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THE *AEGLE MARMELOS* LEAF EXTRACTS AND WHOLE LEAF POWDER INFLUENCING EFFECTS ON EXPERIMENTAL ANIMALS TISSUE ANTIOXIDANTS AND ATPASES DURING CHRONIC ADMINISTRATION

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ABSTRACT

The *Aegle marmelos* plant belonging to the family Rutaceae occupies a distinct position in Indian traditional medicine system for its medicinal properties. The chronic oral treatment study of whole leaf powder (2000mg), aqueous and alcohol extract (400mg) for 90 days in male Wistar rats showed absence of any alterations in tissue lipid peroxidation indicating the absence of any pro-oxidant property. Catalase in the liver and lung was found to be elevated during treatment with *Aegle marmelos*. Tissue glutathione peroxidase is found to be comparatively more in alcohol extract treated group in all the tissues analyzed. The non-enzymic antioxidants GSH and vitamin C in tissues were more after treatment with alcohol extract. Thus a desirable effect on both enzymic and non-enzymic antioxidants by leaf powder extracts of *Aegle marmelos* was seen in most of the tissues studied. Tissue ATPases analyzed in the present study was found to be altered during treatment with the alcohol extract. The aqueous extract and leaf powder have shown the effect only to a little extent in certain tissues. All the ATPases analyzed in brain, liver and lung increased during treatment with alcohol extract. While the alcohol extract increased the Na^+-K^+-ATPase alone, the aqueous extract increased all the ATPases in heart. When the relative percent increase of the enzyme activity is considered, the aqueous extract is found to enhance the activity of Ca_2^+-ATPases more than that of Na^+-K^+-ATPases. In renal tissue, an increase in Ca_2^+-ATPase was seen after aqueous extract treatment. The relevance of the possible enhanced reabsorption of calcium needs to be investigated and from the pharmacological effects identified this study needs further evaluation for their clinical significance as the leaf of *Aegle marmelos* is found to have a multitude of pharmacological effects without any toxic impact during long-term administration.

KEYWORDS

Aegle marmelos, Antioxidants, ATPases, Chronic and Wistar albino rats.

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INTRODUCTION

The *Aegle marmelos* plant belonging to the family Rutaceae grows wild in dry forests on hills and plains of central and southern India, Burma, Pakistan and Bangladesh, also in mixed deciduous and dry dipterocarp forests of former French Indochina. The plant (Tamil: Vilvam, Kuvilam. English: Bael tree, Holy fruit tree. Sanskrit: Bilva,

Vilvah) is considered to be extremely auspicious and cultivated around most of the Hindu temples. The leaves are indispensable offerings to the 'Lord Shiva'. The plant leaves are more preferred for its medicinal properties and occupies a distinct position in Indian traditional medicine system. It is highly valued in ayurvedic medicine and the importance of it from the medicinal point of view has been quoted in the Nighanthas and in the works of Charaka, Sushruta and Mohammed Hossain and more recently of Dymock, Nandkarni, Hooker, Kirtikar and Basu, Fitzpatric, Lanchester and others. Considering the leaves wide usage in the traditional medicine and being safe during long term administration in experimental animals without causing any toxic impact[1], the present study was carried out to find whether the plant (leaf powder and aqueous, alcohol extracts) possessed any property of improving the functional status of the body by improving the tissue antioxidants and ATPases during chronic administration, and also the available scientific literatures on this plant relating to its influencing effect on tissue antioxidants [3,4] and their extent of responsiveness are sparse and were of shorter duration [5,6].

MATERIALS AND METHODS

Collection and identification of the plant

Fresh leaves of *Aegle marmelos* collected locally from single source during the month of July were used for the study. Identification and certification of the leaves of *Aegle marmelos* was done by the taxonomist, Prof. R.Viswanathan at the Centre for Advanced Studies in botany, University of Madras, Chennai, India. A specimen sample was deposited for future reference. The allotted voucher number was CASB H-3. The leaves were washed, kept under shade and air dried.

