

Protective Activity and Antioxidant Potential of *Lippia nodiflora* Extract in Paracetamol Induced Hepatotoxicity in Rats

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ABSTRACT

This study was designed to evaluate the hepatoprotective and antioxidant activity of methanol extract of *Lippia nodiflora* (MELN) in acute experimental liver injury induced by paracetamol (750mg/kg, b.w). MELN at the doses of 200 and 400mg/kg, p.o was administered for 7 days and biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), bilirubin and total proteins with enzymatic and non-enzymatic antioxidant level were recorded. MELN at both doses prevented the increase in liver weight when compared to hepatotoxin treated control. The higher dose (400 mg/kg) of *Lippia nodiflora* extract was found to be more effective than the lower dose (200 mg/kg) in paracetamol induced liver damage. MELN produced significant ($p < 0.001$) hepatoprotective effect by decreasing the activity of serum enzymes such as SGOT, SGPT, ALP, bilirubin and lipid peroxidation while it significantly ($p < 0.001$) increased the levels of total proteins, glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) in a dose dependent manner. The activity of MELN was compared with standard drug silymarin (25mg/kg), which is a well-known natural anti-hepatotoxic drug and the potency of MELN is more or less the same as that of silymarin. It is concluded that the methanol extract of whole plant possesses good hepatoprotective activity probably by its antioxidative potential on hepatocytes.

Keywords: Antioxidant estimation, Biochemical analysis, Hepatoprotective, Hepatotoxicity, *Lippia nodiflora*, Paracetamol, Rats

Lippia nodiflora Mich. (Verbenaceae) is a creeping perennial herb with small white flowers, a weed of wet ground and grassy pastures [1,2]. The plant is distributed throughout India, Ceylon, Baluchistan and Africa. In Ayurveda, the plant is aphrodisiac, useful in diseases of heart, good for ulcers and bronchitis. In Yunani medicine, the plant is used as diuretic, useful in fevers and cold [3]. The herb possess cooling, diuretic and stops knee joints pain [4,5]. The plant made in to a poultice used as maturant for boils [6,7]. Antimalarial activity was reported from the herb [8], leaves of the plant were reported to possess anti-inflammatory, analgesic and antipyretic activity in rodents [9] and Gastroprotective effect were reported [10]. Resin, stigmasterol, β -sitosterol and sugars were reported [11]. Essential oil constituents such as monoterpenes and sesquiterpenes were reported [12,12]. Presence of Lippiflorin A and

Lippiflorin B, flavonoids like nepetin, jaceosidin, hispidulin, flavone monosulphates and flavone disulphates were reported from aerial parts of the plant [13].

The liver plays an important role in drug elimination and detoxification, but in turn, it can be subjected to damage by xenobiotics. Liver damage may also be caused by alcohol consumption, malnutrition, infection, anemia and certain medications. Currently available drugs have little effect on the treatment of liver disorders, which creates a demand to develop new drugs [14].

Prime targets for free radical reactions are the unsaturated fatty acids, which have a role to play in membrane fluidity and receptor alignment and potentially in cellular lyses. Free radical damage to sulphur-containing enzymes and other proteins culminates in inactivation, cross-linking and denaturation. Damage to

