

Evaluation of Antioxidant Activity of *Melia azedarach* On Depression Induced Rat Brain Tissue

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Abstract: In the present study the antidepressant activity of *Melia azedarach* on depression induced rat brain tissues was evaluated. The aqueous extract showed significant increase in the levels of protein, cholesterol and significant decrease in LPO level when administered orally for 30 days to depression induced rats at a dose of 50 and 150 mg/kg body weight. phytochemical studies also revealed the presence of (flavonoids) in aqueous extract which may be responsible for exerting antidepressant activity. Hence it can be used as a drug for depression.

Keywords: Antioxidant, *Melia azedarach*, aqueous extract.

1. Introduction

Depression is a common mental disorder that present with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite and poor concentration. Moreover, depression often comes with symptoms of anxiety (1). Depression is not simply unhappiness. Indeed prolonged depression should be viewed as an illness like any other and no other different than an infection, cancer or heart disease. Depression can be recurrent and chronic, and it can run in families as a result of genetic inheritance (2).

Over the past 50-60 years, enormous studies were published from indices addressing various aspects of this commonly prevalent disorder. In various works the several of depression originating from India were reviewed which conclude that the past life events of an individual were major cause to depression in India (3). *Melia azedarach* is a digenous plant possessing several medicinal properties. *Melia azedarach* belongs to the family meliaceae and is a tree found in India. Its popular name as Indian lilac. Different phytochemicals have been isolated from fruit include melianonin, melianol, melianone, meliandiol, vanillin (4).

The plant traditionally used for the treatment of leprosy, inflammations and cardiac disorders. Its fruits extracts posses ovicidal (5) and larvicidal activity (6).

The leaf extracts also posses antiviral (7) and infertility activity (8). Since no reports are available on antidepressant activity of *Melia azedarach* the present study is focused to evaluate the antioxidant activity of the leaf of *Melia azedarach*. Plant based medicine play an important role in health care programmes worldwide with minimal side effects. World health organization (WHO) also approves the use of plant drug for different disease; including depression *Melia azedarach* .L (family meliaceae) is a deciduous tree that is nature to north western India. The tree members of family *Meliaceae* are good source of folk medications. This fact drew the attention of many scientists around the world to study the potential contribution of those plants to their efforts in finding a

suitable, effective and disease curing and environment friendly product to control or prevent the disease (8). The plant is traditionally used for the treatment of leprosy, inflammations, and cardiac disorders. Its fruit extracts possess ovicidal and larvicidal activity.

2. Materials and Methods

2.1 Procurement of Animals

Young male Sprague Dawley rats strain (50 ±150g) procured from PSG Laboratory Institute of Medical Sciences and Research, Coimbatore, Tamil Nadu, India. The study protocol was approved by Institutional Animal Ethics Committee (IAEC) constituted for the purpose of control and supervision of experiments on animals (CPCSEA No: 158/1999/CPCSEA). The animals were acclimatized and maintained under standard laboratory conditions with controlled temperature and humidity. Food pellets and water were provided *ad libitum*.

2.2 Preparation of Plant Extract

The leaves of *Melia azedarach*.L were shade dried and powdered in a mixer grinder. The dried plant powder was subjected to successive solvent extraction in different solvents. It was then evaporated and vacuum dried as per standard products.

2.3 Phytochemical Analysis

Extract obtained using different solvent were subjected to the phytochemical screening of constituents by standard methods. Carbohydrates were identified by Molisch's test; proteins were identified by Biuret test. Steroids, flavanoids, alkaloids, tannins, glycosides, sponins and fixed oils were identified by Libermann, Burchard test, Draggandroff's test, Braymerr's test, Lavau's test, Haemolysis test, lead acetate test respectively.

2.4 Induction of Depression

Depression was induced in the young male Sprague Dawley rats by following stress procedures (CUMS) as per

2.5 Stress Procedures

Stress Condition/ Groups	Water Deprivation	Food Deprivation	Cage Tilting (45°C - 65°C)	Solid Cage	Spraying Air Freshener	Kept In Rotator Shaker	Cold Water Swimming (4°C)	Light Illumination	Diet	Treatment
Group – I	-	-	-	-	-	-	-	-	Basal	-
Group-II	12 Hours	24 Hours	12 Hours	24 Hours	12 Hours	10 Minutes	5-10 Minutes	2-4 Days	Served As Depression	7 Days
Group-III	12 Hours	24 Hours	12 Hours	24 Hours	12 Hours	10 Minutes	5-10 Minutes	2-4 Days	Standard Drug	7 Days
Group-IV	12 Hours	24 Hours	12 Hours	24 Hours	12 Hours	10 Minutes	5-10 Minutes	2-4 Days	Low Dose Plant Extract	7 Days
Group-V	12 Hours	24 Hours	12 Hours	24 Hours	12 Hours	10 Minutes	5-10 Minutes	2-4 Days	High Dose Plant Extract	7 Days

