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ANTI DIABETIC, HYPOLIPIDEMIC AND ANTI OXIDANT ACTIVITIES OF THE PLANT EXTRACTS OF *Morus alba* Linn.

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ABSTRACT

The present study deals with the preliminary phyto physicochemical evaluation of a well known folklore remedy for diabetic and hyperlipidemic activityi.e *Morus alba Linn*. In preliminary phyto chemical investigation was found that Aminoacids, Tannins, Phytosterols, Flavanoids, Saponins, Triterpenoids and Cardiacglycosides present in the extracts. The plant leaves were collected for this work at Govt. Agriculture College Othakadai, Madurai, Tamilnadu. The leaves were identified and confirmed by Dr.D.Stephen PhD, Botanist, American College of Arts and Science, and then subjected for morphological, microscopical and physicochemical analysis. The oral administration of *Morus alba*.Linn was significantly reduces the Total cholesterol, LDL, Triglycerides, HDL level at the dose of 100mg/kg, 200mg/kg respectively for 30 days. Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. The scavenging capacity of the extract was found to be 62.52%. Extract of Morus alba inhibited FeSo4 induced lipid peroxidation in a dose dependant manner. Lipid per oxidation of the extract was found to be 55.53%. Oral administration of alcoholic leaf extract at 10mg, 50mg, 100mg/kg body weight. Significantly lowered the blood glucose level as compared to the untreated diabetic rats.

Keywords: *Morus alba* Linn, Anti diabetic, Hypo lipidemic , Anti oxidant activities.

INTRODUCTION

A small genus of trees or shrubs distributed in the temperature and sub-tropical regions of the northern hemisphere. Four or five species occur in India.Commonly know as mulberries, a few of the morus speies are valued for their foliage, which constitute the chief feed for mulberry silk worms. Leaves are very variable, ovate or broadly ovate, serrate or crenate-serrate often deeply lobed. Flowers are inconspicuos, greenish, male spikes-lax, flowered, broadly cylindrical or ovoid. Female spikes-ovod, pendunculate.

Fruits are syncarp cosists of many drips enclosed in freshly perianth white to pikish colour. A monoecious , occasionally dioecious shrub or moderate sized tree with a fairly cylindrical straight bole up to 3m height and 1.8m in girth, mulberry is grown extensively for leaves used for rearing silk worms [1,2,3,4,5].

MATERIAL AND METHODS

The plant leaves were collected for this work at Govt.Agriculture College Othakadai, Madurai, Tamilnadu. The leaves were identified and confirmed by Dr.D.Stephen PhD, Botanist, American College of Arts and Science. The voucher specimen was kept at Dept. of Pharmacognosy, K.M.College of pharmacy.

Extraction

The fresh plant material was washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The dried powdered plant material (500gms) was extracted successively with hexane, chloroform, ethylacetate and alcohol by soxhlet for 72hours at a temperature not exceeding the boiling point of the solvent. Table1.

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Hyper Cholesterolemic Animal Model

Animals-Male wistar strain rats weighing about the body weight 180-200gm. Feeding the rats diet containing by weight, 18% casein, 1% cholesterol powder and 0.5% cholic acid induced hyper cholesterimia. The animals were divided into 4 groups. Group I – Normal (cholesterol and cholic acid free), Group II – Only the cholesterol diet, Group III – 100 mg/ kg of extract with cholesterol diet, Group IV – 200 mg/kg of extract with cholesterol diet . *Morus alba*.Linn leaf extract was dissolved in CMC and given orally to rats at a dose of 100mg/kg and 200mg/kg body weight/ day for 30 days using a stomach tube. Six hours after the last dose the rats were decapitated their blood was collected and serum for the measurement of cholesterol level triglycerides LDL, HDL, VLDL [6,7,8]. Table 2

Anti Oxidant Activity
DPPH radical scavenging activity

DPPH scavenging activity was measured by Spectophotometric method

To a methanolic solution of DPPH (100µM, 2.95 ml), 0.05 ml of test compounds dissolved in methanol was added at different concentration (500-8000 µg/ml). Equal amount of methanol was added to the control. Absorbance was recorded at 517 nm at regular intervals of 30 sec for 5 min [9,10,11,12,13,14,15].

The degree of lipid preoxidation inhibition was assayed by estimating the Thiobarbituric acid-reactive substances (TBARS).

The degree of lipid preoxidation was assayed by estimating the tiobarbituric acid-reactive substances (TBARS) by using the standardmethod with minor modifications. Briefly, different concentration of extracts (200-1000µg/ml) was added to the liver homogenate. Lipid peroxidation was initiated by adding 100 µl of 15 mM FeSO4 solution to 3 ml of liver homogenate (final concentration was 0.5mM). After 30min, 100µl of the reaction mixture was taken in a tube containing 1.5ml of 10% TCA. After 10 min, tubes were centrifuged and supernatant was separated and mixed with 1.5ml of 0.67% TBA in 50% acetuc acud. The mixture was heated in a hot water bath at 85 C for 30 min and in a boiling water beth to complete the reaction. The intensity of pink coloured complex formed was measured at 535 nm in a spectrophotometer (Pharmacia Biotech, India). The valued of TBARS were calculated from a standard curve (Absorption against concentration of tetraethoxypropane) and expresses as moles/ mg of protein. Table 3. The percentage inhibition of lipid per oxidation was calculated by comparing the results of the test with those of controls not treated with extracts, as per the following formula:

$$\text{Inhibition}(\%) = \frac{(\text{Control-test}) \times 100}{\text{Control}}$$

