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EFFECT OF *AEGLE MARMELOS* LEAF EXTRACTS AND WHOLE LEAF POWDER CHRONIC ADMINISTRATION IN EXPERIMENTAL ANIMALS

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ABSTRACT

The *Aegle marmelos* whole leaf powder (2000mg) and alcohol extract (400mg) chronic oral treatment for 90 days in male Wistar rats caused elevation of serum transaminases and alkaline phosphatases. This was maximum after 60 days of treatment this effect does not substantiate for a toxic impact on liver, as the levels have been found to come towards normalcy after 90 days of treatment and fluctuations in the serum total proteins were also noticed, but they were found to be within normal range. The blood urea and hematological parameters during different duration of treatment showed absence of fluctuations, which indicates no deleterious impact on vital tissues like liver, kidney and bone marrow; the post mortem observation and histopathology studies confirms this and thus it can be suggested that the fluctuations are due to initial stress and cannot be considered as reflection of any permanent toxic injury. These treatments showed a decrease in serum total cholesterol, triglycerides, VLDL, LDL with an increase in HDL. The β-sitosterol and Rutin are reported to posses' hypocholesterolemic effect and the rutin was identified in the alcohol extract. The acute toxicity study of the leaf powder and extracts were non-toxic up to a dose of 2000 mg/ kg b. wt.

KEY WORDS

Aegle Marmelos; Transaminases, Alkaline Phosphatases, Lipids, Acute, Chronic and Wistar rats.

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INTRODUCTION

Aegle marmelos belonging to the family Rutaceae is an aromatic slow-growing, medium sized tree with strong straight 1-3 cm long axillary spines. The leaves, borne singly or in 2's or 3's, are composed of 3 to 5 oval, pointed, shallowly toothed leaflets, 4-10 cm long, 2-5 cm wide, the terminal one with a long petiole. This plant is more preferred for its medicinal properties than its edible quality and occupies a distinct position in Indian traditional medicine. Many medicinal properties are claimed to be possessed by

the plant, but the available scientific literature on this plant is mostly related to the botanical aspects, isolation of chemical constituents[1-7], assessment of some specific medicinal properties[8-12] and evaluation of certain pharmacological activities[13-16]. Most of the medicinal properties are attributed to the leaf of the plant but reports on safety or toxic impacts during long-term administration are lacking. Considering the medicinal properties reported to be possessed by the leaf of *Aegle marmelos* and its use for various illnesses, a chronic study was carried out to assess for any toxic impact during repeated daily administration of *Aegle marmelos* leaves by studying the influence on the biochemistry, hematology and histopathology in experimental animals.

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 of (Porchelvan and Murali., 2014)[17] and for the whole leaf powder preparation the plant materials were ground manually using grinding stone. The fine powder got 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 acute, chronic toxicity studies, Wistar albino rats were used. Healthy male Wistar albino rats, weighing 175-200 g 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).

Acute toxicity studies in rats

As per the IAEC suggestion acute toxicity study by oral route was carried out in four groups of male Wistar albino rats with 4 animals in each to ascertain for any mortality at the dose level of 2000 mg/kg reported earlier Jagetia *et al,* (2005)[9] as LD10 in mouse. Allocation of the groups for treatment was as follows:

Group I - animals served as control and were treated with distilled water (2ml).

Group II - animals were given 2000 mg/kg body weight of the leaf powder (LP) in 2ml of distilled water.

Group III - animals were given 2000 mg/kg body weight of the aqueous extract (AqE) in 2ml of distilled water.

Group IV - animals were given 2000 mg/kg body weight of the alcohol extract (AlE) in 2ml of distilled water.

Observations were made for any physical manifestation at 1, 2, 4 and 8 h after oral administration. This included observation of skin and fur, eyes and mucous membranes, respiratory pattern, heart rate, autonomic and central nervous system manifestations and changes in behavior. Observation was made for any mortality during the 24 hour following administration of the test materials. Since literature regarding acute toxicity is

available, the animals were not sacrificed but were maintained for another 14 days with a once daily observation.

Sub chronic studies in rats

Sub chronic studies were conducted to get data on safety profile and to find its toxic impact and pharmacological significance of biochemical alterations consequent to repeated daily administration of the *Aegle marmelos* leaf powder or extracts. To correlate pharmacological significance of biochemical changes found during daily treatment.

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 the acute study. 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 every 30-day period, blood was collected in separate test tubes for hematological and biochemical parameters from the retro-orbital plexus under light diethyl ether anesthesia using capillary tubes. The tubes containing blood for biochemical analyses were kept in slant position for 2 h. The exuded serum was decanted and centrifuged at 2500 rpm for 20 min. The clear supernatant serum was subjected to biochemical evaluations. At the end of the 90-day period, blood was collected and 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 immediately on a mono-pan balance. Portions of these organs were fixed in 10% formalin for histopathological examinations.