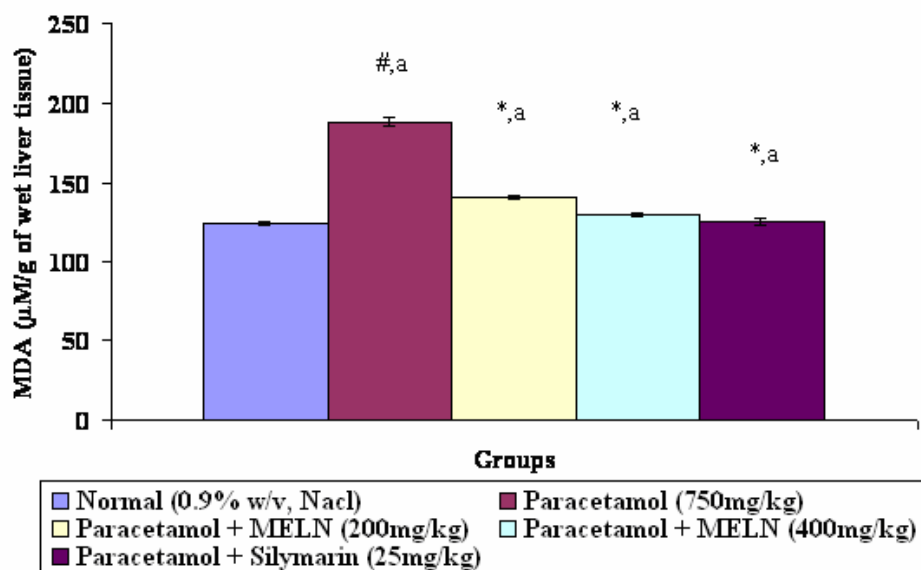


Fig 1. Effect of methanol extract of *Lippia nodiflora* (MELN) on Lipid peroxidation in paracetamol intoxicated rats. Values are mean \pm SEM, n= 6, # compared with normal group, * compared with paracetamol control, ^a $p < 0.001$.

DNA can cause mutation that may be carcinogenic. Oxidative damage to carbohydrates can modify any of the cellular receptor function including those associated with hormonal and neurotransmitter responses [15]. The reactive oxygen species (ROS) such as superoxide anion radical ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot\text{OH}$) have been implicated in the pathophysiology of various clinical disorders, including ischemia, reperfusion injury, atherosclerosis, acute hypertension, hemorrhagic shock, diabetes mellitus and cancer [16]. The inhibition of free radical generation

can serve as facile model for evaluating the activity of hepatoprotective agents.

The whole plant of *Lippia nodiflora* is used traditionally by the local and tribal people of south India for the treatment of diuretic, bronchitis, hypertension and fever. Hence there is no scientific work has been performed so far in hepatoprotective activity of the plant. Therefore, the present study was undertaken to evaluate the hepatoprotective and antioxidant properties of methanol extract of *Lippia nodiflora* in paracetamol-induced intoxicated liver in rats.

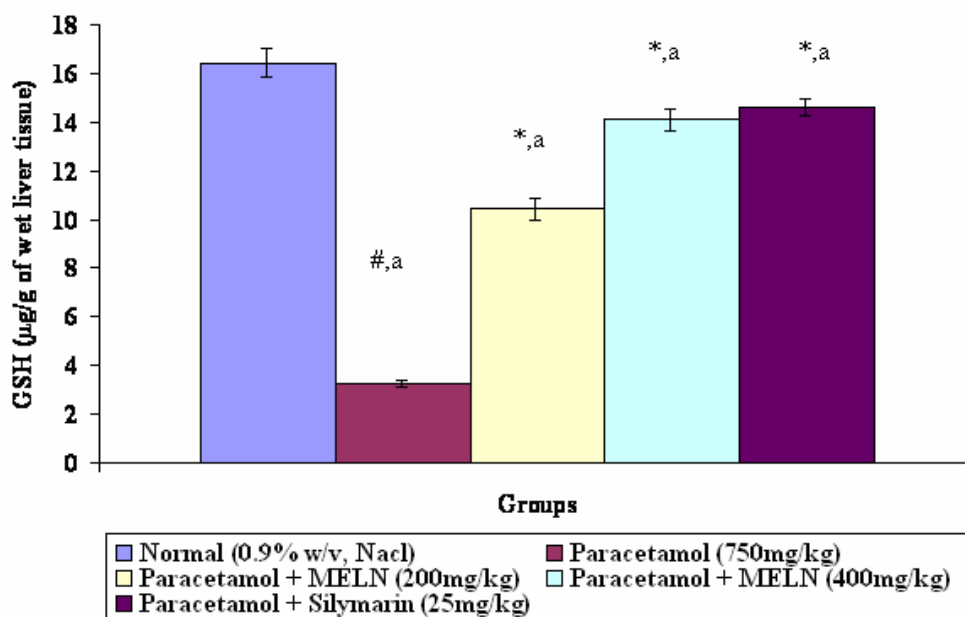


Fig 2. Effect of methanol extract of *Lippia nodiflora* (MELN) on Reduced glutathione level in paracetamol intoxicated rats. Values are mean \pm SEM, n= 6, # compared with normal group, * compared with paracetamol control, ^a $p < 0.001$.

