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Sub-acute toxicity profile of a modified resveratrol supplement

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ABSTRACT

Longevinex, a nutraceutical formulation containing Resveratrol as the main component along with other polyphenolics exhibits diverse health benefits but systemic safety studies are lacking. Hence, to test the safety of Longevinex use for therapeutic purposes, 50 Sprague Dawley rats were randomly divided into five groups ($n = 10$; 5M, 5F) wherein group I as vehicle treated control, group II and group III received 50 mg and 100 mg of plain Resveratrol respectively and group IV and group V received 50 mg and 100 mg of Longevinex respectively for a period of 28 days. All toxicological parameters were analyzed as per OECD-407 guidelines. Results showed treatment with Resveratrol and Longevinex did not result in any mortality of rats neither did they exhibit any clinical signs of toxicity. Hematological and biochemical analysis of serum enzymes and metabolites were not significantly altered between Longevinex and control rats. Likewise, histopathological analysis for various organs did not reveal significant changes in the vital organs of the treated rats. The study revealed that there were no significant treatment related adverse effects in rats exposed to Longevinex for 28 days and considered safe at the given dose where compared to plain Resveratrol.

1. Introduction

The discovery, development and marketing of nutraceutical supplements are currently the fastest growing segments of marketed products around the globe and are of common use in the Western world (Nicoletti, 2012). They are available in various dosage forms (pills, powders, capsules, vials, etc.) containing food bioactive compounds as active principles over the counter. Scientific research including epidemiological studies show a link between the consumption of plant-derived foods and a range of health benefits (Espín et al., 2007). These benefits have been associated, at least partially, to some of the phytochemical constituents, and, in particular, to polyphenols. Amongst the different groups of polyphenols, anthocyanins, proanthocyanidins, flavanones, isoflavones, Resveratrol, Quercitin and Ellagic acid are largely used in the nutraceutical industry currently. Resveratrol, a red wine derived polyphenolic phytoalexin shows diverse health benefits from chemoprevention to cardioprotection including antiaging (Das and Das, 2007; Maulik and Das, 2006). Recently, the hormetic effect of Resveratrol as evidenced by the protective action at lower dose and the detrimental effect at higher doses was studied in comparison with a commercially available Resveratrol supplement, Longevinex (Resveratrol Partners LLC, USA) which interestingly did not exhibit hormesis (Juhasz et al., 2010).

Longevinex a micronized nutraceutical supplement containing Resveratrol, Ferulic acid, Quercitin and rice bran Phytate exhibited cardioprotective effect by converting death signals to survival signals in a relatively shorter period of time (Mukherjee et al., 2010). Further, it has exhibited antiatherosclerotic effect by acting as an antioxidant and hypocholesterlemic agent (Juhasz et al., 2010). Recently, it has also shown impressive results in managing macular degeneration (Kalantari and Das, 2010; Richer et al., 2013). As per regulatory toxicology a repeated dose study for 28 days following OECD guidelines 407 is mandatory for any drug to undergo phase 1 clinical trial. This mooted us to study the toxicity profile of Longevinex in comparison with plain Resveratrol on healthy adult Sprague Dawley rats which could be an initial study to use Longevinex as an antiaging pill in the days to come.

Abbreviations: ACP, Acid Phosphatase; ALP, Alkaline Phosphatase; DMSO, Dimethylsulfoxide; EDTA, Ethylenediaminetetraacetic acid; GGT, γ-Glutamyltransferase; H&E, Hematoxylin and eosin; HGB, Hemoglobin; LDH, Lactate Dehydrogenase; NOAEL, No observed adverse effect level; SD, Sprague Dawley rats; SEM, Standard error means; SGOT, Serum Glutamic Oxaloacetic Transaminase; SGPT, Serum Glutamic Pyruvic Transaminase; ULN, Upper Limit of Normal.

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2. Materials and methods

2.1. Test materials and chemicals

The details of the test materials are given in Table 1. Biochemical kits for the assays were purchased from Spin React Diagnostic kit, Spain and all other chemicals used were of analytical grade from Hi Media, India.

Table 1
Test materials.

Name	Resveratrol	Longevinex
Source	Blue California, USA	Resveratrol Partners, USA
Colour	Half White	Straw Yellow
Taste	Bitter	Bitter
State	Powder	Microencapsulated powder
Storage	Room temperature	Room temperature
Purity	85% extracted from *Polygonum cuspidatum* (Giant knotweed)	Resveratrol-85% extracted from *Polygonum cuspidatum* (Giant knotweed) Quercetin (98%) IP6 phytate from rice bran (70%) Ferulic acid from rice bran (75%)
Ingredients	Resveratrol	Each Capsule contains Resveratrol – 100 mg, Quercetin (98%) – 25 mg, IP6 phytate – 75 mg, Ferulic acid – 10 mg, Vitamin D3 – 25 μg, Plant dextrin and starch for microencapsulation and 40 mg of Candelilla wax as emulsifier

Details given by Resveratrol Partners USA.