2.6 Collection of Serum and Tissues

After the end of the experimental treatment procedure (7 days), the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected by cardiac puncture and the serum was separated by centrifugation at 5000 rpm for 10 minutes. The brain was excised immediately and thoroughly washed in saline before use. The excised brains were treated with formalin for histopathological studies and as sterile dry tissues in 4°C for further analysis.

2.7 Estimation of Antioxidants

Catalase was assayed calorimetrically at 610 nm and expressed as μ moles of hydrogen peroxide (H_2O_2) consumed per minute per mg of protein as described by (9). Glutathione peroxidase (GP_x) activity was measured by the method of (10). Lipid peroxidation was done by method of (11).

2.8 Statistical Evaluation

All results are expressed as Mean \pm SD. Statistical evaluation was done using one way analysis of variance (ANNOVA) followed by student t-test.

3. Results

For all experimental group rats when depression was induced by physical method, a significant increased level of serum antioxidants level (CAT & GP_x) was found in depression group when compared with the control group of animals. Aqueous extract fractions contained more number of plant constituents and secondary metabolites such as alkaloids, steroids, glycosides, saponins, flavanoids and tannins (Table-1).

Table 1: Phytochemical screening of leaf extracts of *Melia azedarach* L.

Extracts & Tests	Aqueous	Ethanol	Benzene	Chloro-Form	Petroleum Ether	Hydro Ethanol	Acetone
Alkaloids	+	-	-	-	-	-	-
Flavonoids	+	Trace	+	+	+	+	-
Saponins	+	Trace	+	-	-	-	+
Carbohydrates	+	+	+	+	Trace	+	+
Proteins	+	+	+	-	-	-	-

Phenols	+	+	+	+	-	+	+
Glycosides	+	+	+	Trace	+	-	-
Tannins	+	+	+	-	-	-	-
Sterols	-	+	-	-	+	+	+
Thiols	+	-	-	+	+	+	-

“+” and “-” symbol indicates the presence and absence of compounds.

Effect of aqueous extract of *Melia azedarach* leaves on enzymic antioxidants Catalase, glutathione and Lipid peroxidase of the animal.

The experimental rats were divided into five group of six animals in each group.

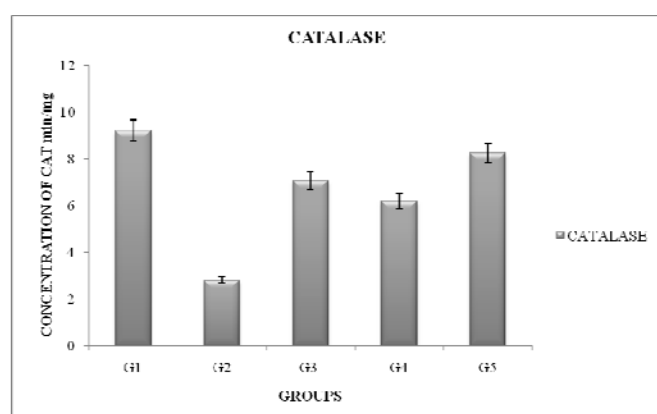
G1=Normal control group

G2=Depression group

G3=Standard drug treated group

G4=low dosage plant aqueous extract treated group

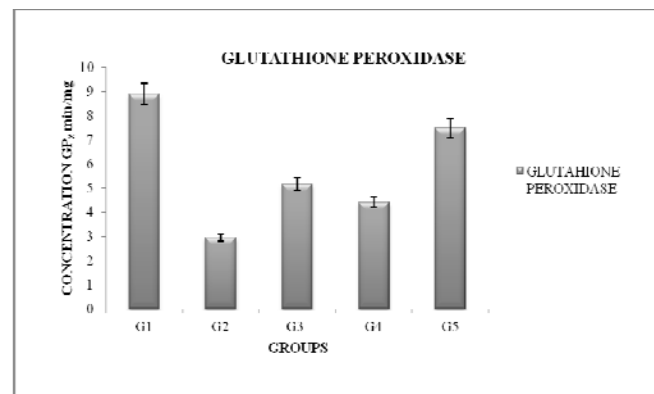
G5=High dosage plant aqueous extract group



Statistical significance

* - Significant ($P < 0.05$) when compare with respective control group.