Biochemical parameters

The activities of alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1) were estimated by the method of Wooten, (1964)[18], alkaline phosphatase (ALP, EC 3.1.3.1) by the method of King, (1965)[19] and Protein content was determined by the method of Lowry *et al*. (1951)[20] using bovine serum albumin as a standard. The serum triglycerides was determined using lipid kit obtained from Biolab Diagnostics (I) Pvt. Ltd., Mumbai and the serum cholesterol was estimated using the kit got from Star Diagnostics Pvt. Ltd., from these results VLDL, LDL and HDL values were calculated.

Hematology

Red blood cell and Total white blood cell count in whole blood was done according to the procedure described by Garvey *et al*, (1977)[21]. Hemoglobin was determined by the method of Hawk *et al*, (1966)[22] using Sahli's hemoglobinometer and blood urea by the method described by Varley, (1976)[23].

Histopathology of tissues

To investigate the histopathological changes in the visceral tissues following the dosage treatments, permanent mounts of the Lung, Heart, liver and Kidney tissue was prepared as detailed by Bancroft and Cook, (1984)[24].

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

Acute toxicity studies

No mortality was observed up to 24 h after treatment during acute toxicity testing. No abnormal behaviour was noticed in the animals treated with the test material (Groups II-IV) and the behaviour was similar to that of the control animals (Group I). No abnormal behaviour or death was noticed during the post treatment observation period of 14 days.

Sub-chronic studies

No mortality was seen during the treatment period in LP, AqE and AlE treated groups. The feed, water consumption and body weight value of the LP, AqE and AlE treated groups were found to be similar to that of control group.

Biochemical parameters

Serum alanine transaminase (ALT)

The results of Table No.1 shows the serum ALT levels of different groups treated with *Aegle marmelos*. Compared with the control, serum ALT was high on day 30 in AlE treated group only ($p<0.01$). On day 60 the increase was seen in all the test groups AlE ($p<0.001$), LP ($p<0.01$) and AqE ($p<0.05$). On day 90 a rise was seen in AlE ($p<0.01$) and LP ($p<0.05$) treated groups. When the effect of duration of treatment was analysed by comparing the 30 days value with other durations, only day 60 of AlE treated group showed a increased level ($p<0.01$).

Serum aspartate transaminase (AST)

Table No.1 shows the serum AST levels of different groups treated with *Aegle marmelos.* Serum AST was found to be high in LP and AlE ($p<0.05$ and $p<0.01$) treated groups on day 30. Treatment for 60 days also resulted in a rise in LP ($p<0.01$) and AlE ($p<0.001$). But on day 90, an increase was seen in AlE treated animals alone ($p<0.05$). When compared with the 30 days value an increase was seen in AlE treated group after 60 days ($p<0.05$) and a decrease after 90 days ($p<0.001$).

Serum alkaline phosphatase (ALP)

In Table No.1 serum ALP was more after treatment for 30 days when ALE was compared to control ($p<0.01$). A higher enzyme level was seen in LP ($p<0.01$) and AlE ($p<0.001$) groups after 60 days. Compared to control group the enzyme level was more in LP ($p<0.05$) and AlE ($p<0.05$) after 90 days of treatment. Compared to the 30 days value no significant difference was seen on 60 or 90 days in all the test groups.

Serum total Proteins

In Table No.1 the serum protein level was comparatively more in LP ($p<0.05$) and AlE ($p<0.01$) treated groups than the control group at the end of 30 days. Similarly after 60 days of treatment also the level was more in LP ($p<0.05$) and AlE ($p<0.001$) groups. However AlE treated group alone showed a higher level ($p<0.05$) when compared with the control group after 90 days. Compared to the 30 days value no significant difference was seen on 60 or 90 days in all the test groups.

Blood urea

In Table No.2 the blood urea level was found to be within normal limits in the control as well as test groups after all durations of treatment. No significant difference was seen between the control and test groups after treatment for 30, 60 or 90 days. Within each group no difference was observed between the different durations of treatment.

Hematology parameters

All the blood parameters in Table No.2 were found to be within normal limits in the control as well as in the test groups after all durations of treatment. No significant difference was seen between the control and test groups after treatment for 30, 60 or 90 days. Similarly no difference was observed within the groups during different durations of treatment.

Lipid parameters

Serum total Cholesterol

Serum total cholesterol was found to be less in the AlE treated group, when compared to the control group after treatment for 30 ($p<0.05$), 60 ($p<0.001$) and
90 ($p<0.001$) days. When compared with 30 days treatment, a decrease was seen after 60 days ($p<0.05$). Though a decrease was seen after 90 days, the difference was not statistically significant (Table No.3).

Serum Triglycerides

No change in serum triglycerides were seen after 30 days of treatment in any of the treated groups. But a lowering effect was seen in AlE treated group ($p<0.05$) after 60 days of its treatment. When compared with 30 days, no statistical significance was seen in any of the treated groups (Table No.3).

