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Research Article

Effect of Ezenus on Aged Rats

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ABSTRACT
Age-related changes in body are known to be related to the development of several disorders. The present study was undertaken to determine the effect of Ezenus on biochemical function and oxidative stress markers in aged rats. Animals were divided into three groups including control, moderate-dose and high-dose Ezenus treated groups. Animals were treated for a period of 90 days (twice daily) and controls were given equivalent volume of vehicle. Blood was withdrawn retroorbitally on days 0, 30, 60 and 90. Biochemical as well as oxidative stress parameters were evaluated in the sera. This included SGPT, SGOT, ALP, Glucose, Total protein, Triglycerides, HDL-C, SOD, catalase and malondialdehyde levels. Modest alteration was observed in the biochemical parameters. It was found that high dose of Ezenus prevented the minor unfavourable changes in SGPT, SGOT and ALP levels in the aged animals. Glucose, total protein, triglycerides and HDL-C levels were found to be comparable between all the groups. Interesting findings were observed in the oxidative stress markers. These parameters revealed that there was an age-dependent decline in antioxidant status of the animals with a concurrent rise in oxidative stress. Changes in oxidative stress and antioxidant levels were found to be significantly different from same groups as compared to baseline values. However, Ezenus therapy was able to significantly prevent the development of oxidative stress and maintained the levels of antioxidant enzymes as well. Ezenus therapy, at high doses, significantly prevented elevation in MDA levels. Hence, it can be concluded that Ezenus is safe at repeated dosing and also eliminates oxidative stress in rats.
Key words: Oxidative stress, SGOT, Ezenus, Rat

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INTRODUCTION:
A major characteristic of an aging organism is its progressive functional decline, including a loss of adaptive responses to stresses, with the passage of time (1). One of the major cause of aging is the concept of oxidative stress (2). Oxidative stress initiates a wide variety of oxidative reactions including per-oxidation of lipids, proteins, nucleic acids and contributes to the process of aging. Oxidative stress is described generally as a condition under which increased production of free radicals, reactive oxygen species (including singlet oxygen and reactive lipid per-oxidation products, such as reactive aldehydes and peroxides), and oxidant-related reactions. There is a growing awareness that oxidative stress (OS) plays a key role not only in the aging process but also involved in the various pathophysiological states. The OS theory, first

proposed by Harman (1956), suggested that age-related biochemical and physiological decline is associated with a chronic state of imbalance between the production of oxidants and the intracellular antioxidant capacity (3). This can trigger deleterious changes in numerous cellular processes, leading to a loss in metabolic function. Reactive oxygen species (ROS), such as hydroxyl radicals ($HO\cdot^{-}$), superoxide anions ($O2\cdot$) and hydrogen peroxide (H_2O_2) are continuously produced endogenously as by-products of normal cellular respiration. Harper et al (2004) have already showed that $O2\cdot^{-}$ leakage was significantly higher in isolated hepatocytes cultured from 30 month old mice, compared to 3 month old mice. Impairment in mitochondrial function and the consequent reduction in ATP-production may explain both the pro-oxidant shift and energy deficit in aging (4).

Ezenus is a polyherbal sugar-free candy intended to exhibit favourable effects on ageing and related problems. The active ingredients present in Ezenus (*Andrographis paniculata* and *Vitis vinifera*) have been previously reported as neuroprotective, hepatoprotective, antioxidant, detoxifier, immunomodulator, anti-oxidant and anti-depressant activities (5-7). The major roles of these ingredients are to reduce the production of inflammatory mediators responsible for the signs of inflammation. They also gives protection from oxidative stress in the brain. It is a strong hepatic detoxifier. Based on these reports, the purpose of this study was to determine the effect of 90 days repeated dose of Ezenus on aged rats.

MATERIALS AND METHODS

Chemicals:

Ezenus candies were procured locally. Thiobarbaturic acid (TBA; 30231), 2,4-Dinitrophenyl hydrazine (DNPH; 52144), Xanthine (03472), Bovine Serum albumin (BSA; 27423), reduced glutathione (GSH; 13679) and other biochemicals were procured from Himedia laboratories Ltd, Mumbai, India and Sigma, St. Louis, MO, U.S.A. Other biochemical parameters (SGOT, SGPT, Glucose, Triglyceride and Cholestrol) were measured by fully automatic biochemistry analyzer using Erba Commercial Kits, Germany. Other reagents, solvents or chemicals used in the study were of analytical grade.