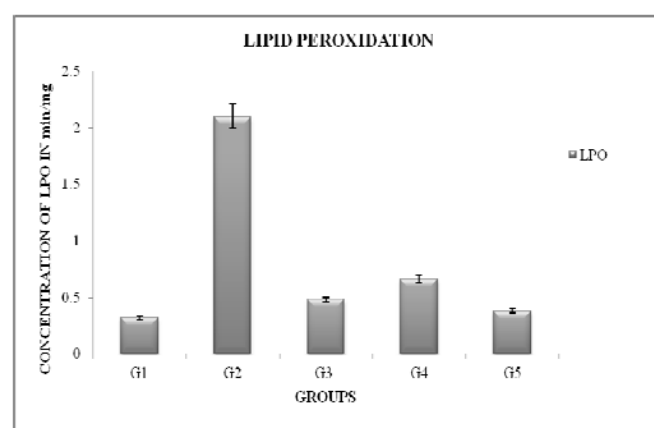
Figure 1: Effect of aqueous extract of *Melia azedarach* leaves on enzymic antioxidant Catalase level of the animal.



Statistical significance

* - Significant ($P < 0.05$)

Figure 2: Effect of aqueous extract of *Melia azedarach* leaves on enzymic antioxidant glutathione level of the animal



Statistical significance

* - Significant ($P < 0.05$)

Figure 3: Effect of aqueous extract of *Melia azedarach* leaves on enzymic antioxidant Lipid peroxidase level of the animal.

The above (Fig: 1&2) shows the Administration of aqueous extract of *Melia azedarach* leaf extracts at two different concentrations of (50 mg/kg and 150 mg/kg) body weight brought about the increase in enzyme activity of catalase and glutathione peroxidase.

The (Fig: 3) shows the significant decrease in the level of serum anti oxidant lipid peroxidation level was found in

depression group rats when compare to control group experimental animals.

3.1 Discussion

Antioxidants are a compound that protects cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxy radical, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has linked to cancer, ageing, atherosclerosis, ischemia injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's) (12). Certain antioxidant enzymes are produced within the body. The most commonly recognized of naturally occurring antioxidants are superoxide dismutase, catalase and glutathione. Superoxide dismutase changes the structure of oxidants and breaks them down hydrogen peroxide. Catalase in turn breaks down hydrogen peroxide into water and tiny oxygen particles or gases. Glutathione is a detoxifying agent, which binds with different toxins to change their form so that they are able to leave the body as water. Other antioxidant agents are found in foods, such as green leafy vegetables. Items high in vitamin A, vitamin E and beta carotene are believed to be most beneficial.

A previous report evaluated the anti-oxidant activity of the *Melia azedarach* was investigated in rats with ethanol-induced erythrocyte damage. Chronic administration of ethanol (20 % w/v, 2g / kg.p.o., daily for four weeks) increased the level of lipid peroxidation (LPO), decreased the activity of superoxide dismutase (SOD) and catalase and reduced the glutathione (GSH). The concurrent treatment of ethanol-administered rats with *Melia azedarach* (500 mg/kg.p.o) prevented the ethanol-induced changes and the effect was compared with combination of vitamin E and C. It can, therefore, be suggested the leaves of *Melia azedarach* possess an erythrocyte protective activity against drug-induced oxidative stress (13).

Oxidative stress in rat brain structures may play a role in the pathogenesis of depression (14). Unpredictable chronic mild stress (UCMS) has been widely used in animals to mimic depression-like disorders, and is regarded as being close to the unexpected stressors of everyday life in humans (15 and 16). Animal stress models are widely used in pre-clinical antidepressant evaluation (17). UCMS-induced oxidative damage has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases, ranging from psychiatric disorders such as depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, impotence, cognitive dysfunctions, and peptic ulceration to hypertension and ulcerative colitis (18). Reactive oxygen species (ROS) may play a role in some neuropsychiatric disorders such as major depression. There is some evidence that the activation of immune-inflammatory process, increased monoamine catabolism, and abnormalities in lipids may cause overproduction of ROS, lipid peroxidation, and reduced antioxidant enzyme activities, and these processes may be related to pathophysiology of depression (19). Oxidative stress in rat brain structures may play a role in the pathogenesis of depression (20). In a previous study

with animal model of repeated restraint stress, it was shown that this model induced an increase in TBARS levels in hippocampus (21).

