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Anti-Stress Effect of *Morus alba* Extract on Biochemical Parameters and Histology of Liver in Stressed Mice Induced by Epinephrine

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Abstract: ***Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. In the present investigation, we examined the Morus alba extract effect on epinephrine induced strees. Twenty four albino mice were divided into three groups as non stressed group and stressed group and extract treated group .The stressed groups were exposed to 2 weeks and 4 weeks of chronic immobilization stress with epinephrine. At the end of the 2 and 4 weeks the mice were sacrificed and blood samples were collected through orbital sinus method. The blood samples of these groups were analyzed for selected biochemical parameters viz. Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), and Bilirubin which were found to be significantly increased in the stressed group when compared to the non stressed Control. The present data indicate that epinephrine induced stress causes the significant alterations in the SGOT, SGPT and Bilirubin levels affecting the normal metabolism in liver. These elevations were found to be significantly reversed in Morus alba extract treated group. Morus alba have a potent capacity to control anti-stress.***

Keywords: *Morus alba*, Liver function test, Epinephrine, Swiss albino mice

Abbreviation: SGPT- Serum Glutamic Pyruvate Transaminase, SGOT- Serum Glutamic Oxaloacetate Transaminase, DM- diabetes mellitus

1. Introduction

1.1 Stress is a condition of highly individualized response of an organism to external and internal challenges which one can control with difficulties or cannot control. It is one of the important factor acting upon a large human population in the entire country. It induces the strain upon both emotional and physical endurance which has been considered the basic factor in the etiology of a number of diseases eg: cardiovascular diseases, cancer, DM etc [1, 2]. In response to stressors, a series of behavioral, petrochemical, and immunological changes occur that ought to serve in an adaptive capacity [2, 3]. However, if those systems become overly taxed, the organism may become vulnerable to pathology. Chronic psychological stress is one of the major factors that contribute to several pathological disorders [4-6]. Immobilization/restraint stress is an easy and convenient method to induce both psychological, an escape reaction and physical stress, a muscle work which leads to restricted mobility and aggression [7, 8]. However, it is well known that intensive stress response results in the creation of reactive oxygen species (ROS) e.g. hydrogen peroxide, hydroxyl radical and superoxide anion radical that cause lipid peroxidation, especially in membranes and can play an important role in tissue injury. It has been suggested that chronic stress and high level of glucocorticoids, the adrenal steroids secreted during stress, affect diverse processes involving ROS and increase ROS by approximately 10% basally [9]. The membrane injury causes disruption of the tissue [10-12].

1.2 Norepinephrine, and the related molecule epinephrine (EPI), also known as adrenaline, are stress hormones that activate the body's adrenoceptors, which includes the receptors that beta blockers antagonize. There are five major types of adrenoceptors in the body: beta1, beta2, beta3, alpha1, and alpha [13-14] most beta blockers target the beta1 and beta2 receptors. Adrenoceptors are G protein-coupled receptors that initiate second messenger signaling processes within cells that bear these receptors. Adrenoceptors are found not only in the brain, but also in nearly all, if not all, organs of the body [14]. NE and EPI have direct access to these organs through localized release by the sympathetic nervous system (SNS), as well as through general release into the bloodstream, as part of the body's "fight or flight" response to psychological stressors.

1.3 The leaves of *Morus alba* Linn. (Family: Moraceae) are used as food for the silkworms, as vegetable and as cattle fodder in different parts of the World. Apart from its use as vegetable, the leaves of the plant are used for the treatment of a variety of disorders traditionally. The medicinal properties attributed to mulberry are extensive. Topically, it is applied for the treatment of wounds [15]. Internally, it is used to relieve insomnia, regulate bleeding during menstruation, treat digestive disturbances, to ease cough and asthmatic breathing, reduce fever and inflammation, as hypolipidemic, antiageing, antifilariasis, diuretic, and antiulcer agent.[16]

1.4 Objective of research- The aim of present work was to asses the anti- stress effect of *Morus alba* on epinephrine induced stress.