Serum HDL

Serum HDL was found to be less in the AlE treated group compared to the control group after treatment for 30 ($p<0.05$), 60 ($p<0.01$) and 90 ($p<0.001$) days. But LP treated group showed a higher value after 60 and 90 days of treatment ($p<0.05$). When compared with the levels of 30 days treatment, values were not statistically significant (Table No.3).

Serum VLDL

Serum VLDL was found to be unaffected in all treated groups after a period of 30 days. While a significant reduction was seen after 60 and 90 days ($p<0.05$) in AlE treated groups. When compared with the level after treatment for 30 days, no statistically significant effect was seen in 60 and 90 days of treatment (Table No.3).

Serum LDL

Serum LDL levels was found to be decreased in LP ($p<0.05$) and AlE ($p<0.01$) groups when compared with control after 30 days of its treatment. Similarly after 60 days the level of LDL was found to be decreased in LP, AlE ($p<0.001$) and AqE ($p<0.05$) treated groups compared with control. The LDL level in LP ($p<0.001$) and AlE ($p<0.001$) treated groups significantly reduced after 90 days. When compared with the 30 days treatment of respective groups, AlE, AqE ($p<0.01$) as well as LP ($p<0.001$) showed a decrease after 60 days of treatment, while the animals treated with LP ($p<0.05$) alone showed significance when compared with 60 days of treatment (Table No.3).

Histopathology

Control as well as animals treated with the leaf powder or extracts of *Aegle marmelos* sacrificed at the end of the experimental period showed no abnormal postmortem finding. There was no morphological change in liver, heart, kidney and lung (data not shown).

DISCUSSION

Administration of the leaf powder and extracts during long-term oral administration was found to cause alteration in the biochemistry of blood and other tissues. Assays for many serum enzymes have been proposed as a measure for hepato-cellular damage. Of these, aminotransferases have proved to be the most practical. AST occurs in all body tissues especially heart, liver and skeletal muscle. ALT occurs primarily in liver and to a lesser extent in kidney and skeletal muscle. Thus in the absence of acute necrosis or ischemia of other organs such as myocardium, high AST and ALT levels suggest liver cell damage. Although many studies have shown the extent and duration of serum enzymes elevation that parallel the extent of liver cell damage, precise quantitative correlation cannot be made in most clinical condition (Isselbacher and Lamont, 1981)[25].

Serum transaminases have been found to be increased during treatment with *Aegle marmelos* whole leaf powder and alcohol extract. The increase has been found to reach a maximum after 60 days of treatment. In case of leaf powder this effect does not substantiate for a toxic impact, as the levels have been found to come towards normalcy after 90 days of treatment and the fluctuations observed were found to be within normal range. However, the increase seen with alcohol extract has been found to be on a higher side, even after 90 days, though there was a decreasing trend compared to 60 days treatment. Thus the active principle responsible for a comparatively sustained effect in this aspect seems to reside in the alcohol extract because no biologically significant change has been noticed with the aqueous extract except for a mild increase and this was found to be within normal range after 60 days of treatment. The greater intensity and longer duration of action of this effect seen with the alcohol extract compared to the leaf powder could be due to the higher concentration of the principle present in the extract. Similar observation has been observed with serum alkaline

phosphatase also with an increase reaching peak after 60 days of treatment and showing a decline afterwards with whole leaf powder and alcohol extract. Serum alkaline phosphatases which hydrolyze synthetic phosphate esters is produced in many tissues like bone, liver and placenta, and excreted in bile. Most of the enzyme present in normal serum is derived from bone. In hepato-biliary disease, there is increased release of this hepatic enzyme into blood stream apparently due to an increased enzyme synthesis. In the absence of bone diseases and pregnancy, an elevated phosphatase level generally reflects impaired hepatic excretory function. Since the elevation in alkaline phosphatase seen after initial treatment has shown a decline after wards, in the present study, it could be inferred that no permanent obstructive impairment in excretion occurs during treatment with *Aegle marmelos*. Several serum proteins are synthesized by liver cells and an extensive liver damage may lead to decreased levels of these proteins. Fluctuations in the serum total proteins are also noticed with whole leaf powder and alcohol extract treatment. However in the present study these fluctuations are within normal limits and are not indicative of any extensive liver damage. The increase in serum transaminases, phosphatases and serum total proteins seen after the initial period of treatment has shown a declining afterwards suggesting that the fluctuations are due to initial stress and cannot be considered as reflection of any permanent toxic injury to liver. The quality and quantity of the biologically active principles residing in the leaf of *Aegle marmelos* seem to determine the extent and duration of the effects. Absence of fluctuations in blood urea during different duration of treatment indicates that the renal functions are not affected. Thus repeated administration of the leaf of *Aegle marmelos* or its extracts for longer durations has been observed to have no permanent toxic impact. Absence of any toxic impact is also reflected in the hematological parameters studied in the present investigation.