Preparation of the Aqueous, Alcohol extracts and Whole leaf powder

The extracts were prepared as in the previous work[2], and for the whole leaf powder preparation the plant materials were ground manually using grinding stone. The fine powder obtained after sieving through a fine mesh was used to treat the animals. Henceforth, the aqueous leaf extract of *Aegle marmelos* will be called as AqE, alcohol extract as AlE and leaf powder LP.

Animals

For this study, Wistar albino rats were used. Healthy male Wistar albino rats, weighing 175-200g were purchased from Tamilnadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The animals were acclimatized to animal house environment prior to the conduct of experiment. The animals were housed in autoclavable polypropylene cages having stainless steel grill lid with provision for water bottle and feed. Dried husk was used for bedding. The bedding material was changed twice a week. The animals were maintained in the animal house with temperature of 25 ± 2 °C, relative humidity of 65 ± 5% and 12 h dark-light cycle. They were provided diet in the form of pellets supplied by M/s. Kamadhenu Agencies, Bangalore and water *ad libitum*. During the experimental period, feed and water consumption by the rats were recorded daily and weight gain was recorded every fortnight. All the procedures were conducted in accordance with national guidelines and protocols, approved by the Institutional Animal Ethical committee (IAES No. 07/011/02).

Experimental studies in rats

Male Wistar albino rats (175-200 g) were randomly divided into four groups (I-IV) with 6 animals in each group so that the mean body weight per group was approximately equal. They were caged not more than 3 animals per cage. Following was the treatment schedule:

Group I (CON) : Treated with distilled water.

Group II (LP) : Treated with whole leaf powder - (2000 mg/ kg body weight) as aqueous suspension in distilled water.

Group III (AqE) : Treated with Aqueous extract - (400 mg/ kg body weight) as aqueous suspension in distilled water.

Group IV (AlE) : Treated with Alcohol extract - (400 mg/ kg body weight) as aqueous suspension in distilled water.

Dose regime was based on the maximum tolerated dose (without any mortality) observed in our previous study[1]. For the group that received LP the maximum dose was taken as such. For the extracts one-fifth of the maximum dose tested and found tolerated i.e. 400 mg/kg was given. Test doses were prepared daily and the dose volume was adjusted to 2 ml. During the treatment period, feed and water consumptions were recorded. The animals were monitored daily for any toxic manifestation. The body weight changes were recorded every 15 days. At the end of the 90-day period, the animals were sacrificed by decapitation. Autopsy was done to see for any morphological change of essential organs Liver, heart, lung, kidney and brain were dissected out immediately. After removing the extraneous tissue, the tissues were blotted free of blood and weighed on a mono-pan balance and were homogenized immediately in cold 50mM Tris-HCl, pH 7.4. The homogenate was centrifuged for 10 min at 2000 rpm to yield a pellet that was discarded and the supernatant obtained was used to determine the effects of *Aegle marmelos* treatment on tissue antioxidants and ATPases.

Biochemical parameters

The status of Lipid Peroxidation in tissue homogenates were estimated based on malondialdehyde formation[7]. The chromic acetate formation was used to estimate activity of Catalase (Hydrogen peroxide: Hydrogen peroxide oxidoreductase - EC.1.11.1.6.)[8], Glutathione peroxidase (Glutathione: Hydrogen peroxide oxidoreductase - EC. 1.11.1.9.) activity is based on Glutathione (GSH) convertion into oxidized Glutathione (GSSG) [9, 10]. The Reduced Glutathione content in the tissue homogenates was estimated with slight modification [11 10], Vitamin - C content according to the method of [13]. The activity of Na^+-K^+-ATPase (ATP: Phosphohydrolase - EC. 3.6.1.3.) in the tissue was estimated with modifications[14, 15], Ca^{2+}-ATPase (ATP: Phosphohydrolase - EC. 3.6.1.3.) [16, 15] and the activity of Mg^{2+}-ATPase (ATP: Phosphohydrolase - EC. 3.6.1.3.) in the tissues was estimated also with modifications[17, 15].

Statistical Analysis

The results obtained were subjected to One way Analysis of Variance (ANOVA) and Tukey's Multiple Comparison Test using SPSS statistical package (Version: 7.5). The results obtained were tabulated and the values are presented as mean ± S.D. *P* value < 0.05 was considered significant.

