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Research Article

Anti-oxidant activity of the aqueous extract of the *Morinda citrifolia* leaves in triton WR-1339 induced hyperlipidemic rats.

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ABSTRACT

Different epidemiological studies have shown that the oxidative stress is one of the major contributor of the development of hyperlipidemia and atherosclerosis. This study was aimed to evaluate the antioxidant activity of aqueous extract from *Morinda citrifolia* leaves against hyperlipidemic rats. *Morinda citrifolia* leaf extract has not shown any side effects during acute toxicity studies Hyperlipidemia was developed by intraperitoneal injection of Triton WR – 1339 400mg/kg.The animals were divided into Normal group, Triton treated group (T), Triton plus Atorvastatin, Triton plus herb extract 150 mg/kg, Triton plus herb extract 300 mg/kg, treated groups. Oral administration of *Morinda citrifolia* leaf extract (150 mg/kg and 300 mg/kg) in both groups At 24 hrs after treatment with TRITON WR 1339 caused a significant decrease in Malondialdehyde levels and significant increase in Superoxide dismutase (SOD), Reduced glutathione, Catalase levels as like in atorvastatin treated groups. Significant reduction in above stated anti-oxidant parameters indicating that this herb may contain products that lowers the oxidative stress and might be beneficial in treatment of hyperlipidemia.

Keywords: *Morinda citrifolia,* Triton WR–1339, Superoxide dismutase, Malondialdehyde, Catalase, Reduced glutathione.

INTRODUCTION

Oxidative stress is an imbalance between oxidants and anti-oxidants in favor of the former has attracted intense research interest recently as the cause of cardiovascular events as well as variety of vascular diseases [1]. Epidemiological studies have clearly shown that the diet rich in plant foods protects human against degenerative diseases such as cardiovascular diseases [2]. Hyperlipidemic an elevation of lipids in the blood steam and other lipids include fats, fatty acids, cholesterol, cholesterol esters, phospholipids and triglycerides, it is also denoted as a major cause of atherosclerosis and coronary heart diseases [3]. A number of epidemiological studies conducted during recent years have clearly demonstrated a link between stress and development of many diseases [4]. Various medicinal properties have been attributed to natural herbs and constitute the main source of a pharmaceutical and health care products [5].

Morinda citrifolia (Noni) is one of the plant most widely utilized by the Polynesians.which is having components like vitamin C, Terpenoids, alkaloids, anthraquinones,amino acids, flavones glycosides, Linoleic acids, rutin and new iridoid glycoside were identified from noni leaves [6].

The present study was aimed to measure the tissue anti-oxidant parameters like SOD, Catalase, Glutathoine, Lipid peroxidation (Malondialdehyde) in livers of the Triton WR 1339 induced hyperlipidemic in rats.

MATERIALS AND METHODS

Animals

Male Wistar albino rats were used for the study. The animals were housed in groups of six and maintained under standard conditions (27±2ºC, relative humidity 44 - 56% and light and dark cycles of 10 and 14 hours respectively) and fed with standard rat diet and purified drinking water ad libitum for 1 week before and during the experiments.

All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupathi and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA Reg No. 1016/a/06/CPCSEA/004/2009), Ministry of Social Justice and Empowerment, Government of India.

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All the experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals [7].

Plant material

The leaves of the plant *Morinda citrifolia* were collected from the S.V.Governement poly technique college campus, Tirupati and auhentified by Dr.N.Yasodamma, Prof of Botany, S.V University, Tirupati.

Preparation of the aqueous extract

The aqueous extract from the leave of *M.citrifolia* was prepared by the same method used in folk medicine with some imporovements.These were then powdered coarsely. The powder was decocted in purified boiling water in the ratio of 1:16. The decoction was then kept for an overnight and filtered. To it 1-2 drops of chloroform was added and stored at 8º C in screwed glass vials. The weight/ml was estimated randomly and used for oral administration to animals.

Chemicals:

Epinephrine, DTNB (sigma), Thiobarbituric acid (TBA) and Trichloroacetic acid, Hydrogen peroxide (SD fine chemicals Ltd). Sodium dihydrogen phosphate, potassium dihydrogen phosphate, Tris buffer and all other reagents used were of analytical grade.

Triton model of hyperlipidemia

Triton WR 1339 (Tyloxapol, Sigma – Aldrich, USA) was dissolved in normal saline (pH 7.4) and administred intraperitoneally to the rats (400mg/kg B.W) in order to develop oxidative stress in animals.

Experimental design:

The experiment was conducted for 28 days. Male Wister rats (n= 30) are divided in to 5 groups as per following. Normal group which Received normal diet. Triton WR 1339 400mg/kg i.p, Extract 150 mg/kg oral +. Triton WR 1339 400mg/kg, i.p, Extract 300 mg/kg oral + Triton WR 1339 400mg/kg i.p, 5mg/kg Atorvastatin + Triton WR 1339 400mg/kg i.p.

Instruments

Absorbances were recorded in analytical UV-Visible spectrophotometer.

Acute Toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines [8].

Preliminary Phytochemical screening

The extract was then subjected for the phytochemical screening [9].

Preparation of homogenate

The animals were sacrificed under mild chloroform anesthesia and their liver tissues were isolated and washed with cold saline and 10% homogenate of the liver tissues were prepared with 0.1M Tris-HCL buffer which is at the pH 7.4. Then the homogenate was used for assaying the enzyme activities.