2. Materials and Methods

2.1 Chemical

Epinephrine, used to prepare stressed model was purchased from the Rohit laboratories (Hindustan) Pvt. Ltd. Surat, India. Commercially available kits for chemical analyses such as SGOT, SGPT and Bilirubin were used with crest coral clinical system, Goa, India. Analytical grade ethanol was purchased from Merck Company.

2.2 Experimental Model

Reared sexually matured 6-8 weeks old age group male and female Swiss Albino mice (Mus musculus) weighing 25-35gm b.w. in the animal house and used in the present study. The animals were housed at controlled environmental conditions 22±2ºC, relative humidity 50±10%, and 12h dark-light cycle. Animals were housed and allowed to free access to food and water. All experimental procedures were conducted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

2.3 Collection & Preparation of ethanolic extract

Aquas leaf extract of *M. alba is* administered after epinephrine exposure. Fresh leaf of *M. alba* was collected from local garden of Allahabad. The identity of the leaf was confirmed by a Botanist.

2.4 Experimental Protocol

Twenty four pathogen-free mice were randomly used during the present study. One group served as control and epinephrine was administered intramuscularly to other groups at the dose of 200nl/kg b.w. respectively. Epinephrine administered group was followed by aquas leaf extract of *M. alba* administration @ 50 mg/kg. b.w. for two and four weeks. Animals were sacrificed after two weeks and four weeks of treatment and blood samples were collected by orbital sinus puncture method followed by serum collection.

2.5 Clinical Analyses

Serum glutamate pyruvate transaminase and Serum glutamate pyruvate transaminase were performed using standard kit (Coral) based on the methods of Reitman and Frankel (1957). Serum total bilirubin was evaluated using again the standard kit (Coral) based on the method of Jendrassik and Grofs (1938).

2.6 Statistical Analysis

Results are presented as mean ± S.D and total variation present in a set of data was analysed through one-way analysis of variance (ANOVA). Difference among means has been analysed by applying Dunnet's t test at 95 % ($p<0.05$) confidence level. Calculations were performed with the GraphPad Prism Program (GraphPad Software, Inc., San Diego, USA).

3. Results and Discussion

The increase in levels of SGOT and SGPT in epinephrine treated Swiss albino mice is given figure. The levels of SGOT and SGPT increased after two weeks and four weeks in epinephrine treated mice as compared to control in **table no-1**. The percentage increase in SGOT and SGPT level in the two weeks was found to be 85.12%, 75.12%. After four weeks, the levels of SGOT and SGPT increased to 80.55%, 80.59% as compared to control. These results were found to be significantly increased ($P\leq0.05$). The levels of SGOT and SGPT in epinephrine treated Swiss albino mice which were dosed with aqueous extracts of *M.alba* (81.11%, 90.56%). The increased level of SGOT and SGPT enzymes in stress induced animals is due to the reason that stress increases hypatholamo-pitutaryoxis (HPA), and sympathetic system stimulation resulting in libration of catecholamine, gluco-corticosteroids, which inhibit the immune system at multiple sites like liver, kidney **(Goodman and Gilman, 2006)**, similarly SGOT and SGPT levels have also been reported to increase in wistar rat **(Rai *et al.*, 2003).** A similar result has been reported by **Semwal *et al.*, (2008)** in wistar rat, other hand treatment with an herbal dose of *M. alba* (50 mg/kg b.w) the SGOT and SGPT levels significantly decreased ($P\leq0.05$) as compared to epinephrine treated mice.

Table 3.1: SGOT and SGPT of control and experimental groups of Swiss albino mice

Parameter	Control	Treated two week	Treated Four week	*M.alba* Treated
SGOT (IU/ml)	24±5.96	30.00±5.177	38.30±5.083	32.15±3.677
SGPT (IU/ml)	21.45±5.810	51.00 ±5.553	53.00±4.050	37.16±4.912

