Acute oral toxicity evaluation of antioxidant rich plant based nutraceutical formulation

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Abstract

Antioxidants are important molecules that can protect the organism from free radical-induced oxidative stress. Natural antioxidants are obtained mostly from plants, and their effectiveness varies according to plant species, diversity, extraction, processing methods and growth circumstances. The prepared product was tested for nutritional analysis, heavy metal analysis, and microbiological analysis, which yielded positive findings. The acute toxicity was monitored for 14 days in albino rats, and no toxicity signs were seen throughout that time.

Keyword: *Plant based, Nutraceutical, Acute toxicity*

Introduction

The concept of free radicals of oxygen has been known for more than 50 years; however, it is only in the last two decades that their role in disease development has been identified, and thus the positive advantages of antioxidants have been extensively researched. (Rana, 2010; Liu 2019). Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress (Qusti, 2010; Zehiroglu et al 2019). The antioxidant activity in plants is notable since plants are rich in compounds that have an important role in free radical-scavenging activity. Antioxidant based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stoke, diabetes, Alzheimer's disease and cancer (Kotha et al 2022). Nutraceutical foods provide medical or health benefits, including the prevention and treatment of diseases (Costa 2017).

Materials and Methodology

Selection of nutraceuticals

High potential Antioxidant nutraceutical will be selected for the study. Phytosterol from plants, Lycopene from Tomoto, Oligomeric Proanthocyanin from grapes, Curcumin from Turmeric and Xanthones (Alpha mangostin) from mangosteen fruits was selected.

Formulation of supplement

The High quality Lycopene, Curcumin, Oligomeric proanthocyanin, Lutein, piperine will be purchased by comparing the Certificate of Analysis (COA). By reviewing the research literature the composition of the product and percentage of each ingredient was finalized.

Phytosterol is extracted from plants, phytosterols are a group of naturally occurring compounds found in plant cell membranes. Because phytosterols are structurally similar to the body's cholesterol, when they are consumed they compete with cholesterol for absorption in the digestive system. Curcumin is extracted from turmeric; Curcumin happens to be a potent antioxidant that can neutralize free radicals due to its chemical structure. Lutein is extracted from carrot; research has shown that the benefits of lutein go beyond vision and eye health. Lutein also supports brain function and enhances memory and learning.

Lycopene is extracted from tomatoes; **t**here are numerous studies on the use of lycopene in cancer and cardiovascular disease, as well as its antioxidant activity. Human epidemiological evidence indicates that diets high in tomatoes may reduce the risk of cervical, colon, oesophageal, and stomach cancers. Proanthocyanin is extracted from grape seeds, protect against oxidative damage. It inhibits enzymes that produce histamine and help to ease of allergies. Piperine is extracted from peppers; Piperine may help to increase the availability of key nutrients.

The developed nutraceutical is a combination of phytosterol 50 mg, curcumin 150 mg, lycopene 50 mg, proanthocyanin 100 mg, Lutein 10 mg and piperine 5 mg

Nutritional Analysis of Total Antioxidant

Determination of proximate composition was carried out in accordance with A.O.A.C. methods. Proximate composition of a substance constitutes the different classes of nutrients

present in the samples such as carbohydrates, protein, fat, as well as caloric value calculated from values of carbohydrate, fat and protein.

Heavy Metals Analysis

Heavy metals have been widely acknowledged to adversely affect the nutritive values of agricultural produce on account of their deleterious effect on human beings. Therefore, national and international regulations on food quality have set the maximum permissible levels of toxic metals in human food. As such, an increasingly important aspect of food quality assurance has been to control the concentrations of heavy metals in food (Sobukola, *et al* 2010). Organic matter in sample was digested by wet digestion or dry digestion or high pressure microwave digestion and determine the amount of heavy metals, i.e. arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) by using graphite furnace atomic absorption spectrophotometer and flow injection analysis system - atomic absorption spectrophotometer.