Administration of *Aegle marmelos* leaf powder and extracts on daily repeated administration has been found to cause no change in hematological parameters. RBC, WBC counts and hemoglobin content of blood recorded during various intervals of the experimental period have been found to be within normal ranges. Thus by correlating with biochemical parameters it could be said that *Aegle marmelos* leaf powder and extracts on chronic administration have been found to have no deleterious impact on vital tissues like liver, kidney and bone marrow. Results of the post mortem observation and histopathology studies of liver, heart, kidney and lung are confirmative of the absence of any toxic impact.

Though no toxic effect of the plant leaves has been reported earlier, centrilobular congestion and hydropic degeneration have been reported in Sprague-Dawley rats fed orally with the fruit powder (Arseculeratne *et al*., 1985)[8]. The histopathological assessment in the present study has not shown any hepatic lesion. Treatment (i.p) with the alcohol and aqueous leaf extracts for 14 days in rats has been reported to cause no adverse hepatic lesions (Veerappan *et al*., 2007)[26]. Both the leaf powder and leaf extracts have not caused any structural alteration in liver, heart, kidney and lung. In all these tissues the normal architecture has not been disturbed and no specific lesion was seen except for a mild interalveolar congestion in some of the animals treated with alcohol extract or aqueous extract. These observations assessed in conjunction with the results of the biochemical parameters, indicates that repeated administration of the plant material produces no toxic impact on essential tissues.

While having no toxic impact in general, the leaf of *Aegle marmelos* seems to possess pharmacological effects that can have clinical relevance as indicated by the biochemical alterations observed in the serum lipid profile. Long term administration of the leaf of *Aegle marmelos* or its extract has been found to have an influence on lipid metabolism. The whole leaf powder as well as alcohol extract have been found to reduce the serum cholesterol level. While the whole leaf powder decreased the serum cholesterol from 60 days onwards, the alcohol extract caused a reduction in serum cholesterol from 30th day onwards. The extent of reduction seen with alcohol extract was

more compared to the leaf powder treated group during corresponding duration of assessment. Strikingly an increase has been noticed in HDL levels; in groups treated with alcohol extract and whole leaf powder. The increment seen seems to depend on duration of exposure as the increase corresponds to the duration of exposure. A time dependent decline has been noticed in serum TGL during treatment with the alcohol extract. The decrease in triglyceride and VLDL levels has been noticed only with alcohol extract treated group, which has shown the effect from 60 day of treatment onwards. However LDL cholesterol is found to be decreased in alcohol extract and whole leaf powder treated groups during all the period of assessment when compared with control group. It is to be noticed that the effects on serum lipoproteins have been found to be altered by treatment with whole leaf powder and alcohol extract only. A reduction in LDL with aqueous extract is seen only after 60 days and this effect seems to be a transient, as such a reduction is not seen after 90 days of treatment. Thus the reduction seen with the LDL in powder and alcohol extract treated groups are not related to duration of treatment whereas, the effect on the HDL has shown an increasing trend. Thus the alcohol extracts and to certain extent in whole leaf powder are found to decrease total cholesterol, triglycerides, VLDL, LDL with an increase in HDL. Thus the plant seems to posses bioactive principles that have an effect on the lipid metabolism.

Though all the values recorded in tests and control are found to be within normal range, a significant difference has been noticed in groups treated with extracts/powder, when compared with control group. These observations has provided an additional confirmation about the previous work of Kesari *et al.*, (2006)[10] who reported that, oral administration of *Aegle marmelos* aqueous seed extract for 14 days to severe diabetic rats caused a reduction in enhanced levels of total cholesterol, LDL and triglycerides but increased HDL levels. Ponnachan *et al*., (1993)[12] have reported a reduction in serum cholesterol levels, with administration of *Aegle marmelos* leaf aqueous extract to alloxan induced diabetic rats. In studying isoproterenol induced myocardial infarction, Rajadurai and prince (2005)[13] reported a reduction in serum lipids and lipoproteins levels in rats pretreated with aqueous leaf extract of *Aegle marmelos*. It has to be pointed out that Aegeline, an alkaloidal amide present in leaf of *Aegle marmelos* has been reported to cause a decrease in total cholesterol, triglycerides and free fatty acids with an increase in HDL in dyslipidemic hamsters models after oral administration for 7 days (Narender *et al*., 2007)[11]. Hypercholesterolemia has been recognized as a risk factor and led to the development of drugs that reduce cholesterol levels (Mahley and Bersot, 2001)[27]. Hyperlipidemia is a major cause of atherosclerosis and atherogenic-associated conditions, such as coronary heart diseases (CHD), ischemic cerebrovascular diseases and peripheral vascular diseases. CHD are stated to be reduced by as much as 30-40% and its non fatal events are also reduced when hypercholesterolemic patients treated with moderate doses of hypolipidemic drugs (Scandinavian simvastatin survival study group, 1994)[28]. Hyperlipidemia with elevated levels of triglycerides or cholesterol and reduced HDL-C levels are consequence of several factors that affect the concentration of the various plasma lipoproteins. These factors include life style, behaviour, genetic or metabolic conditions that influence plasma lipoprotein metabolism. VLDL are produced in the liver and are synthesized when triglycerides production is stimulated by an increased flux of free fatty acids or by increased *de novo* synthesis of fatty acids by the liver (Mahley and Bersot, 2001)[27]. LDL particles arise from catabolism of IDL and have a half-life of 1-2 days, which accounts for the higher plasma concentration of LDL than of VLDL and IDL, the latter lipoproteins having lesser plasma half-life. In accordance with this the serum LDL in the control and treated animals was found to be higher than that of VLDL in our study. Plasma clearance of LDL particles is primarily by LDL receptors and to a smaller proportion by non receptor clearance mechanism (Brown and Goldstein, 1986)[29]. The liver expresses large complements of LDL receptors and removes approximately 75% of