Animal Husbandry:

Total 30 male sprague dawley rats were taken for the study. The animals were selected as per their age (13-17 weeks). The animals were further divided into 3 groups (n=10 per group). They were housed in animal facility of Venus Medicine Research Centre, Baddi, H.P. in individual polypropylene cages. Rats were provided with commercially available feed and water ad libitum. The experimental room was air conditioned with temperature 22 ± 3°C, humidity 55 ± 5%, and with artificial fluorescent light (12:12 h of light and dark cycle). Experiment was carried out after approval from the institutional animal ethical committee (IAEC) and all the procedures were in accordance with the CPCSEA guidelines, Ministry of Environment and Forests, Govt. of India.

Groups and Treatment:

G-I : Control- treated with water.

G-II: Moderate dose- treated with Ezenus (387.5 mg/Kg body weight, b.i.d) i.e equivalent to three candies of 2.5 grams each divided in two equal doses per day.

G-III: High dose treated with Ezenus (775 mg/kg body weight, b.i.d) i.e equivalent to six candies of 2.5 grams each divided in two equal doses per day.

Doses:

The animal doses for treatment were calculated based on equivalent human dose. The dose single Ezenus candy of 2.5 g for an adult human (60 Kg), equals to 258 mg/kg for rats based on body surface area factor. Higher doses are calculated accordingly. The respective doses of Ezenus were given to animals through oral route, to each group of animals twice daily for 90 days. Blood samples were collected through retro-orbital plexus on 0, 30^{th}, 60^{th} day and at the end of experiment (90^{th} day), for measurement of biochemical, oxidative stress (lipid peroxidation) and anti-oxidant status (superoxide dismutase and catalase) parameters in serum samples of all the groups.

Biochemical Analysis:

Clinical biochemistry determinations to investigate effect of Ezenus on age-related biochemical profile and oxidative stress markers were performed on blood samples obtained from all animals on the terminal day of the study. Overnight fasting of the animals prior to blood sampling was done. Investigations of serum included glucose, total protein, triglycerides, HDL-C, enzymes indicative of hepatocellular effects (SGPT and SGOT), antioxidant enzymes (SOD and catalase) and marker of oxidative stress i.e. lipid peroxidation (MDA). All biochemical parameters were measured in the serum as per previously reported standard methods and commercial kits (8-10).

Statistical analysis:

All values were expressed as Mean ± SEM. Means were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test to determine statistical difference between control *vs* moderate and high dose Ezenus treated groups. $P<0.05$ was considered to be statistically significant.

RESULTS

The biochemistry parameters of control, moderate and high dose treated groups on 0, 30th, 60th and 90th day presented in figures 1 to 10.

SGOT and SGPT:

The SGPT and SGOT values did not show any significant changes in moderate as well as high dose treated groups. However, it was observed that the liver enzymes of the control group was slightly increased which is a physiological phenomenon in old aged rats. Ezenus treatment showed modestly lowered values of these enzymes probably due to mild improvement of liver function as compared to respective controls on 90 days (Figures 1-2).

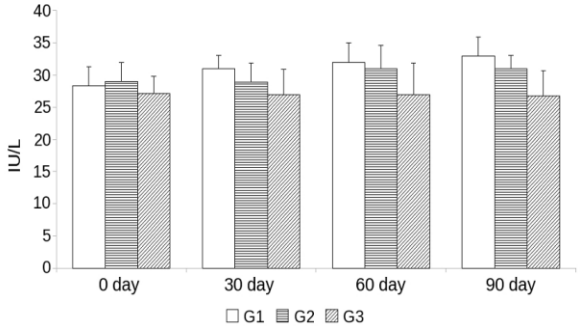

Figure 1: SGPT levels in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose, G3-High dose.