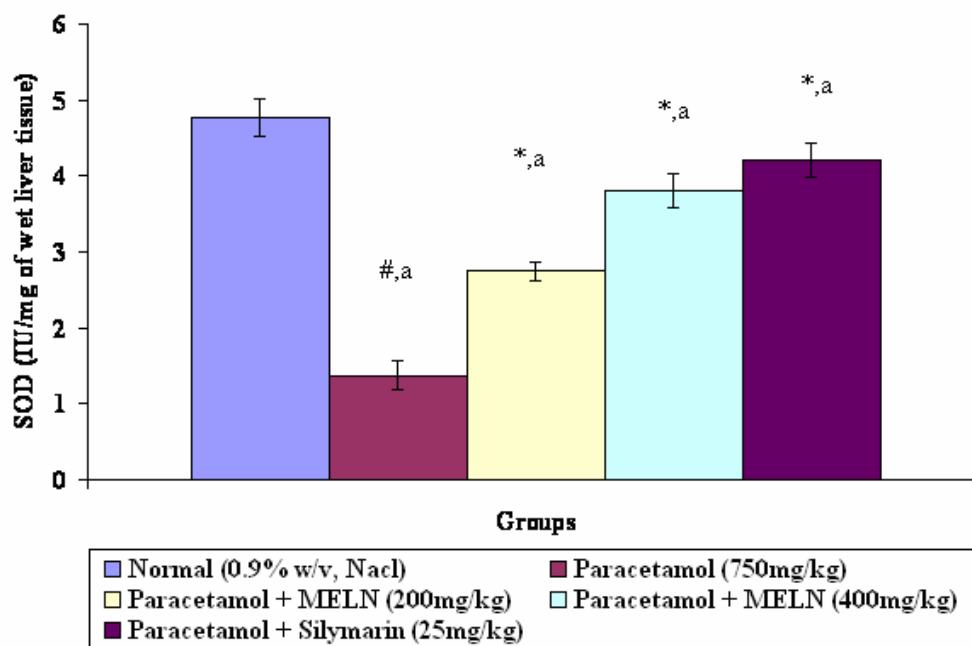


Fig 3. Effect of methanol extract of *Lippia nodiflora* (MELN) on Superoxide dismutase in paracetamol intoxicated rats. Values are mean \pm SEM, n= 6, # compared with normal group, * compared with paracetamol control, ^a $p < 0.001$.

MATERIALS AND METHODS

Collection of the herb

The plants were collected during January 2005 from wet areas of Mallasamudram, Namakkal district of Tamilnadu, India and identified by H.O.D, Department of Botany, Kuvempu First Grade College, Channapatna, Karnataka. A voucher specimen (DAK/JU04/2005) was deposited at the Departmental laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kol-

kata for future reference.

Preparation of plant extract

Three hundred and fifty grams of the whole plant of *Lippia nodiflora* was air dried, powdered and extracted with petroleum ether (60-80^o C) and methanol using soxhlet apparatus. The petroleum ether and methanol extract were filtered, concentrated to dryness under reduced pressure and yielded 2.91% and 21.42% respectively. The MELN was selected for the present study