RESULTS

Lipid peroxidation, Enzymic and Non-Enzymic Antioxidants

Lipid peroxidation (LPO)

Among all the treated groups only liver of the LP treated group showed an increase ($p<0.05$) in lipid peroxidation. While lung, heart, kidney and brain tissues showed no changes in their lipid per oxidation levels (Table No. 1).

Catalase (CAT)

The liver of AlE treated group showed a significant rise ($p<0.05$) in the catalase activity. The activity of catalase in brain and lung was raised ($p<0.01$). The heart tissue of AqE and LP treated group's brain and lung showed an increase in enzyme activity ($p<0.05$) (Table No.1).

Glutathione peroxidase (GPx)

The levels of glutathione peroxidase in liver and lung showing a rise in enzyme activity ($p<0.01$) in the AlE treated and ($p<0.05$) LP treated groups. The heart tissue showed a rise ($p<0.001$) and ($p<0.01$) in AlE and AqE treated groups, while the kidney and brain of the AlE treated group and brain of LP treated group showed ($p<0.05$) rise in enzyme activity (Table No.1).

Reduced glutathione (GSH)

Reduced glutathione showed a rise ($p<0.01$ and $p<0.05$) in the AlE and LP treated groups liver, lung and brain. While a rise ($p<0.01$) and ($p<0.05$) was seen in heart tissue of AlE and AqE treated groups (Table No.2).

Vitamin-C

The liver of AlE treated group and LP treated group showed a rise ($p<0.01$ and $p<0.05$) in the Vit - C levels. The heart of AlE and LP treated groups also showed a similar rise ($p<0.05$). A rise of ($p<0.01$) and ($p<0.05$) in AlE and AqE, LP treated groups

were seen in Lung tissue; while the brain showed a rise ($p<0.05$) in AlE treated group (Table No.2).

ATPase parameters

Na^+-K^+-ATPase

The liver and lung tissues of AlE treated group showed increase ($p<0.01$) in the activity of Na^+-K^+-ATPase, while the heart tissue showed a rise ($p<0.05$) in AlE and AqE treated groups. Brain tissue of AlE and LP treated groups showed increased activity ($p<0.05$) (Table No.3).

Mg^{++} ATPase

The liver and brain tissues showed a rise in activity ($p<0.05$) in AlE treated groups, similarly the heart tissue showed a rise ($p<0.05$) in AqE treated group. While the lung tissue of AlE and LP treated groups showed increased activity ($p<0.01$) and ($p<0.05$) (Table No.3).

Ca^{++} ATPase

The liver and lung tissues showed increased activity ($p<0.01$ and $p<0.05$) in AlE and LP treated groups. AqE treated groups showed a rise ($p<0.01$ and $p<0.05$) in Heart and kidney tissues. The brain tissue showed an increase in activity ($p<0.05$) in AlE treated group (Table No.3).