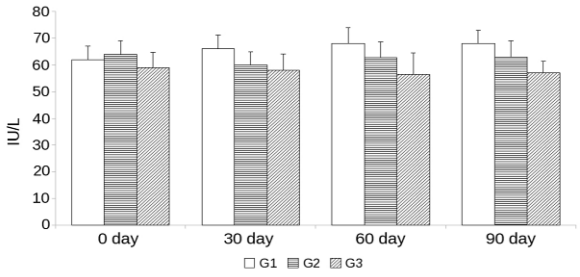

Figure 2: SGOT levels in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose, G3-High dose.

ALP:

The results suggested that there is a minor age related elevation in the serum ALP in the control as well as treated animals. However, such a change was not found to be statistically significant and values in both the age groups were found to be well within the normal range. It could be observed that the higher dose treated groups exhibited mildly better values as compared to control group. The changes were insignificant irrespective of the groups (Figure 3).

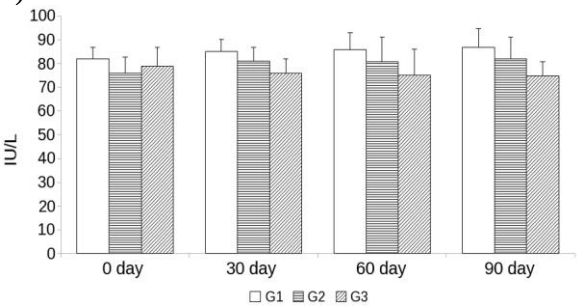

Figure 3: ALP levels in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose, G3-High dose.

Glucose, Total Protein, Triglyceride and HDL:

The values of Glucose, Total Protein, Triglyceride and HDL suggested that major fluctuations were not observed in the different groups. Age-related changes were also found to be insignificant. It could be inferred from the data that aging did not show any significant changes in these biochemical parameters (Figures 4-7).

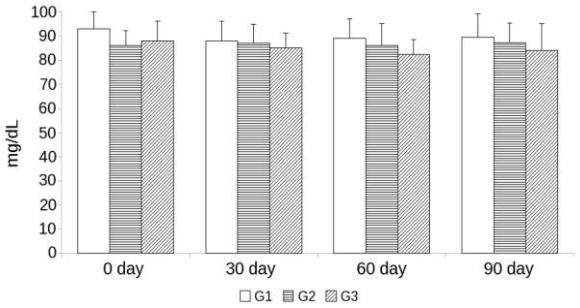

Figure 4: Glucose levels in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose, G3-High dose.

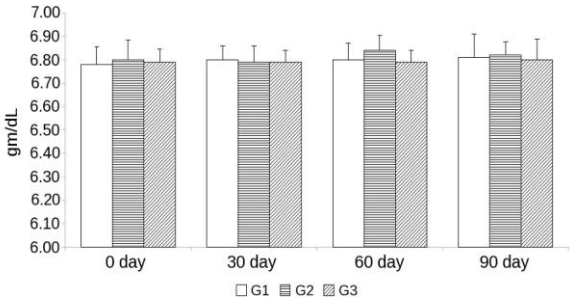

Figure 5: Total protein levels in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose G3-High dose.

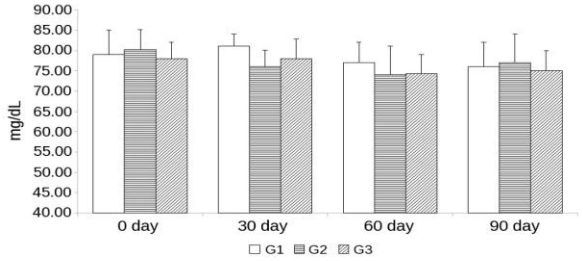

Figure 6: Triglyceride levels in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose G3-High dose.