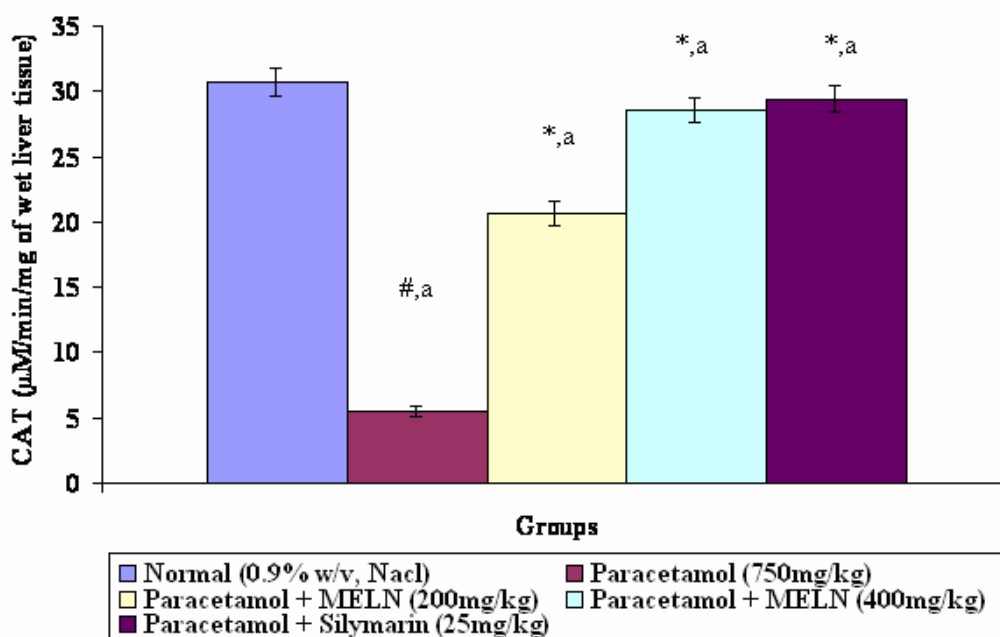


Fig 4. Effect of methanol extract of *Lippia nodiflora* (MELN) on Catalase level in paracetamol intoxicated rats. Values are mean \pm SEM, n= 6, # compared with normal group, * compared with paracetamol control, ^a $p < 0.001$.

and administered as a suspension in water directly in to the stomach using a gastric tube.

Experimental animals

Male albino rats (Wister strain) of weighing 150-200g were obtained from Indian Institute of Chemical Biology, Kolkata, India. They were placed in polypropylene cage with wire-net floors in a controlled room environment ($25^{\circ}\pm 2^{\circ}$ C), were provided with standard laboratory food supplied by Hindustan Lever Limited, Mumbai and water *ad libitum*. They were maintained at a natural day-light cycle. The animals were fasted for 24 hrs before experimentation but allowed free access to water.

Chemicals used

Silymarin (Sivylar) was purchased from Ranbaxy laboratories, Indore was used as standard drug. 1-Chloro-2, 4-dinitrobenzene (CDNB), Bovine serum albumin obtained from Sigma chemical St. Louis, MO, USA, Thiobarbituric acid (TBA), Nitro blue tetrazolium chloride (NBT) from Loba Chemie, Bombay, India, 5,5'-dithio bis-2-nitrobenzoic acid (DTNB) obtained from Sisco Research Laboratory, Mumbai, India and all the other reagents obtained were used of analytical grade.

Experimental procedure

The animals were divided into five groups of six rats each. A suspension of paracetamol was prepared in distilled water and administered orally at the dose of 750mg/kg body weight. Silymarin and extracts were also administered on a similar way. Group I, untreated rats served as a control. Group II rats were similarly treated as group I. Group III and IV received 200mg/kg and 400mg/kg b.w of MELN and group V received standard drug silymarin (25mg/kg b.w) for 7 days. On day 7, rats of groups II, III, IV and V received toxic doses of paracetamol (750mg/kg b.w) [17].

Estimation of biochemical parameters

Twenty four hours after paracetamol intoxication (On the 8th day), rats of each group were anaesthetized using anesthetic ether and then blood samples were collected by direct cardiac puncture, and centrifuged at 2500 g at 30° C for 15 minutes to separate the serum for different biochemical analysis such as Serum glutamate oxaloacetate (SGOT) and Serum glutamate pyruvate transaminase (SGPT) [18], SALP (Serum alkaline phosphatase) [19], Serum bilirubin [20], total protein, albumin and albumin/globulin (A: G) ratio [21], and amount of cholesterol, phospholipids and cholesterol/phospholipids ratio [22, 23] were estimated.