DISCUSSION

While having no toxic impact in general during chronic administration [1], the leaf of *Aegle marmelos* seems to possess pharmacological effects that can have clinical relevance as indicated by some of the biochemical alterations seen during long-term oral administration. Lipid peroxidation is frequently used as an indication of tissue oxidative stress as a result of free radical attack on the cell membrane[17]. *Aegle marmelos* does not seem to affect the free radicals production as indicated by the results of lipid peroxidation assessed in various tissues except for a mild increase in liver after treatment with the leaf powder. Among the tissues studied maximum lipid peroxidation was observed in brain, though no difference between the control and *Aegle marmelos* treated groups was seen. Free radicals produced in a number of biological processes are necessary for life, as in the case of intracellular killing of bacteria by neutrophils. Production of oxidants is efficiently handled by the immune system, by making the oxidizing species to have a lethal effect on the pathogens. This is especially seen in activated phagocytes, which produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) [18]. Free radicals have also been reported to play an important role in certain cell signaling processes[19]. As it has been mentioned earlier, free radicals have not been influenced by *Aegle marmelos* in the present study. Both the leaf powder as well as extracts *per se* have not caused any increase in the lipid peroxidation in most of the tissues analyzed indicating the absence of any pro-oxidant property. Thus absence of any alteration in the lipid peroxidation in *Aegle marmelos* treated group compared to the control group could be construed as noninterference in the normal beneficial mechanisms in which the free radicals are involved. In addition to the beneficial roles, free radicals have got the potential to damage the biological tissues, and can be of considerable threat to health. Oxidative stress results from the metabolic reactions that use oxygen, and it is a disturbance in the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells. Because of their reactivity with the tissues, free radicals can participate in reactions that can result in cell damage. Lipid peroxidation appears to be a highly significant consequence of oxidative stress in injured human arterial wall, contributing to the development of atherosclerosis [20]. Free radical induced oxidation has been implicated in ageing and atherosclerosis. Similarly reports related to involvement of free radicals in Parkinson's disease, Schizophrenia and Alzheimer's disease are available and more intense stresses may cause necrosis [21]. Though free radicals are essential for life, the body has also got mechanisms to minimize the free radical induced damage such as the production of enzymes which include Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx). In addition, antioxidants like vitamin C, E, etc., also play a vital role in this regard. The *Aegle marmelos* leaf extracts as well as leaf powder have been found to have an influence on the tissue antioxidant status. Though the type of influence on the tissue

antioxidants was almost similar in all the tissue studied, the intensity seems to differ between the different tissues. Hydrogen peroxide (H_2O_2) is mostly a product of enzymatic reactions and a constant cellular concentration of H_2O_2 is maintained in tissues. In plants and animal cells SOD produce H_2O_2 by the dismutation of $O_2^{\cdot -}$, thus resulting in lowering of oxidative reaction. H_2O_2 is a harmful byproduct of many normal metabolic processes and must be converted into less harmful product to prevent tissue damage. The enzyme Catalase found in the living organism catalyzes the decomposition of H_2O_2 to H_2O and O_2 [22]. Thus the dismutase and catalase combination removes H_2O_2 and thus possess a cellular antioxidant activity. It has been suggested that dietary restriction may reduce free radical levels by retarding age-related declines in rat liver catalase activity [23]. In liver, lung and brain the enzymic antioxidant catalase has been found to be comparatively more in alcohol extract treated group compared to the control. Treatment with the leaf powder has resulted in an increase in brain and lung catalase. Unlike the leaf powder and alcohol extract, the aqueous extract has been found to cause an increase in the catalase in heart. It has been reported that catalase activity varies greatly from tissue to tissue. Liver and kidney with higher activity and connective tissues with lower activity have also been reported [24]. Observations made in the present study is in accordance with this and the order of the enzyme activity seen was Kidney>Liver>Lung>heart>brain. However, after treatment with *Aegle marmelos* the order of presentation was; Liver>Lung>Kidney>heart>brain. Thus catalase in liver and lung has been found to be more responsive to treatment with *Aegle marmelos*. The observation recorded in this study with reference to lipid peroxidation and enzymic antioxidants are in accordance with the available reports. In this regard an increase in catalase and GPx accompanied by a decrease in lipid peroxidation in liver of mice orally treated with the hydro alcoholic leaf extract of *Aegle marmelos* for 14 days have been reported[5]. Similarly, kar group have reported hepatic lipid peroxidation decrease as well as an increase in catalase and SOD 15 days after oral treatment with aqueous extract in a study on the alteration of thyroid hormone by medicinal plants in male mice[6]. Whether rutin, a flavonoid reported to be present in *Aegle marmelos* leaf [2, 25] could be responsible for these effects needs to be investigated since it has been reported that polyphenols and flavonoids can stimulate catalase and decrease malondialdehyde [26]. The cytoplasmic and mitochondrial enzyme Glutathione peroxidase is important for detoxifying H_2O_2 in most cells and protects the organisms from oxidative damage. GPx present in significant concentration in cytoplasm of cells detoxifies free H_2O_2 to water through the oxidation of GSH [27]. *Aegle marmelos* has been found to have an influence on this enzyme antioxidant also. Tissue glutathione peroxidase has been found to be comparatively more in alcohol extract treated group compared to the control in all the tissues analysed. Treatment with the leaf powder has resulted in an increase in liver, brain and lung GPx. The aqueous extracts increased the GPx in heart alone. Thus variability in the level of antioxidant enzymes in different tissues has been observed after treatment with *Aegle marmelos*.