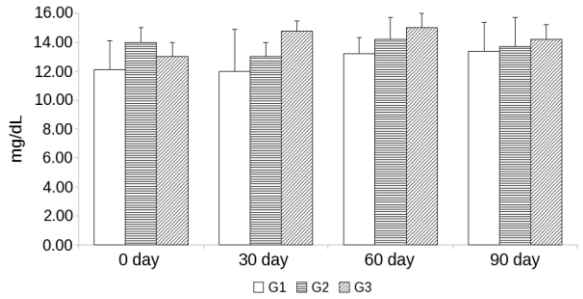

Figure 7: HDL-C levels in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose, G3-High dose**.**

Antioxidant enzymes:

Superoxide-dismutase activity

The SOD values were modestly increased in Ezenus treated rats as compared to control group at the end of 90 days. This suggested that Ezenus was able to prevent aging-related decline in antioxidant status Control animals showed lower SOD levels as compared to the Ezenus treated groups. It was observed that, at the end of 90 days SOD levels in the high-dose treated group were significantly higher (P<0.05) as compared to control (Figures 8).

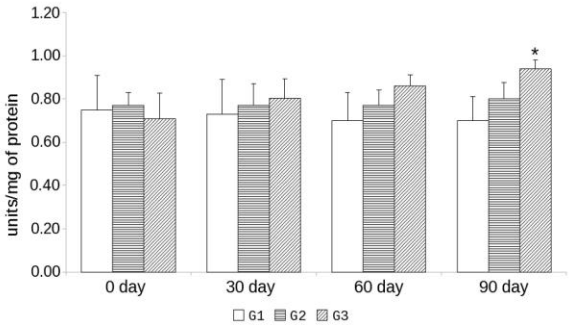

Figure 8: Effect of Ezenus on superoxide dismutase levels in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose G3-High dose.
* represents P<0.05 as compared to control after 90 days of treatment.

Catalase activity

The Catalase values were found to be increased in Ezenus treated groups as compared to the control group. The high dose group showed significant increase in catalase activity (P<0.05) as compared to control animals after 90 days of treatment(Figure 9).

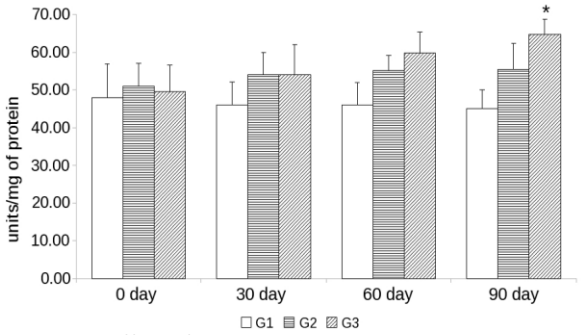

Figure 9: Effect of Ezenus on Catalase levels in all groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose, G3-High dose. * represents P<0.05 as compared to control after 90 days of treatment.

Lipid peroxidation activity (Malondialdehyde levels)

Age-related increase in lipid peroxidation was witnessed through malondialdehyde (MDA) levels. The data suggested that the control animals exhibited increase in MDA levels on days 0, 30, 60 and 90. However ,it was observed that Ezenus treated groups were able to mitigate rise in MDA levels to a significant extent. In fact, the Ezenus treated animals from the high-dose group significantly lowered MDA levels (P<0.05), suggesting a profound prevention of lipid peroxidation with Ezenus. Data is shown in figures (Figure10).

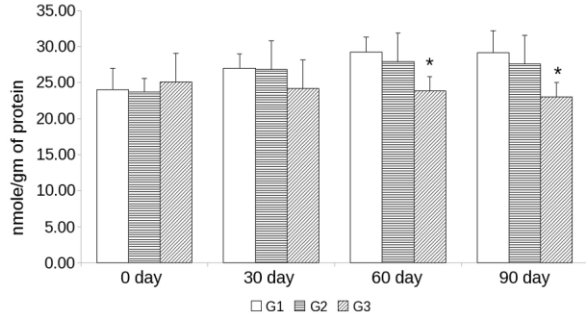

Figure 10: Effect of Ezenus on lipid peroxidation in all the groups. All data are mean ± SEM (n=10). G1-Control, G2-Moderate dose G3-High dose. * represents P<0.05 as compared to day-matched control group after 60 and 90 days of treatment respectively.