After collection of blood samples, the rats were sacrificed and the liver was excised, rinsed in ice cold normal saline, dried and weighed. A 10% w/v of homogenate was prepared in 0.15 M Tris- HCl buffer and processed for the estimation of lipid peroxidation [24]. A part of homogenate after precipitating proteins with Trichloro acetic acid (TCA) was used for the estimation of glutathione [25]. The rest of the homogenate was

centrifuged at 1500 g for 15 minutes at 4° C. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD) [26], catalase (CAT) [27] activities and protein estimation [21].

Statistical analysis

The experimental results were expressed as the Mean \pm SEM (Standard Error Mean). The One way ANOVA followed by Dunnett's test was used to make a statistical comparison between the groups. Results with $p<0.001$ and $p<0.05$ as considered statistically significant.

RESULTS

This study examines the hepatoprotective effects of methanol extract of *Lippia nodiflora* (MELN) in paracetamol induced liver toxicity. Acute administration of paracetamol (750mg/kg p.o) leads to increase in SGOT, SGPT, ALP, serum bilirubin concentration and decrease in the total protein level, which are used as reliable markers of hepatotoxicity. Oral administration of whole plant of MELN extract at a doses of 200mg/kg and 400mg/kg body weight, p.o markedly prevented the paracetamol- induced elevation of SGOT, SGPT, ALP, Serum bilirubin and also increased the level of total proteins. There were significant restoration of enzyme levels on administration of MELN at both doses (Group III and IV) and silymarin (Group V). The enhanced concentration of serum albumin, nonsignificant changes in serum globulin level and abnormal albumin:globulin (A:G) ratio was observed. Moreover treatment of rats with paracetamol produced an increase in the serum level of cholesterol, decrease in the level of phospholipids and a subsequent increase in the cholesterol to phospholipids ratio from 0.55 ± 0.02 in normal rats to 1.26 ± 0.05 in paracetamol treated rats. Cholesterol/ phospholipids ratio of 0.64 ± 0.01 , 0.51 ± 0.01 and 0.54 ± 0.01 were observed for MELN at 200, 400mg/kg and standard drug silymarin respectively (Table 1).

It was observed that the size of the liver was enlarged in paracetamol intoxicated rats but it was normal in extract treated group. A significant reduction in liver weight at 200mg ($p<0.05$) and 400mg/kg ($p<0.001$) supports this finding (Table 1).

The effects of MELN on rat liver lipid peroxidation, glutathione, and antioxidant enzyme (SOD and CAT) levels were determined (Table 2). TBARS level (expressed in term of malondialdehyde (MDA) formation) are significantly increased in paracetamol treated group compared with the normal group ($p<0.001$). Treatment with MELN (200mg and 400mg/kg) significantly prevented the increase in MDA levels ($p<0.001$) and brought back near to normal level. Liver enzymatic and nonenzymatic antioxidant levels were significantly altered in paracetamol treated rats compared with normal group ($p<0.001$). GSH, SOD and CAT levels were significantly increased ($p<0.001$) in MELN treated group. The effects of MELN were comparable to that of silymarin (25mg/kg, b.w.).

Table 1. Effect of methanol extract of *Lippia nodiflora* (MELN) on liver weight and serum biochemical parameters. Values are mean \pm S.E.M, (n= 6). # compared with normal group, *compared with paracetamol control, ^a $p < 0.001$, ^b $p < 0.05$ are considered statistically significant