Non-Enzymic Antioxidants in tissues have also been found to be influenced by *Aegle marmelos* treatment. The extent of responsiveness was observed to differ in various tissues. No influence on these antioxidants has been noticed in the kidney. Glutathione, the base material for the antioxidant enzyme glutathione-peroxidase, functions as a non-enzymatic reducing agent and also prevents oxidative stress in most cells by helping to trap free radicals. A deficiency of intracellular glutathione has been reported to be associated with various conditions including alcohol-induced liver disease, some forms of cancer and treatment with certain drugs. The haloperidol causes oxidative stress due to increased turnover of dopamine with excessive production of hydrogen peroxide and loss of glutathione[28], similarly the efficacy of glutathione (GSH) in the prevention of cisplatin induced neurotoxicity also been demonstrated[29]. In the present study, GSH in liver, lung, brain and heart

has been found to be more after treatment with alcohol extract. Such an effect was seen in liver, lung and brain of groups treated with the leaf powder while an increase was seen only in heart with aqueous extract. Thus *Aegle marmelos* seems to have the potential for enhancing tissue GSH, a property that can be used in conditions that deplete this vital antioxidant. This observation can have clinical significance since it has been reported that dietary glutathione is not a major determinant of circulating glutathione as it is hydrolysed by intestinal and hepatic gamma- glutamyltransferase [30]. Treatment with the leaf powder and extracts of *Aegle marmelos* resulted in an increase in tissue vitamin C. Many species that can oxidize vitamin C have been found to be involved in human diseases [31]. With vitamin C, the reactive free radical is reduced, and the ascorbyl radical formed in its place is less reactive. Ascorbate is therefore a good free radical scavenger due to its chemical properties [32]. Many disease conditions caused or exacerbated by oxidant stress are also associated with low plasma and tissue vitamin C concentrations. Vitamin-C concentrations have been reported to be low in patients with myocardial infraction [33]. Vitamin-C has been found to be more in liver, lung, brain and heart after treatment with alcohol extract and aqueous extract treatment has resulted in an increased Vitamin-C in lung only. With leaf powder such an effect was seen in liver, lung and heart. Thus the plant under investigation seems to have the potential to increase the tissue Vitamin-C. In a human double-blind study[34], the Vitamin-C supplementation maintained reduced glutathione concentrations in blood and also improved the overall antioxidant protection capacity of blood. Such a mutually beneficial interaction between these two antioxidants is possible by treatment with *Aegle marmelos;* since the leaves of *Aegle marmelos* have been found to increase the tissue GSH as well as Vitamin-C in this study. The pro-oxidant conditions with low plasma vitamin C concentrations include smoking [35] and diabetes mellitus [36]. In a study on the effect of aqueous extract of the *Aegle marmelos* fruits in streptozotocin diabetic rats, the extract caused an elevation in plasma GSH and Vit-C[3]. Similarly a decrease in lipid peroxidation with an increased GSH in liver and kidney of irradiated mice pre-treated with hydro alcoholic fruit extract has also been reported[4]. Thus a desirable effect on both enzymic and non-enzymic antioxidants by leaf powder extracts of *Aegle marmelos* was seen in most of the tissues studied. The alteration in tissue ATPases recorded after treatment with the leaf or its extracts is an expression of the impact on tissue biochemistry which needs to be analysed for the pharmacological and clinical significance. ATPases, the enzymes that catalyse dephosphorylation of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a free phosphate ion, play a vital role in cellular functions as the energy released during the reaction is utilized for other chemical reactions. Tissue ATPases analysed in the present study was found to be altered during treatment with alcohol extract. The aqueous extract and leaf powder have shown the effect only to a little extent in certain tissues. In most animal cells the major ion-translocating ATPase is the sodium pump or Na^+-K^+-ATPase and hepatocellular Na^+-K^+-ATPase is an important driving force for bile secretion [37].Though a study reported a relatively higher activity of Na^+-K^+-ATPase in the brain and kidney compared to the other tissues in female albino rat[38], the result of the present investigation showed higher activity in lung, heart and kidney than in brain of control animals. In the present investigation, a significant increase in Na^+-K^+-ATPase, Mg_2^+ and Ca_2^+-ATPases has been seen after treatment with alcohol extract, whereas treatment with leaf powder increased the Na^+-K^+-ATPase alone in the brain. Na^+-K^+-ATPase is essential for cellular excitability and is decreased in experimental and human epilepsy. By assessing the *in-vitro* effects of some guanidine compounds (GC) on the activity of Na^+-K^+-ATPase in the synaptic plasma membrane of young rats cerebral cortex, were they significantly inhibited Na^+-K^+-ATPase activity and this inhibitory effect of GC on Na^+-K^+-ATPase was suggested to be related to the brain dysfunction observed in hyperargininemia[39]. In a study to understand the neurological dysfunction