all LDL from the plasma (Dietschy *et al*., 1993)[30]. Consequently, manipulation of hepatic LDL receptor expression is a most effective way to modulate plasma LDL and cholesterol levels. The dietary control by decreased consumption of saturated fats and cholesterols, and pharmacological treatment with statins of hypercholesterolemia act by enhancing hepatic LDL receptor expression (Woollett and Dietschy, 1994)[31]. Thus the probability of decreased LDL levels recorded in the alcohol extract and leaf powder treated groups due to an effect on the LDL receptor expression needs to be studied. Since LDL become atherogenic when they are modified by oxidation (Steinberg, 1997)[32] the possibility of this plant having the therapeutic potential for use in prevention of atherosclerosis deserves to be explored. HDL is protective lipoproteins that decrease the risk of coronary heart diseases; thus, high levels of HDL are desirable. This protective effect may result from the participation of HDL in reverse cholesterol transport, the process by which excess cholesterol is acquired from the cell and transported to the liver for excretion. HDL also may inhibit oxidative modification of LDL through the action of paraoxonase, an HDL-associated antioxidant protein. Thus the higher serum HDL recorded in alcohol and powder treatment is an added advantage of the plant's therapeutic potential to be used in cases of hyperlipidemia and atherosclerosis. Hyperlipidemia may be secondary to several drug therapies resulting in elevated lipid levels requiring appropriate modification of drug therapies. Hence usefulness of *Aegle marmelos* in suppressing drug-induced hyperlipidemia needs to be studied further as it can reduce gross modifications in such therapies. β-Sitosterol and Rutin have been isolated from *Aegle marmelos* (Patra *et al*., 1979 and Sharma *et al*., 1980)[6,7] as phytoconstituents. β-Sitosterol has been reported to have effect on lipids. Hypocholesterolemic effect which included a reduction of plasma total cholesterol, triglycerides, LDL-C associated with increase in HDL-C levels was observed in a low-dose formulation of soy proteins supplemented with isolated β-sitosterol in a ratio of 4:1 in moderate hypercholesterolemic subjects (Cicero *et al*., 2002)[33]. Smith *et al*, (2000)[34] reported that feeding hamsters with high saturated fat and cholesterol diet, supplemented with β-sitosterol for 28 days, caused a decrease in levels of total cholesterol, triglycerides and total cholesterol/HDL-cholesterol ratio by 33%, 49% and 48% respectively and suggested a decreased absorption of cholesterol and lower incorporation in chylomicrons and VLDL + IDL as possible mechanisms. In addition to this Park *et al*, (2002)[5] reported that, rutin and tannic acid when supplemented with a semisynthetic diet to rats significantly lowered the plasma lipid and hepatic cholesterol levels by promoting the excretion of fecal sterols and also by decreasing absorption of dietary cholesterols. Rutin on intraperitoneal administration to Wistar rats caused a reduction of cholesterol and triacylglycerol levels (Santos *et al*., 1999)[35]. Thus there is a possibility of β-sitosterol and Rutin reported to be present in *Aegle marmelos* may be responsible for observed alteration in the serum lipids. In this regard rutin has been identified in the alcohol extract (Porchelvan and Murali., 2014)[17] of the present investigation.