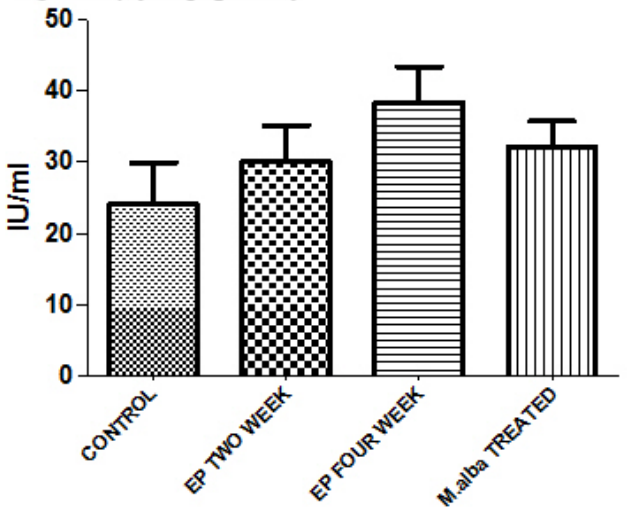


Figure 3.1: Effect of *M.alba* on epinephrine induced group showing SGOT levels (n= 6, Values are mean ± S.D)

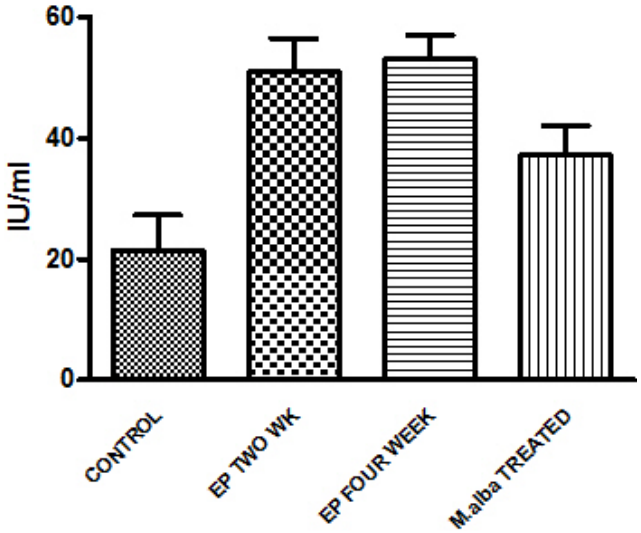


Figure 3.2: Effect of M.alba on epinephrine induced group showing SGPT levels (n= 6, Values are mean ± S.D).

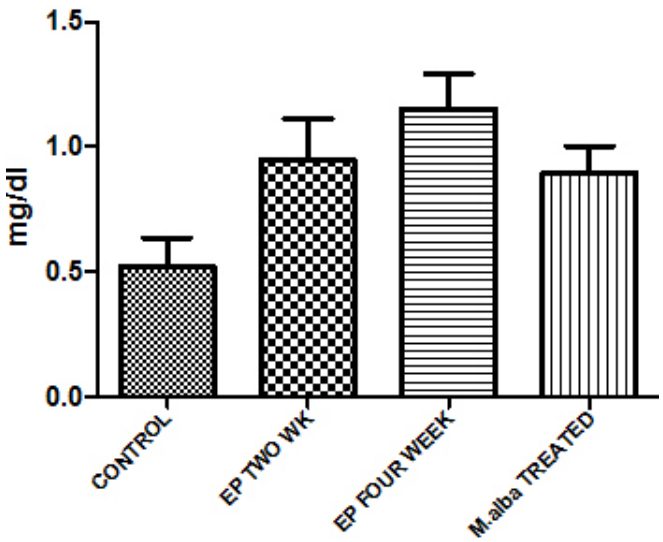


Figure 3.3: Effect of *M.alba* on epinephrine induced group showing Bilirubiin levels (n= 6, Values are mean ± S.D).