of patients affected by prop ionic academia rats injected s.c. with propionic acid showed significantly reduction of Na^+-K^+-ATPase activity but not the Mg_2^+-ATPase activity in cerebral cortical synaptic plasma membranes[40]. The observation of increased Na^+-K^+-ATPase activity after treatment with *Aegle marmelos* thus provides its possible therapeutic use in conditions where the activity of the ATPase is reported to be decreased.

In lung tissue all the ATPases analysed have found to be increased with alcohol extract. While the aqueous extract did not caused any alteration, an increase in the Mg_2^+ and Ca_2^+-ATPase activity has been noticed after treatment with leaf powder. The role of Na^+-K^+-ATPase in cellular function has been recognized and its importance in the lung has been reported [41]. Lung edema clearance is mostly dependent on active Na^+ transport out of the alveoli. The alveolar epithelial Na^+-K^+-ATPase has an important role in regulating cell integrity and homeostasis. The importance of the alveolar epithelial Na^+-K^+-ATPase function is reflected in the changes in the lung's ability to clear edema when the Na^+-K^+-ATPase is inhibited or increased [42]. Studies in alveolar epithelial cells suggest that impairment of lung edema clearance may be due to decreased function of epithelial Na^+-K^+-ATPase [43, 44]. Since alcohol extract seems to possess the active principle that can enhance the activity of Na^+-K^+-ATPase, the clinical relevance of this activity possessed by the extract needs further exploration. Unlike other tissues, treatment with alcohol extract resulted in increase of Na^+-K^+-ATPase alone in heart. However the aqueous extract was found to increase all the ATPases in this tissue. However when the relative percent increase of the enzyme activity is considered, the aqueous extract has been found to enhance the activity of Ca_2^+-ATPase more than that of Na^+-K^+-ATPase. Leaf powder did not produce any significant alteration in the activity of tissue ATPases. This may due to the principle responsible for enhancing the ATPase activity present in the aqueous extract not being present in adequate concentration in the leaf powder in the dose administered. In liver the alcohol extract increased all the ATPases analyzed whereas an increase in Ca_2^+ ATPase alone has been noticed with the leaf powder. The aqueous extract did not cause any alteration. Reduction in oxygenation induces inhibition of Na^+-K^+-ATPase in a number of cells and tissues, including hepatocytes. When not reversed, decrease in Na^+-K^+ pump activity leads to a gradual Na^+ accumulation, cell swelling and death [45]. The possible usefulness of the plant in reversing toxic responses with suppression of Na^+-K^+-ATPase needs to be investigated. When interpreted in association with the biochemical profile of increased serum transaminases and alkaline phosphatase indicating hepatic damage during early phase of treatment which has been proposed as an initial stress response as the levels of these enzymes (transaminases and phoshatases) have shown to return back to normalcy on day 90 [1], the increase in tissue Na^+-K^+-ATPase could be regarded as an indication of a compensatory biochemical response associated with cellular regeneration or increased activity. Na^+-K^+-ATPase is not distributed uniformly in the kidney and is highest in the outer medulla, intermediate in the cortex, and lower in the inner medullary papilla [46]. The basolateral segment of the rat renal tubular plasma membrane possesses Ca_2^+dependent ATPase activity which are independent of Mg_2^+ [47] and the ATPases measured in this study are from renal tissue homogenates and does not allow quantitative correlations with the enzyme functions as they are postulated to mediate in various nephron segments. Because of its selective location in the basolateral membrane, it is has been postulated that the Ca_2^+- Mg_2^+-ATPase participates in the tubular reabsorption of calcium [48]. The relevance of the possible enhanced reabsorption of calcium needs to be investigated and from the foregoing discussion the pharmacological effects identified in this study needs further evaluation for their clinical significance as the leaf of *Aegle marmelos* is found to have a multitude of pharmacological effects without any toxic impact during long-term administration.