Table No.1: Effect of *Aegle marmelos* on serum parameters in Wistar albino rats

Serum parameters	DAY 30				DAY 60				DAY 90			
	CON	LP	AqE	AlE	CON	LP	AqE	AlE	CON	LP	AqE	AlE
ALT (IU/L)	30.18 ± 5.59	36.81 ± 7.46	31.53 ± 4.97	43.21 ± 5.20 a**	29.25 ± 5.65	40.79 ± 4.47 a**	38.05 ± 4.20 a*	53.98 ± 6.64 a*** b**	29.42 ± 4.01	38.60 ± 2.36 a*	35.66 ± 3.91	41.17 ± 3.78 a c**
AST (IU/L)	43.14 ± 6.82	54.18 ± 4.90 a*	47.16 ± 6.96	59.85 ± 7.66 a**	44.26 ± 6.01	56.77 ± 4.72 a**	52.14 ± 3.63	71.14 ± 5.83 a*** b*	42.04 ± 3.49	49.97 ± 7.72	46.54 ± 5.93	53.24 ± 6.9 a* b***
ALP (IU/L)	346.97 ± 13.65	374.93 ± 35.78	369.96 ±30.50	418.83 ± 33.63 a**	351.76 ± 21.88	401.04 ± 34.21 a**	375.96 ± 18.92	429.83 ± 23.11 a***	355.25 ± 19.49	394.31 ± 25.52 a*	369.04 ± 17.87	397.22 ± 24.20 a*
Total proteins (mg/100ml)	5.27 ± 0.53	6.15 ± 0.50 a*	5.91 ± 0.57	6.58 ± 0.45 a**	5.32 ± 0.43	6.29 ± 0.48 a*	5.80 ± 0.59	6.74 ± 0.65 a***	5.30 ± 0.37	5.88 ± 0.54	5.56 ± 0.42	6.19 ± 0.35 a*

n=6. Values are mean ± S.D. CON – Control, LP – Leaf Powder 2000mg/Kg , AqE - Aqueous leaf extract 400mg/Kg, AlE – Alcohol leaf extract 400mg/Kg. Route – Oral. a :Comparisons made with control (CON) group, b :Comparisons made with 30 days value of respective group, c :Comparisons made with 60 days value of respective group. * $p < 0.05$ **$p < 0.01$ ***$p < 0.001$.

Table No.2: Effect of *Aegle marmelos* on hematological parameters in Wistar albino rats

Blood parameters	DAY 30				DAY 60				DAY 90			
	CON	LP	AqE	AlE	CON	LP	AqE	AlE	CON	LP	AqE	AlE
Erythrocyte counts (millions/ml)	6.19 ± 0.74	6.62 ± 0.90	6.78 ± 0.83	6.49 ± 1.05	6.72 ± 0.84	6.91 ± 0.67	7.09 ± 0.83	7.19 ± 0.99	7.14 ± 0.90	7.20 ± 1.02	7.55 ± 0.82	6.99 ± 0.65
Leuckocyte counts (leuckocytes/ml)	6729.03 ± 20.02	7755.50 ±774.86	7551.77 ±663.88	6945.70 ±1075.52	6602.63 ±749.76	6946.70 ±680.00	6827.27 ±900.61	6622.28 ±701.36	6840.15 ±856.44	7028.88 ±706.88	6824.32 ±690.32	6815.67 ±782.79
Hemoglobin (gms/100ml)	11.82 ±0.62	11.28 ±0.85	11.06 ±0.96	11.08 ±0.75	11.67 ±0.34	11.54 ±0.701	11.35 ±0.55	11.77 ±0.67	12.23 ±1.06	12.25 ±0.90	11.43 ±0.78	11.87 ±0.71
Urea (mg %)	29.43 ± 4.53	30.48 ± 4.48	33.21 ± 3.96	27.58 ± 6.06	29.91 ± 4.50	31.11 ± 4.65	33.85 ± 3.95	27.25 ± 5.84	27.83 ± 2.71	28.68 ± 3.63	31.20 ± 5.15	30.06 ± 4.50

n=6. Values are mean ± S.D. CON – Control, LP – Leaf Powder 2000mg/Kg, AqE - Aqueous leaf extract 400mg/Kg, AlE – Alcohol leaf extract 400mg/Kg. Route – Oral. a :Comparisons made with control (CON) group, b :Comparisons made with 30 days value of respective group, c :Comparisons made with 60 days value of respective group.

Table No.3: Effect of *Aegle marmelos* on Serum lipid parameters in Wistar albino rats