Parameters	Group I (0.9%, NaCl w/v, 5ml/kg)	Group II (Paracetamol 750mg/kg)	Group III (MELN 200mg/kg+ Paracetamol)	Group IV (MELN 400mg/kg+ Paracetamol)	Group V (Silymarin 25mg/kg+ Paracetamol)
Liver weight (Wt/100g b.w)	3.23 \pm 0.06	6.49 \pm 0.26 ^{#,a}	5.60 \pm 0.11 ^{*,b}	4.56 \pm 0.14 ^{*,a}	3.38 \pm 0.15 ^{*,a}
SGOT (U/L)	51.00 \pm 1.78	117.50 \pm 2.55 ^{#,a}	99.16 \pm 1.62 ^{*,a}	76.00 \pm 2.39 ^{*,a}	64.66 \pm 2.40 ^{*,a}
SGPT (U/L)	41.33 \pm 1.90	133.33 \pm 2.26 ^{#,a}	93.16 \pm 2.83 ^{*,a}	66.33 \pm 3.82 ^{*,a}	52.66 \pm 2.41 ^{*,a}
ALP (U/L)	59.66 \pm 1.42	120.50 \pm 3.91 ^{#,a}	94.83 \pm 4.07 ^{*,a}	75.16 \pm 2.21 ^{*,a}	65.66 \pm 2.32 ^{*,a}
Bilirubin (mg %)	1.05 \pm 0.11	2.91 \pm 0.01 ^{#,a}	2.26 \pm 0.10 ^{*,a}	1.60 \pm 0.01 ^{*,a}	1.31 \pm 0.01 ^{*,a}
Total proteins (g/dl)	7.38 \pm 0.14	4.16 \pm 0.01 ^{#,a}	5.58 \pm 0.18 ^{*,a}	6.95 \pm 0.13 ^{*,a}	7.10 \pm 0.10 ^{*,a}
Albumin (g /dl)	4.53 \pm 0.10	3.04 \pm 0.10 ^{#,a}	4.51 \pm 0.03 ^{*,a}	5.00 \pm 0.03 ^{*,a}	4.42 \pm 0.10 ^{*,a}
Globulin (gm/dl)	2.72 \pm 0.11	5.06 \pm 0.06 ^{#,a}	3.89 \pm 0.06 ^{*,a}	3.32 \pm 0.07 ^{*,a}	2.84 \pm 0.10 ^{*,a}
Albumin / Globulin (A: G)	1.67 \pm 0.04	0.60 \pm 0.02 ^{#,a}	1.16 \pm 0.02 ^{*,a}	1.50 \pm 0.03 ^{*,a}	1.56 \pm 0.06 ^{*,a}
Cholestrol (mg/dl)	32.02 \pm 0.83	52.35 \pm 1.00 ^{#,a}	47.80 \pm 0.55 ^{*,b}	45.77 \pm 0.77 ^{*,a}	32.59 \pm 0.84 ^{*,a}
Phospholipids (mg/dl)	58.30 \pm 0.84	41.66 \pm 1.14 ^{#,a}	77.99 \pm 1.86 ^{*,a}	89.23 \pm 0.85 ^{*,a}	59.73 \pm 1.58 ^{*,a}
Cholestrol / Phospholipids (C: P)	0.55 \pm 0.02	1.26 \pm 0.05 ^{#,a}	0.64 \pm 0.01 ^{*,a}	0.51 \pm 0.01 ^{*,a}	0.54 \pm 0.01 ^{*,a}

DISCUSSION

Paracetamol, a widely used non-prescription analgesic-antipyretic drug, is safe when used within therapeutic doses. When taken in large doses, it may cause hepatotoxicity, leading to fulminant hepatic and renal tubular necrosis, which is lethal to humans and many species of animals [28,29,30]. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates [31,32]. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P₄₅₀ [33]. Induction of cytochrome P₄₅₀ or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity. Therefore the anti-hepatotoxic activity of the drug may be due to: inhibition of cytochrome P₄₅₀, promotion of glucuronidation, stimulation of hepatic regeneration, activation of the functions of the reticulo endothelial systems or inhibition of protein biosynthesis [34].