DISCUSSION

During the process of metabolism that takes place in our body at cellular level, oxygen is continuously converted to free oxygen radicals. Free radicals are a group of unstable reactive oxygen (RO) compounds that are highly toxic often leading to body cell damage and death (2-3). As production of free oxygen radicals in our body is a continuous process and requires immediate elimination, our body is equipped with molecules called antioxidants that act as scavengers by neutralizing these free radicals. An

unhealthy lifestyle and improper diet may lead to deficiency of antioxidants leading to an imbalance between free radicals and antioxidants in our body causing accumulation of toxic free radicals that form a "vicious cycle of toxic overload" or "oxidative stress". Oxidative stress also occurs over a period of time during the physiological aging process. The repercussions of oxidative stress includes damage to vital organs like heart, liver and nervous system thus increasing the risk of lifestyle diseases like cancer, arteriosclerosis, diabetes and neurological diseases (4, 11). There is accumulation of toxins and by-products of oxidative stress in the blood leading to increased impurities in blood. Circulation of such impure blood leads to development of problems related to skin and internal organs as well. The present study was carried out to identify the effect of age on various biochemical and oxidative stress markers in aged rats and also to evaluate the effect of Ezenus on aged animals so as to understand its role in age-related oxidative stress.

The present study demonstrated that different doses of Ezenus administered to aged animals did not result in any clinically significant adverse change in the biochemical parameters studied herewith. Thus, tests of liver function like SGPT and SGOT, ALP levels, glucose levels, total protein, triglycerides as well as HDL-C levels remained within the accepted laboratory range. It was observed that, control animals did show minor decrease in the liver function, which may be considered as manifestation of age-related change. Though, this change was not found to be statistically significant from baseline values at day 0. However, Ezenus showed betterment of liver function. This indicated that since there is significant built-up of toxins in the blood due to oxidative stress, improvement in liver function could be a useful strategy towards elimination of such toxins and hence purification of blood from toxins. Since a preventive effect on triglyceride elevation was observed in the Ezenus group, it can be assumed that improved liver function increases the recycling of chylomicrons thus maintaining the normal serum lipid status. Further, no significant difference was found between glucose, total protein and HDL-C from either control or any Ezenus treated groups throughout the period of the study. Additionally, it is worth mentioning that hepatic transaminases and ALP activities remain fairly normal with repeated Ezenus therapy for 90 days. Other parameters like glucose, total protein and HDL-C did not show any changes with Ezenus administration. Thus, it is evident that the use of high doses of Ezenus for longer periods is safe and is not associated with any type of toxicity.

On the other hand, interesting findings were observed in relation to the oxidative stress markers. It was observed that as the study progressed, control animals showed an increase in oxidative stress, observed from a minor decrease in SOD and catalase activity with a modest increase in lipid peroxidation. This can also be correlated with depletion in the levels of glutathione, tocopherols or other antioxidants in the body. Widespread alkylation and peroxidation occurs leading to decrease in the purity of the blood. This may be attributed to age-related increase in oxidative stress. These changes may be a direct result of the insult mediated by free radicals on different tissues in the body. Ezenus treatment, albeit the higher dose, was able to prevent any such toxic insult in the experimental animals and thus it may be speculated that Ezenus exhibits antioxidant effect and purifies blood by increasing liver function. Further, it has been reported that ingredients of Ezenus: *Andrographis paniculata* and *Vitis vinifera*, show blood purifying activities (5, 12). The antioxidant effects of these ingredients are well documented suggesting that these effects might be responsible for the anti-oxidant effects observed in the present study (7, 13-14).

From the study it can be evidenced that Ezenus therapy was able to prevent any age-related effect upon hepatic enzymes, and does not affect other lipid parameters, blood glucose or total protein levels. Antioxidant status was improved with a reduction in oxidative stress in the Ezenus treated groups as compared to controls. The blood purification activity can thus be ascribed to be an extension of the hepatoprotective and antioxidant activity of Ezenus. Moreover, administration of Ezenus affects biochemical profile and antioxidant status in a favorable manner suggesting that repeated administration of high doses of Ezenus for prolonged periods is safe, in fact favorable to maintain homeostasis of the body. Hence, it can be concluded from the study that, Ezenus is safe on repeated dosing in aged rats, and displays a favorable effect in aged animals.

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