Lipid Parameters	DAY 30				DAY 60				DAY 90			
	CON	LP	AqE	AlE	CON	LP	AqE	AlE	CON	LP	AqE	AlE
Total Cholesterol (mg/dl)	89.00 ± 5.90	83.25 ± 4.73	88.27 ± 4.86	80.74 ± 4.32 a*	87.24 ± 5.61	78.38 ± 5.02 a*	82.36 ± 4.40	71.90 ± 5.67 a*** b*	89.47 ± 6.68	80.57 ± 4.15 a*	87.26 ± 4.20	74.76 ± 6.35 a***
Triglycerides (mg/dl)	147.56 ± 11.16	144.03 ± 9.91	146.26 ± 10.99	135.59 ± 11.12	144.37 ± 11.89	142.61 ± 9.85	140.60 ± 10.33	126.43 ± 10.85 a*	144.64 ± 12.69	134.43 ± 8.95	142.52 ± 10.34	125.63 ± 13.14 a*
HDL (mg/dl)	20.61 ± 2.13	21.79 ± 1.77	20.71 ± 2.23	24.27 ± 1.80 a*	20.81 ± 1.94	23.65 ± 0.82 a*	21.75 ± 2.35	24.76 ± 1.20 a**	20.89 ± 2.66	24.17 ± 1.15 a c*	21.67 ± 2.09	26.39 ± 1.72 a***
VLDL (mg/dl)	29.51 ± 2.23	28.80 ± 1.98	29.25 ± 2.19	27.11 ± 2.22	28.87 ± 2.38	28.52 ± 1.97	28.12 ± 2.06	25.28 ± 2.17 a*	28.93 ± 2.53	26.88 ± 1.79	28.50 ± 2.07	25.12 ± 2.63 a*
LDL (mg/dl)	38.87 ± 3.68	32.64 ± 2.88 a*	38.30 ± 2.91	29.35 ± 5.25 a**	37.55 ± 1.84	26.20 ± 2.39 a b***	32.49 ± 2.20 a* b**	21.85 ± 2.20 a*** b**	39.64 ± 1.61	29.51 ± 1.28 a***	37.09 ± 2.31 c*	22.41 ± 1.79 a*** b**

n=6. Values are mean ± S.D. CON – Control, LP – Leaf Powder 2000mg/Kg, AqE - Aqueous leaf extract 400mg/Kg, AlE – Alcohol leaf extract 400mg/Kg. Route – Oral. a :Comparisons made with control (CON) group, b :Comparisons made with 30 days value of respective group, c :Comparisons made with 60 days values respective group. * $p < 0.05$ **$p < 0.01$ ***$p < 0.001$.

CONCLUSION

The results of the current study showed that the whole leaf powder and the leaf extracts of the Aegle marmelos chronic treatment had no toxic impact during repeated daily administration and are found to be safe for long-term administration. The alteration in serum lipid profile with a decrease in triglycerides and increase in HDL can have therapeutic utility in hypercholesterolemia and dyslipidemia.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Chakravarti R N, Dasgupta B. β-sitosterol from the leaves of *Aegle marmelos Correa*, *J Indian Chem Soc*, 35(3), 1958, 194-6.
2. Chatterjee A, Bose S, Srimany S K. Studies on the constitution, stereochemistry, and synthesis of Aegeline, an Alkaloidal-Amide of *Aegle marmelos* Correa, *J Org Chem*, (24), 1959, 687-90.
3. Chatterjee A, Majumder R. Structure of Aegelenine, the minor alkaloid of *Aegle marmelos* Correa, *Indian J Chem*, 9, 1971, 763-66.
4. Govindachari T R, Premila M S. Some Alkaloids from *Aegle marmelos*, *Phytochem*, 22(3), 1983, 755-57.
5. Park S Y, Bok S H, Jeon S M, Park Y B, Lee S J, Jeong T S, Choi M S. Effect of rutin and tannic acid supplements on cholesterol metabolism in rats, *Nutr Res*, 22, 2002, 283-95.
6. Patra A, Mukhopadhyay A K, Ghosh A, Mitra A K. Constituents of *Aegle marmelos*: Carbon-13NMR spectra of Aurapten and Marmin, *Indian J Chem*, 17B, 1979, 385-87.
7. Sharma B R, Rattan R K, Perveen sharma. Constituents of leaves and fruits of *Aegle marmelos*, *Indian J Chem*, 19B, 1980, 162.
8. Arseculeratne S N, Gunatilaka A A, Panabokke R G. Studies of medicinal plants of Sri Lanka. Part 14: Toxicity of some traditional medicinal herbs, *J Ethnopharmacol*, 13(3), 1985, 323-35.
9. Jagetia G C, Venkatesh P, Baliga M S. *Aegle marmelos* (L.) Correa inhibits the proliferation of transplanted Ehrlich ascites carcinoma in mice, *Biol Pharm Bull*, 28(1), 2005, 58-64.
10. Kesari A N, Gupta R K, Singh S K, Diwakar S, Watal G. Hypoglycemic and antihyperglycemic activity of *Aegle marmelos* seed extract in normal and diabetic rats, *J Ethnopharmacol*, 107(3), 2006, 374-9.
11. Narender T, Shweta S, Tiwari P, Papi Reddy K, Khaliq T, Prathipati P, Puri A, Srivastava A K, Chander R, Agarwal S C, Raj K. Antihyperglycemic and antidyslipidemic agent from *Aegle marmelos*, *Bioorg Med Chem Lett*, 17(6), 2007, 1808-11.
12. Ponnachan P T, Paulose C S, Panikkar K R. Effect of leaf extract of *Aegle marmelos* in diabetic rats, *Indian J Exp Biol*, 31(4), 1993, 345-7.
13. Rajadurai M, Prince P S. Comparative effects of *Aegle marmelos* extract and alpha-tocopherol on serum lipids, lipid peroxides and cardiac enzyme levels in rats with isoproterenol-induced myocardial infarction, *Singapore Med*, 46(2), 2005, 78-81.
14. Rao V V, Dwivedi S K, Swarup D, Sharma S R. Hypoglycaemic and antihyperglycaemic effects of *Aegle marmelos* leaves in rabbits, *Curr Sci*, 69(11), 1995, 932-33.
15. Seema P V, Sudha B, Padayatti P S, Abraham A, Raghu K G, Paulose C S. Kinetic studies of purified malate dehydrogenase in liver of streptozotocin-diabetic rats and the effect of leaf extract of *Aegle marmelose* (L.) Correa ex Roxb, *Indian J Exp Biol*, 34(6), 1996, 600-2.
16. Sharma S R, Dwivedi S K, Swarup D. Effect of *Aegle marmelos* leaves on pancreatic β cells and

