{**}
6.	Diabetic + ChF(100mg/kg)	124.45±1.07 ^{**}	68.33±1.12 ^{***}	39.48±1.94 ^{***}	59.51±3.05 ^{**}	16.33±0.91 ^{**}
7.	Diabetic + BtF(100mg/kg)	124.58±1.12 ^{**}	64.33±0.80 ^{***}	47.06±3.44 ^{***}	53.06±4.03 ^{**}	14.49±0.32 ^{**}

All value are expressed as mean ± SEM (n=6). ***P<0.001, **P<0.01, as compared to diabetic control ^{###}P<0.001, ^{##}P< 0.01, as compared to untreated control. One-way ANOVA followed by Bonferroni multiple comparison tests.

Discussion

Traditional Chinese medicines have been used by over one-fifth of the world population since ancient times and is a potential source of pharmaceutical remedies. Now a major challenge to curing liver and kidney injuries are to find novel chemical entities with less toxicity and greater effectiveness than those used in current chemotherapy. Diabetes is associated with profound alterations in plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease.

Insulin and sulphonyl urea drugs (glibenclamide) causes hypoglycemia when taken in excessive doses and overt hypoglycemia is the most worrisome effect of these drug .However Biguanides donot cause hypoglycemia even when taken in excessive doses. MA also did not cause hypoglycemic effect. One of the Biguanide, Metformin does not stimulate insulin secretion and act by reducing hepatic glucose production through inhibition of gluconeogenesis and to a lesser extent by enhancing insulin sensitivity in the tissues. In the present study MA extract/fractions did not influence serum levels of insulin in diabetic as well as normo-glycemic rats. Further the extract/fractions reduced serum lipid.

Other parameters of diabetes such as body weight and urine sugar were also affected on treatment with MA. Treatment with MA inhibits the reduction in body weight and urine sugar by diabetes as the treatment altered the body weight and urine sugar of diabetic animals towards normalcy.

Hyperlipidemia is a recognized complication of DM characterized by elevated levels of cholesterol, triglycerides and changes in

lipoprotein composition. The results of our present study clearly show that *morus alba* leaves has a lipid lowering effect on serum TG, TC, VLDL, LDL, There is a substantial evidence that lowering the total cholesterol (TC), particularly LDL level will lead to a reduction in the incidence of coronary heart disease (CHD), which is still a leading cause of death in diabetic patients.

Since lipid abnormalities accompanying with premature atherosclerosis is the major cause of cardiovascular disease (CVS) in diabetic patient therefore ideal treatment for diabetes in addition to glycemic control, should have a favorable effect on lipid profile.

The dose of ethanolic extract EtOH (100,200mg/kg), Chf, BtF (100mg/kg) not only lowered the TC, TG, VLDL and LDL levels but also enhanced the cardio protective lipid HDL in normal and diabetic rats after 21 days of treatment. In present study the extract/fractions not only decrease the TC level but also enhance the HDL cholesterol significantly. High level of triglycerides and more importantly LDL cholesterol is major cause of coronary risk factors. Administration of leaves of extract/fractions to diabetic rats for 21 days lowered TC and LDL cholesterol level respectively. This is the important finding of this study as diabetes is associated with coronary complications.

Conclusion drawn from the present study on *Morus Alba* (MA) leaves demonstrated that the ethanolic extract/fractions of RS ameliorate the decreased blood glucose, improve lipid metabolism, maintain bodyweight and urine sugar in STZ induced diabetic rats.

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REFERENCES:

Melanin concentrating hormone receptor antagonism and antiobesity effects of ethanolic extracts from morus alba leaves in diet induce obese mice, **Oh k.S, Ryu S.Y, Lee.S, Seo.H.w, Oh B.k, kim y.s, Lee B.H**, journal of ethanopharmacology, volume 122, issue 2, 18 march 2009, 216-220

Morus Alba, patent analgesic and anaesthetic effects, Indian journal of pharmacology, 1980, 17, 237-241

Medicinal aromatic plant abstract, 1991, no 3615

Hypolipidemic and antioxidant effects of morus alba l root bark fractions supplementation in cholesterol-fed rats, Hesham A.El-Beshbishy, Abdel Nasser, B.singab, Jari sinkkonan and Kaleni pihlaja, journal of life sciences, volume 78, issue 23, 1 may 2006, 2724-2733

Mulberry leaves and their major flavonol quercetin 3-(6-malonylglycoside) attenuate atherosclerosis lesion in LDL receptor deficient mice, Byambaa Enkhmaa, Kuninori shiwaku, Takuya katsube, Keiko kitajima, Erdembileg annurad, Masayuki Yamasaki and yosuke Yamane, the journal of nutrition 135, 1 april 2005, 729-734

Regulation of obesity and lipid disorders by herbal extracts from morus alba, melissa officinalis and artemisia, capillaries in high fat diet induce mice, Jinmi Lee, Kyungsil chae, Juran ha, Byung-young park, Hee suk lee, sunhyo jeong, Min-young kim and Michung yoan, journal of ethanopharmacology, volume 116, issue 3, 28 march 2008, 576.

Mulberry extracts inhibits the development of atherosclerosis in cholesterol-fed rabbits, chang che chen, Li-kaung lin, Jeng dong hsu, Hui pei huang, Mon-yuan yang and chan jong wong, journal of food chemistry, volume 91, issue 4, aug 2005, 601-607

Hypoglycemic effect of Egyptian morus alba root bark extract; effect on diabetes and lipid peroxidation of streptozocin-induced diabetic rats, Abdel Nasser, B.singab, Hesham A.El beshbishy, Makiko Yonekawa, Tazo Namura and Toshio fukai, journal of ethanopharmacology, volume 100, issue 3, 14 sep 2005, 333-338.

Antihyperglycemic, antioxidant and antiglycan activities of mulberry leaves extract in streptozocin induced chronic diabetic rats, Jarinyaporn Naowaboot, Patchareewan pannangpetch, veerapol kukong viriyapan, Bunkerd kongying yoes and Upa kukong viriyapan, journal of plants food for human nutrition, volume 64, no 2, june 2009.

In vivo hypoglycaemic effects of phenolics from the root of morus alba, Mizhang, Man chen hangqing zhang, Shisun, Bing

xia, Feihua wu, journal of fitoterpia, volume 80, issue 8, December 2009, 475-477

Ginsenoside Re of Panax ginseng possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats **Leung W N Albert, Cheng H K Christopher, Kevin K M Yue**, Euro J Pharmacol. 2006 ;(550):173-179.

Effect of Bauhinia forficata aqueous extract on the maternal-fetal outcome and oxidative stress biomarkers of streptozotocin induced diabetic rats **Volpato GT, Damasceno DC, Rudge MVC, Padovani CR, Calderon IMP..** J Ethnopharmacol .2008; (116):131-7.

Anaesthetics used in laboratory animals, Fundamentals of experimental pharmacology, **Ghosh M N**, 3rd edition, Kolkata. S.K.Ghosh&other; 2005.15-19.

Organization for economic co-operation and development guidelines for testing chemicals. Acute oral toxicity. Paris: OECD; 1992. P. 98-101

Antidiabetic activity of alcoholic stem extract of Coscinium fenestratum in streptozotocin-nicotinamide induced type 2 diabetic rats, **Shirwaika A, Rajendram K, Punitha ISR** J Ethnopharmacol. 1995 ;(127): 26-33.