BIO-CHEMICAL PARAMETERS

Estimation of Lipid peroxidation (Malondialdehyde (MDA)

Malondialdehyde formation was estimated by the method of Slater and Sawyer, absorbance was measured at 535 nm[10].

Estimation of Superoxide dismutase (SOD)

SOD was estimated by the method of Misra and Fridovich [11].

Estimation of Catalase [CAT]

Catalase was estimated by Aebi method [12].

Reduced glutathione (GSH)

Reduced glutathione was determined by the method of Moran et al [13].

Statistical analysis

All the data was expressed as mean ± SEM. Statistical significance between more than two groups was tested using one way ANOVA followed by the Bonferroni test using computer based fitting program (Prism, Graph pad.). Statistical significance was determined at $P < 0.05$.

RESULTS:

Preliminary phytochemical screening:

Preliminary phytochemical screening of the aqueous extract of *M.citrifolia* leaves was shown that the presence of alkaloids, carbohydrates, Glycosides, steroids, flavonoids, tannins and resins.

After 24 hrs of the induction of hyperlipidemia with Triton WR 1339 at the dose of 400 mg/kg i.p., caused a marked decrease in SOD, Catalase, GSH levels ($p<0.01$& $p<0.05$, $p<0.05$) was noted. The MDA concentrations was increased significantly ($p<0.001$) than the normal levels.

Table 1: Effect of the aqueous extract of the *Morinda citrifolia* on rat tissue (liver) anti-oxidant parameters

S.No.	Groups	SOD	Catalase	GSH	MDA
I	Normal	24.7 ± 2.11	29.6 ± 3.56	23.05±3.873	6.692± 2.13
II	Triton	7.49 ± 2.09**	4.94 ± 1.50*	6.99 ± 1.54*	22.19±1.32 ***
III	TRI + ATR	23.9 ± 2.90**	40.3±7.35***	27.38 ±1.54**	9.003 ± 1.53**
IV	TRI +150 mg + E	24.5 ± 3.82**	27.3 ±3.87*	28.88 ± 2.11*	12.61 ± 2.7*
V	TRI+300mg/kg +E	23.3 ± 0.62**	29.5 ±5.49*	23.21±3.089**	8.6 ± 2.18**

All values shown are mean ± SEM and n = 6

- *Comparisons were between normal Vs Triton treated groups, Remaining All groups were compared to Triton treated group.*
- ** p < 0.05, ** p<0.01,*** p < 0.001. (TRI = Triton, E=Extract)*

Units;

1. SOD activity was expressed as units/mg protein change in optical density/min. 50% inhibition of epinephrine to adrenochrome transition by enzyme is taken the enzyme unit.
2. Catalase - µ moles of H2O2 decomposed min/mg protein
3. GSH - µg of GSH consumed min/mg protein
4. MDA – m moles of MDA formed/min mg protein

The serum anti-oxidant levels of rats treated with aqueous extract of *M.citrifolia* are shown in table 1.

Importantly the elevated MDA concentrations ($p<0.001$) produced by Triton administration after 24hrs were significantly suppressed ($p<0.05$ & $p<0.01$) by the test drug in both 150mg/kg and 300 mg/kg treated groups.

Aqueous extract of *M.citrifolia* caused a significant increase ($p<0.01$) in the SOD levels and a significant increase ($p<0.05$) in catalse levels and also produced a significant increase ($p<0.05$, $p<0.001$) in GSH levels in the Triton WR 1339 treated rats when compared to the animals treated with triton alone.

DISCUSSION

Hyperlipidemia, which is an important risk factor in the initiation and progression of atherosclerosis [14].oxidative stress, is one of the causative factor that links hypercholesterolemia [15]. Tyloxopol is an compound which produces hypercholesterolemia [16].Lipid peroxidation is a free radical chain reaction which is triggered by hydroxyl radical and leads to membrane break down and leading to produce more number of free radicals [17]. Treatment with test drug at the 150 mg/kg and 300 mg/kg reduce the level of lipid peroxides indicating the effective anti-oxidant property of the test drug.Inhibition of lipid peroxidation may be due to the reduced GSH levels also. Because it was stated that GSH levels also inhibits lipid peroxidation [18].Test drug extract produced higher levels of the superoxide dismutase and catalase in both doses when compared with the group treated with Triton only. This may be due to decrease in the conversion of super oxide anions to hydrogen peroxide (H_2O_2) which is caused due to the catalysis produced by the SOD, Which is an enzyme prevents the further generation of free radical [19].

Catalase is an heme protein which is an enzyme catalyses the reduction of H_2O_2 and protects the tissues from hydroxyl radicals [20]. Otherwise reduction of the above SOD an d Catalase enzymes activity inactivates hydrogen peroxide [21] And also results in the accumulation of superoxide and H_2O_2 [22].Aqueous extract of the *Morinda citrifolia* leaves produced a significant increase in reduced glutathione levels. This is considered to be functions as scavenger of free radical and also participates in the repair of biological damage which is produced by free radicals [23].Flavonoids which we have observed in the preliminary screening of the test drug extract also may be one of the reasons for its antioxidant activity, since it was stated that Flavonoids are well known for their anti-oxidant activity. [24].In conclusion, when we compared the antioxidant parameters in rat liver between the hyperlipidemic groups treated or not treated with *M.Citrifolia*.We have observed the reduction in Malondialdehyde levels and increase in SOD, Catalase, GSH levels. From the present study we can conclude that the aqueous extract of the *Morinda citrifolia* leaves may possess anti-oxidant activity against hyuperlipidemia and atherosclerosis.Further studies are needed to be purify the bio active compounds in the test extract.

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