oral glucose tolerance in diabetic rats, *Indian J Animal Sciences*, 67(9), 1997, 827-28.
17. Porchelvan V, Venkatakrishnamurali R. The Aegle marmelos leaves cationic salts composition and phytochemical screening, *Asian Journal of Phytomedicine and Clinical Research*, 2(2), 2014, 100-104.
18. Wooten I D P. In: Microanalysis in medical biochemistry, *J and A Churchill Ltd, London*, 1964, 101-3.
19. King J. The phosphohydrolases - acid and alkaline phosphatases, In: Practical clinical enzymology, *D.Van Nostr and Co Ltd, London*, 1965a, 191-208.
20. Lowry O H, Rosebrough N J, Farr A L and Randall R J. Protein measurement with the Folin phenol reagent, *J Biol Chem*, 193(1), 1951, 265-75.
21. Garvey J S, Cremer N E, Sussdorf D H. Methods in immunology, *W.A.Benjamin, Inc., U.S.A*, 3rd edition, 1977, 1-545.
22. Hawk P B, Oser B L, Summerson W H. Practical physiological chemistry, *Churchill, London*, 14th edition, 1966.
23. Varley H, Gowenlock A H, Bell M. Hormones, vitamins, drugs and poisons, In, Practical clinical biochemistry, *William Heinemann Medical Books Ltd, London*, 5th edition, Vol-II, 1976, 222-3.
24. Bancroft J D, Cook B C. Manual of histological techniques, *Churchill Livingstone, Edinburgh*, 1984.
25. Isselbacher K J, Lamont J T. Diagonistic procedures in liver disease. In: Harrison's Principles of internal medicine, *McGraw-Hill International Book Company*, 9th edition, 1981, 1451-70.
26. Veerappan A, Miyazaki S, Kadarkaraisamy M, Ranganathan D. Acute and subacute toxicity studies of *Aegle marmelos* Corr., an Indian medicinal plant, *Phytomedicine*, 14(2-3), 2007, 209-15.
27. Robert W. Mahley and Thomas P. Bersot, Drug therapy for hypercholesterolemia and dyslipidemia, In: The pharmacological basis of therapeutics, Eds: Joel G. Hardman, Lee E, Limbird, Alfred Goodman Gilmans, *Mcgraw-Hill, Newyork*, 10th edition, 2001, 971-995.
28. Lancet. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S), *Lancet*, 344(8934), 1994, 1383-9.
29. Brown M S, Goldstein J L. A receptor-mediated pathway for cholesterol homeostasis, *Science*, 232(4746), 1986, 34-47.
30. Dietschy J M, Turley S D, Spady D K. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans, *J Lipid Res*, 34(10), 1993, 1637-59.
31. Woollett L A, Dietschy J M. Effect of long-chain fatty acids on low-density-lipoprotein-cholesterol metabolism, *Am J Clin Nutr*, 60(6 Suppl), 1994, 991S-996S.
32. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance, *J Biol Chem*, 272(34), 1997, 20963-6.
33. Cicero A F, Fiorito A, Panourgia M P, Sangiorgi Z, Gaddi A. Effects of a new soy/beta-sitosterol supplement on plasma lipids in moderately hypercholesterolemic subjects, *J Am Diet Asso*, 102(12), 2002, 1807-11.
34. Smith D, Montoro A E, Jimenez F P, Botet J P, Pereperez J J, Ordovas J M. Effect of a high saturated fat and cholesterol diet supplemented with squalene or β-sitosterol on lipoprotein profile in F1B hamsters, *Nutrition Research*, 20(9), 2000, 1309-18.
35. Santos K F, Oliveira T T, Nagem T J, Pinto A S, Oliveira M G. Hypolipidaemic effects of naringenin, rutin, nicotinic acid and their associations, *Pharmacol Res*, 40(6), 1999, 493-496.

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