Microbial Analysis

Microbiological analysis is the use of biological, biochemical, molecular or chemical methods for the detection, identification or enumeration of microorganisms in a material (e.g. food, drink, environmental or clinical sample). The microbial analysis for total antioxidant was done using the test method of The Ayurvedic Pharmacopoeia of India Part - II (Formulations) Volume - II First Edition Appendices 1 to 5. Total Bacterial Count, Total Yeast and mold, Escherichia coli, Salmonella sp. And S.aureus were analysed.

Grow separately the test strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in fluid soyabean-casein digest medium and *Escherichia coli* and *Salmonella typhimurium* at 300 to 350 for 18 to 24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to make test suspensions containing about 103 viable micro-organisms per ml. Mix equal volume of each suspension and use 0.4 ml (approximately 102 micro-organisms of each strain) as an inoculum in the test for *E. coli, Salmonella*, *P. aeruginosa* and *S. aureus*, in the presence and absence of the preparation being examined, if necessary. A positive result for the respective strain of micro-organism should be obtained.

Acute toxicity study

Experimental analysis: Adult wistar mice, aged 8 to 12 weeks were selected. The animals were kept in an animal room where room temperature and relative humidity will be maintained from 19°C to 25°C and from 30% to 70% respectively. The test room will be provided with 12 hrs under light and 12 hrs under dark conditions throughout the experiment. They were provided with food and water for 1 week to acclimatize them before starting the experiment.

Administration dose: Animals were fasted over-night prior to dosing. Following dosing, animals were fasted for further period for 3-4 hrs. During fasting water was provided to animals. Animals were weighted and formulated combination of antioxidant was administered orally at single dose by oral gavages using a suitable oral intubation cannula. The acute toxicity test is performed according to the Organization of Economic Co-operation and Development (OECD) guidelines for testing of chemical (OECD, 2001).

Observation: Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. All observations were systematically recorded with individual records being maintained for each animal.

Body weight: Body weight of each animal was recorded on acclimatization day, prior to the dosing of the test item (day 1 of treatment) and thereafter weekly once.

Toxicity Signs: All treated animals were observed individually at 30 min, 1, 2, and 4 hrs after dosing and thereafter once daily for 14 consecutive days. The appearance and disappearance of clinical signs of toxicity was recorded throughout the experimental period. Observation includes changes in skin and fur, eyes and mucous membranes, circulatory, autonomic and central nervous system, somatomotor activity and behavioral patterns.

Mortality/Morbidity: All animals were observed for Morbidity/Mortality minimum once daily, throughout the experimental period. Animals found in a moribund condition or showing severe pain or severe distress was humanely killed.

Pathology: All test animals should be subjected to gross necropsy. All gross pathological changes should be recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours may also be considered because it may yield useful information.

Results and Discussion

Qualitative study of the supplement

Carbohydrate

Carbohydrates in total antioxidant capsules were estimated by The Association of Official Analytical Chemists International (AOAC) method. In this analysis the developed nutraceutical was taken for estimation. The developed nutraceutical was a combination of phytosterol, curcumin, Lutein, lycopene, proanthocyanin and piperine. The total volume of the sample was 365 mg. The analysis shows the result for the carbohydrate was 20.80%.

TABLE - 1 Shows the nutritional analysis of total antioxidant

SI. No	Parameters	Results
1	Carbohydrates	20.80%
2	Protein	5.60%
3	Fat	18.09%
4	Energy	268.41 Kcal

Fat

The Association of Official Analytical Chemists International (AOAC) method (922.06) was used for the total fat analysis. The total fat content was determined by gravimetric method. The result shows 18.09% of fat was present in the combined nutraceutical product.

Protein

Protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor was based on the fact that most protein contains 16% nitrogen. Protein was determined by kjeldahl method. The result shows 5.60% of protein was present in the combined nutraceutical product.

Energy

The amount of energy present in the formulated product was found to be 268.41 kcal. Energy was calculated from the values of carbohydrates, protein and fat.