The abnormal high level of serum biomarker enzymes and bilirubin observed in this study are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Oral administration of MELN exhibited a significant reduction in paracetamol induced levels of serum GOT, GPT, ALP and bilirubin and also increase the levels of total protein value remarkably to the normal group that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage. This is further evidence for the protective effect of MELN extract and maintains the functional integrity of hepatic cells [35]. The above alterations can be considered as an expression of the functional improvement of hepatocytes, which may be

caused by an accelerated regeneration of parenchyma cells. Effective control of bilirubin level and alkaline phosphatase activity points towards an early improvement in the secretory mechanism of the hepatic cell. The silymarin with a dose of 25mg/kg, b.w has provided a better inhibition of the elevated level of SGOT, SGPT, ALP and serum bilirubin and also increased the protein content. The changes observed in the albumin and globulin level may be because of decrease in the number of hepatocytes, which in turn may result in to the decreased hepatic capacity to synthesize glycogen and proteins as well as damaged cellular integrity of hepatocytes. The treatment of MELN shows significant increase in total serum proteins along with decreased concentration of serum albumin as a result of correction in A:G ratio. This results indicate that the antihepatotoxic activity of the MELN probably through the correction of cellular integrity of hepatic cell and its regeneration.

Administration of paracetamol to rats increases erythrocyte membrane peroxidation, which may also lead to haemolytic changes. It has been indicated that micro viscosity of a membrane increases markedly with increase in cholesterol to phospholipids ratio thus leading to cellular rigidity [36]. Paracetamol induced toxicity in rats may have altered membrane structure and function as suggested by the increases in cholesterol and subsequent decreases in phospholipids concentrations, hence increased cholesterol to phospholipids ratio. This result is an indication of membrane rigidity caused by paracetamol. Alteration of bio-membrane lipid profile disturbs its fluidity, permeability, activity of associated enzymes and transport system. However, pretreatment of rats with MELN inhibited the alteration of lipid

membranes and fluidity hence prevented alterations in the levels of cholesterol and phospholipids *in vivo* [37].

Oxidative stress is considered to be associated with many diseases, including cell damage, but diet plays an important role in human health and in the prevention of certain diseases. Lipid peroxidation has been postulated as being a destructive process in liver injury caused by paracetamol administration [38]. The coincidence of antioxidant activity and protective effect on liver tissues after paracetamol administration suggest that both free radical generation and lipid peroxidation may be involved in this type of drug injury process [39]. In our study, elevations in the levels of end products of lipid peroxidation in liver of rat treated with paracetamol were observed. The increase in MDA level in liver suggests provoked lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Treatment with MELN significantly reversed these changes. Hence it may be possible that the mechanism of hepatoprotection of MELN is due to its antioxidant effect.

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals and maintenance of membrane protein thiols and as a substrate for glutathione peroxidase (GPx) and GST [40]. In our present study the decreased level of GSH has been associated with a provoked lipid peroxidation in paracetamol treated rats. Administration of MELN significantly increased the level of glutathione in a dose dependent manner.

Increase in the serum activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury [41,42]. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide, hence diminishing the toxic effect caused by this radical. In the present study, it was observed that the MELN caused a significantly increased in the hepatic SOD activity of the paracetamol induced liver damage in rats. This indicates MELN can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Catalase (CAT) is a key component of the antioxidant defense system, widely distributed in tissue and the highest activity is found in the red cells and in liver. Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [43]. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of MELN increases the activities of SOD and CAT in paracetamol

induced liver damage rats to prevent the accumulation of excessive free radicals and protects the liver from paracetamol intoxication.

Reduced activities of superoxide dismutase (SOD) and catalase (CAT) in paracetamol treated rats confirms the hepatic damage to the rats [44]. Treatment with MELN to rats in which hepatic damage was induced by paracetamol treatment caused significant increase in SOD and CAT activities. Hence antilipid peroxidative and/or adaptive nature of the system as brought about by the MELN against the damaging effects of free radical produced by the paracetamol.

Free radical reactions are implicated in the progression of cancer, inflammation, Atherosclerosis, hepatocellular damage and the biological process of aging. The hepatoprotective action combined with antioxidant activity has a synergistic effect to prevent the process of initiation and progress of hepatocellular diseases [45]. Although the precise mechanism of MELN has not been elucidated, it can be safely assumed that the lowering of enzyme levels is responsible for cell injury and enhancing the enzymes responsible for antioxidant activity. It can be concluded that the MELN possess good hepatoprotective and definite antioxidant activities either through stabilization of cellular membrane or antiperoxidase activity. Further investigation is needed to pinpoint the exact phytoconstituents responsible for the activity.

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