Table No.1: Effect of *Aegle marmelos* on tissue Lipid peroxidation, Enzymic Antioxidants of Wistar albino rats

S.No	Parameters	Tissues	Groups			
			CON	LP	AqE	AlE
1	Lipid Per oxidation (nmol of MDA formed/mg protein)	BRAIN	13.803±0.421	13.712 ± 0.471	13.685±0.397	13.517±0.512
		LUNG	3.372 ± 0.163	3.631 ± 0.169	3.436 ± 0.188	3.384 ± 0.125
		HEART	2.414 ± 0.142	2.466 ± 0.059	2.545 ± 0.103	2.604 ± 0.157
		LIVER	3.539 ± 0.089	3.993 ± 0.356 *	3.597 ± 0.165	3.800 ± 0.322
		KIDNEY	3.879 ± 0.220	3.849 ± 0.121	3.664 ± 0.154	3.768 ± 0.144
2	Catalase (µmol of H_2O_2 utilized/min/mg protein)	BRAIN	92.44 ± 10.67	111.12 ± 9.46 *	97.68 ± 8.39	120.70 ± 6.09**
		LUNG	266.10 ± 6.55	317.69 ± 30.88 *	301.05±28.20	338.02 ± 6.22**
		HEART	168.11 ± 7.46	197.24 ± 22.02	204.58 ± 18.2 *	196.37 ± 15.59
		LIVER	289.39 ± 6.29	331.01 ± 30.04	324.08 ± 31.7	343.35 ± 34.71*
		KIDNEY	292.07 ± 1.57	297.39 ± 25.10	261.80 ± 30.6	302.68 ± 31.45
3	Glutathione Peroxidase (µg of GSH utilized/min/mg protein)	BRAIN	45.36 ± 4.64	55.34 ± 5.53 *	48.81 ± 9.11	57.12 ± 4.16 *
		LUNG	51.88 ± 5.39	62.95 ± 6.47 *	56.95 ± 7.18	66.02 ± 7.23 **
		HEART	37.08 ± 3.69	40.33 ± 4.94	48.03 ± 4.84 **	50.86 ± 5.90 ***
		LIVER	56.88 ± 4.18	66.33 ± 6.51 *	56.03 ± 6.30	70.52 ± 5.88 **
		KIDNEY	41.10 ± 3.18	46.65 ± 4.00	42.86 ± 3.97	49.12 ± 4.97 *

Values are mean ± S.D, n=6 rats/group.
CON – Control, AqE - Aqueous leaves extract 400mg/Kg b.w., p.o
LP – Leaf Powder 2000mg/Kg b.w., p.o AlE – Alcohol leaf extract 400mg/Kg b.w., p.o.
Comparisons made with control group. *p < 0.05, **p < 0.01, ***p < 0.001.

Table No.2: Effect of *Aegle marmelos* on tissue Non-enzymic Antioxidants of Wistar albino rats