Antidiabetic Activity

Normal healthy rats weighing about 100-125 gms were used for this study. The animals were maintained with adequate quantity of laboratory feed and water. The animals were divided into 4 groups. Group I – Control, Group II drug 10 mg/ kg, Group III drug 50 mg/ kg , Group IV 100 mg/kg .Diabetes was induced by the intravenous administration of Alloxan 120 mg/ kg for 3 days. Forty eight hours later the blood 1ml was collected and the serum was separated by centrifugation at 3500 rpm for 10 min and immediately used for biochemical assays. The animals that consisted of a daily administarion of pretreatment with 10 mg/ kg,50 mg/ kg, 100 mg/ kg alcoholic extract of *Morus alba*.Linn. In another set of experiments non diabetic normal rats were administered daily CMC as vehicle for 30 days. One hour after the last administration of blood was collected for biochemical measurements [16,17,18]. Table 4.

RESULTS
Hyper Cholesterolemic Animal Model

The serum total cholesterol, LDL, triglycerides, HDL, was significantly increased in the experimentally induce hypercholesterolemic rats. The oral administration of *Morus alba*.Linn was significantly reduces the Total cholesterol, LDL, Triglycerides, HDL level at the dose of 100mg/kg, 200mg/kg respectively for 30 days.

Anti oxidant Activity of *Morus alba* Linn

Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. The scavenging capacity of the extract was found to be 62.52%. Extract of Morus alba inhibited FeSo4 induced lipid peroxidation in a dose dependant manner. Lipid per oxidation of the extract was found to be 55.53%.

Antidiabetic activity of *Morus alba* Linn

The blood glucose levels were significantly elevated in diabetic rats by the induction of Alloxan compared to normal rats. Oral administration of alcoholic leaf extract at 10mg, 50mg, 100mg/kg body weight. Significantly lowered the blood glucose level as compared to the untreated diabetic rats.

Table 1. Yield of Extracts

S.No	Solvent	Yield of extract
1.	Hexane	12gm
2.	Chloroform	8.3gm
3.	Ethyl Acetate	7.2gm
4.	Ethanol(60%)	62.7gm

Table 2. Hypolipidemic Activity of *Morus alba* Linn

Treatment	Total cholesterol	Triglycerides	HDL	LDL
Group I (Normal)	50.6 ± 2.1	50.6 ±12.1	43.7 ±1.5	6.9 ±1.1
Group II (cholesterol control)	105.75 ± 2.135 *	81.025 ± 0.56 *	45.975 ± 0.63*	91.2 ± 2.45 *
Group III 100mg/kg extract	86.8 ± 4.96 *	60.98 ± 7.65 *	42.8 ± 1.07 *	28.8 ± 3.08 *
Group IV 200mg/kg extract	67.6 ± 1.03 *	34.66 ± 1.24 *	40.06 ± 0.413 *	14.42 ± 0.897 *

Table 3. Free radical scavenging activity of *Morus alba* Linn

Samples (100µg/ml)	Free radical scavenging activity by DPPH reduction (%)	Lipid peroxidation inhibition (%)
Morus alba.Linn	62.52 ± 1.32	55.53 ± 2.24

DISCUSSION

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule, due to it's odd electron, the methanolic solution of DPPH shows a strong sbsorption band at 517 nm. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses colour stoichometrically with the number of electrons taken up. Such reactivity has been widely used to test the ability of compounds/plant extacts to act as free radical scavengers. Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517 nm. The scavenging capacity of the extract was found to be 62.52%.

Initiation of lipid preoxidation by ferrous sulphate takes place either through ferryl perferryl complex or through OH radical by Fenton reaction. Ferryl- perferyl complex can also initiate lipid peroxidation in a similar manner as OH although it is less reactive than OH, in iron inducedlipid peroxidation, role of OH is not significant because little effect of tris and mannitol has been reported on this system. Extract of *Morus alba* inhibited FeSo4 induced lipid peroxidation in a dose dependant manner. Lipid per oxidation of the extract was found to be 55.53%.

Unregulated cholesterol levels lead to serious pathological conditions. It is widely understood that cholesterols, especially LDL cholesterol and its oxidized derivatives play an important role in the pathogenesis of atherosclerotic conditions. We investigate the effects on hypercholesterolemic of *Morus alba*.Linn. While the rat is fed with the hypercholesterolemic diet showed high serum concentrations of cholesterol as compared to rats given the normal diet, oral administration of *Morus alba* extract reduced the high levels of cholesterol, LDl, HDL, and Triglycerides.

Alloxan induces the diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia. *Morus alba* Linn leaf extract to diabetes rats reversed their blood glucose levels. The possible mechanism by which the *Morus alba* Linn leaf extract brings about its hypoglycemic action may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from βcells of islets of langerhans or its release from the bound form

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