Heavy Metals Analysis

In this study, the content of heavy elements arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) were determined in the Total Antioxidant. The sample prepared for testing, As and Hg - 100 ppm sample in 1 mol/L Hcl and for Cd - 100 ppm sample in 1 mol/L HNo_3.

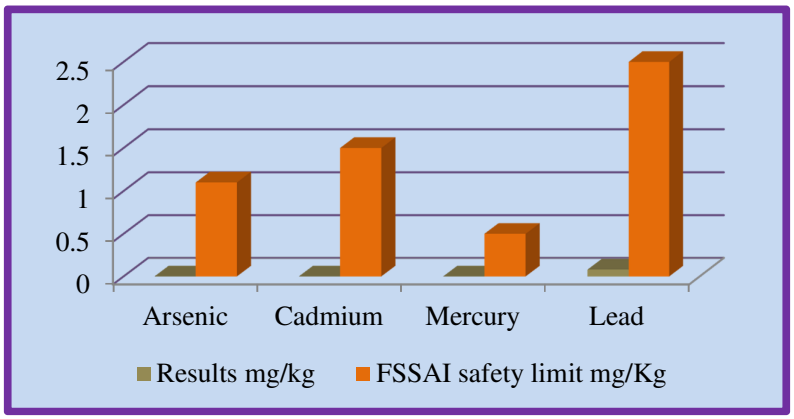

Figure 1 - Heavy metal analysis of total antioxidant

Finally, in the total antioxidant heavy metals like, cadmium, arsenic, mercury and lead were found to be below detection limit. The heavy metal content in Total Antioxidant was given in Table 2.

TABLE - 2 Shows the heavy metal analysis of total antioxidant

SI. No	Heavy metals	Results
1	Hg (mg/kg)	BDL
2	As (mg/kg)	BDL
3	Cd (mg/kg)	BDL
4	Pb (mg/kg)	BDL

BDL – Below Detection Limit

Microbial Analysis

The total bacterial count is found to be 600 CFU/g which is under the limit. The total yeast and mold is <10 CFU/g, it is under the permissible limit. The *Escherichia coli, Salmonella sp.* and *S.aureus* are absent.

TABLE - 3 Shows the microbial testing of total antioxidant

SI. No	Parameters	Specification	Results	Test method
Microbiology Test				
1	Total Bacterial Count	10^5 CFU/g	600 CFU/g	The Ayurvedic Pharmacopoeia of India Part - II (Formulations) Volume - II First Edition Appendices 1 to 5
2	Total Yeast and mold	10^3 CFU/g	< 10 CFU/g	
3	*Escherichia coli*	Absent	Absent	
4	*Salmonella sp.*	Absent	Absent	
5	*S.aureus*	Absent	Absent	

Acute toxicity study

Acute toxicity of the formulated combination of antioxidant nutraceutical had been studied. The toxicity of this compound, when administered orally to a set of Wistar strain rats under controlled conditions and the observations made during the study form the base of this study. They did not exhibit any signs of toxicity during the 14 days of observation. No lethality was observed for any dose during the course of administration of the extract. There was no evidence of deviations for physiological responses and neuro behavioural changes between the control and any of the treated groups. There were no significant rise or fall of body weight of the animals under extract and their food and water consumption were least deviating from the controlled group of normal animals. No macroscopical abnormalities were detected in the examined organs. The organ weights though indicated some changes from that of the normal in particular the weights of liver and kidney slightly decreased but it is non-significant.

There were no behavioural changes or mortality observed in formulated antioxidant among rats. There was no significant change in their body weight. LD50 cut-off for rat was found and the drug came under the category according to Globally Harmonized Classification System (OECD 423 guidelines). This study concludes that in this acute toxicity study, no mortality or toxicology signs were observed.

Conclusion

The combination of nutraceutical products were formulated which has a good antioxidant potential. The nutritional analysis, heavy metal analysis and microbiology analysis showed better results. The formulated supplement was analysed short term toxic effects and the acute toxicity study was carried out. There is no sign of toxicity changes were observed during 14 days of observation.

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