In animal studies, antidepressants of different classes have been shown to replenish, to varying degrees, the glutathione depletion seen in the inescapable shock behavioural paradigm of depression (22). Venlafaxine was associated with the correction of several depression-specific oxidative markers in the rat cortex. A proteomic study using rats has found multiple protein modulations in the hippocampus after venlafaxine or fluoxetine administration. Antioxidant and anti-apoptotic proteins were among those identified (23). In another animal study, lamotrigine, aripiprazole and escitalopram were all shown to improve depression related GSH-Px, glutathione and Vitamin C depletion, and lipid peroxidation increase. An invitro study of rat cerebrocortex neuronal and astroglial cultures showed that moclobemide could attenuate cell death induced by anoxia and glutamate, a process involving oxidative stress pathways (24). The monoamine oxidase inhibitor phenelzine was able to attenuate the loss of differentiated rat PC12 cells exposed to chemical oxidative stress, and demonstrated antioxidant effects including the reduction of ROS formation and the scavenging of the pro-oxidant hydrogen peroxide (25). Most data demonstrating oxidative disturbances have examined indirect measures of oxidative status, such as peripheral and brain levels of antioxidants, oxidative enzymes and products. Similarly, studies involving blood assays of intrinsic antioxidants have collectively demonstrated significantly altered antioxidant activities. Deficiency of glutathione, the major intracellular antioxidant, in its reduced form (GSH), has been observed and suggested to be of pathophysiological significance in schizophrenia as early as 1934 (26), although differences did not reach statistical significance in that study. Reduced levels of the major antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) have also been found in patients with schizophrenia compared with controls (27). Others have reported unchanged levels for these three enzymes (28), or altered concentrations of individual enzymes (29). A strong negative correlation between blood GSH-Px and structural measures of brain atrophy was also reported by an early study (30).

In general, antidepressants that are used today are effective in treating depression regardless of whether they primarily affect serotonergic or noradrenergic neurotransmission or both. It has been suggested that there may be differences in efficacy of antidepressants among certain patients. Evidence has demonstrated significantly greater remission rates with the SNRI venlafaxine compared with SSRIs (31). Evidence demonstrates that 5-HT and NA are involved in both the pathogenesis and recovery from depression. Preliminary results showed that dual acting antidepressants may have an advantage over single acting agents in terms of treating patients to remission. Dual acting agents may also be preferable for the treatment of chronic painful conditions and somatic symptoms (32). Venlafaxine inhibits the reuptake of both 5-HT and NA, thus combining two therapeutic mechanisms in one agent. The anticholinergic and cardiotoxic side effects are not as

common compared with tricyclic antidepressants. Venlafaxine treatment stimulated expression of brain derived neurotrophic factor protein in the frontal cortex and inhibited long-term potentiation in the hippocampus (33). In another study, the relationship between the antidepressant effect of venlafaxine and its ability to protect animals against stress-induced oxidative lipid peroxidation and DNA damage was investigated. It was reported that long-term venlafaxine treatment using effective antidepressant doses can protect against stress-induced oxidative cellular and DNA damage. It was concluded that this action may occur by antagonizing oxidative stress and enhancing antioxidant defense mechanisms (34).

In the present study indicates the decrease in the level of LPO level was found in depression induced group rats when compared with the normal control group of experimental animals. And also reports the oral administration of aqueous extract of *Melia azedarach* leaf extracts at two different concentrations brought about the increase enzyme activity of CAT and GPx and decreased LPO near to normal values in dose dependent manner.

4. Conclusion

The present study revealed aqueous leaf extract of *Melia azedarach* exerts antioxidant activity level in depression induced rats. The cellular organization of brain depression and after treatment the brain tissues of depression induced group along with control groups were dissected. In the histopathological examination, it was found that the damaging effects found in depression before the treatment was cured by the administration of plant leaf extract at low and high dosage.

Aqueous leaf extract of *Melia azedarach* showed the presence of flavanoid content. The phytochemical constituents present in the plant may be responsible to exert such medicinal properties. Future study that involves the isolation and purification of the bioactive compound that may elucidate its mechanism of anti-depressant action and pharmacological effects.

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