2.2. Experimental animals

Healthy, 6–8 week old Sprague Dawley (SD) rats, with body weight ranging between 130 and 160 g purchased from Venkateshwara Enterprises, Bangalore, India were used in this study. Upon arrival, the thirty rats were marked using 0.5% picric acid and allocated randomly into five treatment groups. It was ensured that mean body weight in each treatment group was approximately the same. The experimental animals in each group were housed in individual polypropylene cages for the duration of the study. Animals had access to sterile pelleted feed containing all macro and micro nutrients and purified water *ad libitum*. The room was well ventilated with 100% fresh air. The temperature and relative humidity were set to standard levels of 23 ± 2 °C and 50–70% respectively. The SD rats were exposed to alternating 12 h of light and dark cycles. The experimental animals were acclimatized for 2 weeks in the animal house before the start of the study.

The acclimatization and all succeeding phases of this investigation were conducted in accordance with Good Laboratory Practice Regulations. In addition, the study design and use of experimental animals were reviewed and approved by the Institutional Animal Ethical Committee (PU/IAEC/11/11). All treatment related animal handlings were executed in the morning to minimize the effects of circadian rhythm.

2.3. Selection of doses and study design

Dose of the test materials were selected based on the proposed human doses (conversion was done on the basis of equivalent body surface area of humans to rats). The test materials were freshly prepared by suspending the required amount of drugs in dimethylsulfoxide (DMSO). The vehicle and test materials were administered orally via gastric intubation with ball – tipped oral dosing needles which were affixed to the syringe of appropriate size.

The toxicity study consisted of five groups wherein group-I as vehicle treated control, group-II and group-III received 50 mg and 100 mg of plain Resveratrol respectively and group-IV and group-V received 50 mg and 100 mg of Longevinex respectively for a period of 28 days. Each group includes both male ($n = 5$) and female ($n = 5$) rats caged according to group and sex. The experimental animals were observed for 30 min after treatment, followed by observation twice daily for the entire study period.

2.4. Mortality checks and clinical observations

All rats were observed daily for mortality and signs of toxicity such as changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions, autonomic activity, changes in gait, posture and response to handling, as well as the bizarre behaviour (OECD, 1998) during the entire period of the study.

2.5. Body weight, food and water intake

The body weight of each rat was measured once every week. Based on the new body weight of the experimental rats, the quantities of test drugs to be given were calculated again to ensure administration of fixed dose. About 50 g of pelleted feed was usually laid in the food tray (~8 g/rat) everyday. However, the measure of food left in the tray at the end of the day was calculated to find the food intake. Similarly, the volume of water placed in the bottle was 200 ml per day. The level of the water consumed was also measured daily.

2.6. Hematology and clinical biochemistry

In order to identify the hematological and biochemical alterations, if any, after 14 days and after the last dose of 28 day toxicity study of Resveratrol and Longevinex, the rats were fasted overnight (18 h) and anesthetized by ether inhalation. Blood was collected by retro orbital puncture from each rat. The blood samples were collected in Ethylenediaminetetraacetic acid (EDTA) tubes for hematological analysis and Tri sodium citrate containing tube for clinical biochemistry determinations. The blood for hematological assay was immediately analyzed for hemoglobin (HGB) concentration, number of Neutrophils, Lymphocytes, Eosinophils and Monocytes. The blood was centrifuged for 10 min at 3000 rpm in a refrigerated centrifuge to separate plasma and stored at −20 °C until analysis for clinical biochemistry measurements using the Spin React Diagnostic kit in a semi auto-analyzer (Spain).

Clinical blood marker enzymes such as Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), γ-Glutamyltransferase (GGT) and Lactate Dehydrogenase (LDH) and metabolites such as blood glucose, bilirubin, cholesterol, triglycerides, protein, albumin, urea, creatinine and globulin were analyzed using a semiautoanalyser with SPIN React kits.

2.7. Organ weight and histopathology

All tested rats were subjected to elaborate gross necropsy. The brain, heart, lungs, liver, spleen, kidneys, pancreas and ovaries/testes were removed and the adherent tissues (if any) were carefully cropped. The wet weights of the organs were registered right away. The dissected organs were fixed in 10% neutral buffer formalin and processed adequately. The tissues were then embedded in paraffin wax and sections of 6 μm were stained with hematoxylin and eosin (H&E) for scrutiny under the light microscope.

2.8. Statistical analysis

The data collected on body weight, food and water intake, hematology, serum biochemistry and organ weights of the experimental animals were subjected to statistical analysis and expressed as mean ± SEM (standard error mean) and the statistical significance of differences between groups was analyzed using Student T-test. Value of $p < 0.05$ was considered significant.

3. Results

3.1. Mortality and clinical observation

There were no treatment-related mortality and clinical signs of toxicity in the 28 day repeated dose sub-chronic study. Treated and concurrent control groups were similar in clinical demonstration (Table 2).

3.2. Body weight, food and water intake

The body weight of the control and the treatment rats are as presented in Table 3. Though there were gradual increase in the body weights, statistically significant changes were not observed in the mean body weights of the treatment groups compared to the control group.

Feed intake was found to be normal with no drastic change in the amount consumed by the drug treated groups. However, a constant increase in the quantity of food consumed by the rats was noticed in all groups over the study duration (Table 4). The water intake of the treatment rats was also not substantially different from the control rats throughout the study (Table 5).

3.3. Hematology and clinical biochemistry

The normal values of hematological parameters in the control group and quantitative changes of hematological parameters in rats fed with either Resveratrol or Longevinex after 28 days were are shown in Table 6. There was no significant ($p > 0.05$) difference between the three subject groups in any of the hematological