S.No	Parameters	Tissues	Groups			
			CON	LP	AqE	AlE
1	Reduced Glutathione (Mg/mg protein.)	BRAIN	1.028 ± 0.057	1.155 ± 0.048 *	1.018 ± 0.080	1.187 ± 0.086 **
		LUNG	0.562 ± 0.057	0.678 ± 0.056 *	0.572 ± 0.044	0.698 ± 0.068 **
		HEART	1.053 ± 0.051	1.067 ± 0.069	1.172 ± 0.076 *	1.228 ± 0.086 **
		LIVER	1.672 ± 0.084	1.889 ± 0.135 *	1.708 ± 0.044	1.907 ± 0.144 **
		KIDNEY	0.962 ± 0.061	0.987 ± 0.090	1.002 ± 0.056	1.078 ± 0.078
2	Vitamin-C (µg/mg protein.)	BRAIN	0.418 ± 0.015	0.440 ± 0.034	0.425 ± 0.014	0.462 ± 0.028 *
		LUNG	0.447 ± 0.037	0.516 ± 0.027 *	0.506 ± 0.032 *	0.535 ± 0.050 **
		HEART	0.497 ± 0.024	0.550 ± 0.033 *	0.507 ± 0.019	0.563 ± 0.041 *
		LIVER	0.595 ± 0.027	0.658 ± 0.042 *	0.608 ± 0.025	0.670 ± 0.039 **
		KIDNEY	0.502 ± 0.010	0.518 ± 0.040	0.512 ± 0.021	0.527 ± 0.026

Values are mean ± S.D. n=6 rats/group.
CON - Control AqE - Aqueous leaf extract 400mg/Kg b.w., p.o
LP – Leaf Powder 2000mg/Kg b.w., p.o lE –Alcohol leaf extract 400mg/Kg b.w., p.o.
Comparisons made with control group. *p < 0.05, **p < 0.01.

Table No.3: Effect of *Aegle marmelos* on Tissue ATPases in Wistar albino rats

S.No	Tissues	Na^+-K^+-ATPase				Mg^{++} ATPase				Ca^{++} ATPase			
		CON	LP	AqE	AlE	CON	LP	AqE	AlE	CON	LP	AqE	AlE
1	Brain	0.351 ± 0.011	0.372 ± 0.005*	0.359 ± 0.018	0.373 ± 0.013*	0.438 ± 0.012	0.441 ± 0.020	0.432 ± 0.010	0.464 ± 0.017*	0.429 ± 0.031	0.470 ± 0.042	0.410 ± 0.025	0.483 ± 0.029*
2	Lung	0.673 ± 0.013	0.698 ± 0.020	0.681 ± 0.007	0.713 ± 0.024**	0.830 ± 0.033	0.891 ± 0.036*	0.846 ± 0.030	0.906 ± 0.047**	0.799 ± 0.044	0.860 ± 0.030*	0.813 ± 0.039	0.878 ± 0.041**
3	Heart	0.609 ± 0.027	0.634 ± 0.023	0.648 ± 0.028*	0.649 ± 0.015*	0.761 ± 0.047	0.837 ± 0.048	0.842 ± 0.040*	0.824 ± 0.055	0.753 ± 0.029	0.773 ± 0.034	0.845 ± 0.044**	0.796 ± 0.054
4	Liver	0.402 ± 0.009	0.427 ± 0.028	0.405 ± 0.021	0.463 ± 0.033**	0.517 ± 0.024	0.530 ± 0.034	0.521 ± 0.019	0.568 ± 0.027*	0.471 ± 0.008	0.512 ± 0.010*	0.487 ± 0.037	0.523 ± 0.014**
5	Kidney	0.551 ± 0.017	0.553 ± 0.012	0.576 ± 0.015	0.545 ± 0.010	0.731 ± 0.027	0.765 ± 0.034	0.741 ± 0.029	0.767 ± 0.041	0.667 ± 0.042	0.699 ± 0.022	0.721 ± 0.031*	0.681 ± 0.025

n=6. Values are mean ± S.D. ATPases expressed as µmoles of Pi liberated/min/mg protein.
CON – Control, LP – Leaf Powder 2000mg, AqE - Aqueous leaf extract 400mg, AlE – Alcohol leaf extract 400mg.
Route – Oral. a :Comparisons made with control (CON) group. * p < 0.05 **p < 0.01.

CONCLUSION

The leaf of *Aegle marmelos* during repeated daily administration was found to increase tissue antioxidants this could have a beneficial role in therapeutics, as many pathological lesions are related to free radical production and similarly the property of increasing tissue ATPases can have clinical application in conditions where these enzymes are suppressed.

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CONFLICT OF INTEREST

None declared.

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