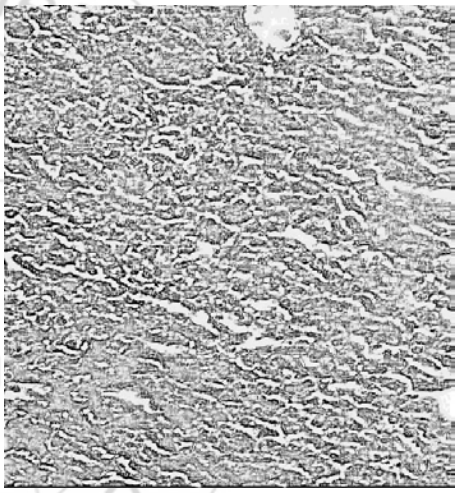
Bilirubin is the break down product of hemoglobin, protein byproduct that is normally converted into urea by the liver before being excreted by kidneys. In the present study Bilirubin level was increased (P≤0.05) in epinephrine induced Swiss albino mice. The level of bilirubin increased after two weeks and four weeks in epinephrine treated mice showed in **table no-2**. The bilirubin level also increased (P≤0.05) when mice treated with *M. alba* (0.89±0.10) plant extract as compared to 2-4 weeks. The percentage increase in bilirubin levels in the two weeks was found to be 11.86%, after four weeks, the levels of bilirubin increased to 18.75% as compared to control.

Thus it is evident from the present study that if any medicinal plant shows restoration on some parameters of LFT that means it is not universal that it restores all parameters in same manner. Thus it is evident that bilirubin was not restored to greater extent after administration of medicinal plants given. That means either production or maintenance of bilirubin in body is disturbed through degeneration of spleen and liver. Thus it is quite clear that these medicinal plants show restorative impact on biochemical parameters of liver while it is not effective on spleen as reported by **Reena, (2009),** due to which bilirubin level is not maintained by these medicinal plant extract.

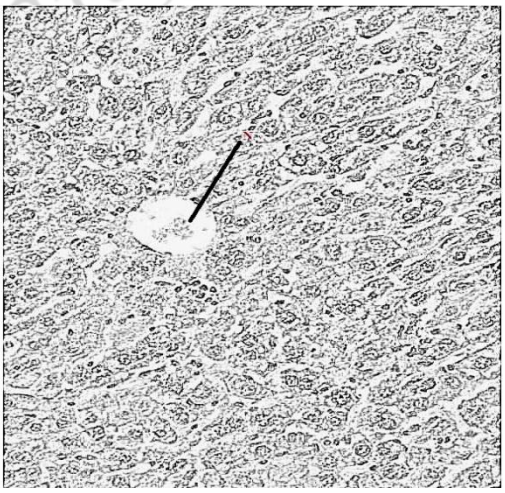
Table 3.2: Bilirubin level of control and experimental groups of Swiss albino mice

Parameter	Control	Treated two week	Treated Four week	*M.alba* Treated
Bilirubin (mg/dl)	0.52±0.1143	0.94±0.1661	1.15±0.1400	0.89±0.10

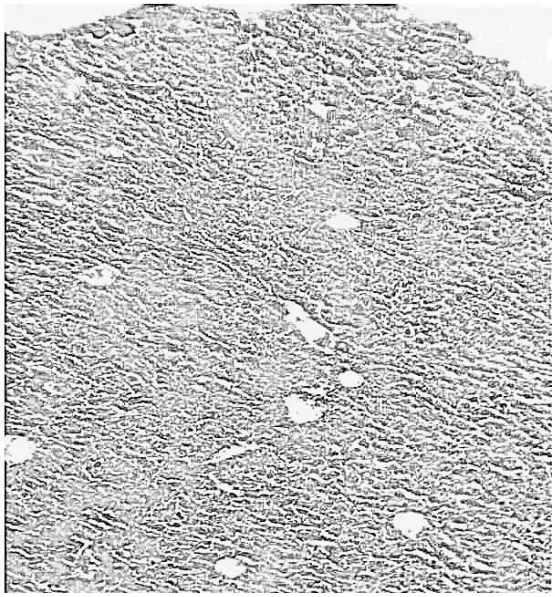
4. Histopathology Study



Restoration in hepatic cells. Cytoplasmic materials are clearly visible with least vacuolization



Section of liver showing normal architecture of CV



Hepatic cells with well distributed

5. Conflicts of interest

All authors have no conflict of interests.

6. Acknowledgement

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