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Acute toxicity study in rats based on physiological examination using aqueous extract of *Eugenia uniflora* (L.) leaves

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Abstract
The present study aims to investigate the acute toxicity study in aqueous leaf extract of *Eugenia uniflora* leaves in rats by examination of physiological parameters. Male Wistar albino rats weighing 170-200g were used for the study. The animals were maintained in a ventilated room with temperature 23± 2ºC, humidity 60-70% and 12h light/dark cycle. Animals were fed with standard pellet and water *ad libitum*. All the studies were conducted in accordance with committee for the purpose of control and supervision of experiments on animals (CPSEA) norms and the National Institute of Health Guidelines "Guide for the care and use of laboratory animals". The animals were observed daily for physiological and behavioral changes. Body weight were taken daily before the administration of extract for 28 days. After the end of experimental regimen the animals were sacrificed and blood was collected by cervical decapitation under mild chloroform anaesthesia. From the physiological parameters examined the extract showed non toxic nature even at the highest dosage. However, more studies should be conducted for further understanding.

Keywords: *Eugenia uniflora*, aqueous leaf extract, acute toxicity, physiological examination

Introduction

Herbal medicines are also known as phytoremedies which have been always used to heal human sufferings since time immemorial. Since ancient time plants have been the major source of medicine. Not only as foods, plants have been used as therapy against several diseases. The phytochemical present in plants plays a major role in decreasing many diseases and can boost up various organs. Phytochemicals in plants acts as antioxidants. 70-80% of people rely on traditional and herbal medicine to meet their health care needs.

Use of traditional medicines is important in Indian culture and was practiced since ancient times. Nature itself has bestowed with immense therapeutic knowledge with different varieties of medicinal plants (1). When compared to the synthetic medicines which are regarded as unsafe, traditional medicines shows safety especially to use in human beings (4). No drug should be used clinically without its clinical trials and toxicity studies. Acute oral toxicity studies of herbal medicines are essential to identify the safety and the determination of dose level that could be used subsequently. It also helps in the investigation of the therapeutic index of drugs and xenobiotics (3).

Eugenia uniflora L. is a widely distributed tree in South American countries, mainly in Brazil, Argentina, Uruguay, and Paraguay (4). Its leaves are used in popular medicine as infusion in the treatment of fever, rheumatism, stomach diseases, disorders of the digestive tract, hypertension, yellow fever, and gout. It may also reduce weight, blood pressure, and serve as a diuretic (5). Pitanga fruits, also known as Brazilian cherry or Suriname cherry, contain various volatile compounds that are also found in the essential oil of pitanga leaves (6, 7). Like the leaves, pitanga fruits may also have health benefits. In the Brazilian food industry, pitanga fruits have mostly been used to produce juice and frozen pulp. Pulp production has high economic potential because the product has consumer appeal and high concentrations of antioxidant compounds, such as anthocyanins, flavonols, and carotenoids (8).

Scientific classification

Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Eugenia
Species: *E.uniflora*
Binomial name: *Eugenia uniflora* L.

In our preliminary study, phytochemical constituents and *in vitro* antioxidant potentials of aqueous leaf extract of *Eugenia uniflora* were carried out. From the previous studies it was clearly evident that the aqueous extract is potential to act as a source of useful drugs because of the presence of various primary and secondary metabolites. The current study is formulated to find out the safe dose level of the extract in rats. The physiological examination was carried out and documented in this study.

Materials and Methods

Selection of animals

The Institutional animal ethics committee (Reg.no.659/02/a/CPSEA), Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India approved the experimental design. Male Wistar albino rats weighing 170-200g were obtained from Small Animal Breeding centre, Kerala Veterinary College, Mannuthy, Thrissur, Kerala. The animals were maintained in a ventilated room with temperature 23± 2ºC, humidity 60-70% and 12h light/dark cycle. Animals were fed with standard pellet and water *ad libitum*. All the studies were conducted in accordance with

committee for the purpose of control and supervision of experiments on animals (CPSEA) norms and the National Institute of Health Guidelines "Guide for the care and use of laboratory animals".

Acute toxicity study of aqueous extract of *Eugenia uniflora* leaves

Rats were divided into six groups with six animals in each group with body weight of 170-200g. Aqueous extract of *Eugenia uniflora* leaves was administered to groups II (100mg/kg b.wt), III (200mg/kg b.wt), IV (300mg/kg b.wt), V (400mg/kg b.wt), VI (500mg/kg b.wt) via oral route for 28 days, while group I is the control group received normal saline. All animals were supplied with standard pellet food and water *ad libitum* during the testing periods.

Physiological examination

The animals were observed daily for physiological and behavioral changes. Body weight were taken daily before the administration of extract for 28 days. After the end of experimental regimen the animals were sacrificed and blood was collected by cervical decapitation under mild chloroform anaesthesia. Liver and kidney were excised and washed in ice cold saline, blotted dry and weighed. The tissues were weighed and organ indices were calculated according to the formula explained by Zhang *et al*., 2003(9) as,

Tissue Index = (Tissue weight/ animal weight) ×100

Result and Discussion

Physiological examination in animals during treatment with different dosages of aqueous extract of *Eugenia uniflora* leaves

Changes in body weight in rats during the treatment with different doses of aqueous extract of *Eugenia uniflora* leaves is presented below,

Table 1: Changes in bodyweight of control and induced rats during treatment with different dosages of aqueous extract of *Eugenia uniflora* leaves

Groups	Dose	Initial body weight (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)	Day 28 (g)
G I	Control	175.15 ± 0.05	178.05 ± 0.08	178.89 ± 0.09	180.02 ± 0.05	182.56 ± 0.05
G II	100 mg/kg b.wt	175.05 ± 0.09	178.25 ± 0.07	178.25 ± 0.04	180.88 ± 0.06	182.06± 0.07
G III	200 mg/kg b.wt	175.56 ± 0.08	178.89 ± 0.12	178.07 ± 0.02	180.37 ± 0.19	182.23± 0.11
G IV	300 mg/kg b.wt	175.89 ± 0.05	178.17 ± 0.06	178.03 ± 0.06	180.45 ± 0.08	182.34 ± 0.05
G V	400 mg/kg b.wt	175.26 ± 0.10	178.26 ± 0.13	178.74 ± 0.17	180.66 ± 0.06	182.56 ± 0.07
G VI	500 mg/kg b.wt	175.65± 0.06	178.44 ± 0.04	178.90 ± 0.05	180.23 ± 0.16	182.83 ± 0.08

Values are expressed as mean ± SD of six animals, Units: Grams

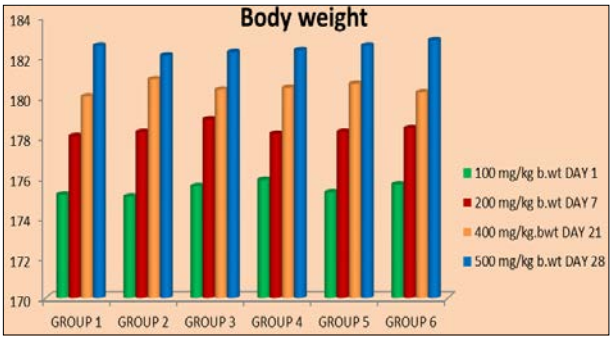

Fig 1: Changes in bodyweight of control and induced rats during treatment with different dosages of aqueous extract of *Eugenia uniflora* leaves

Body weight is an important factor to monitor the health of the animal. The loss of body weight is the first indicator of the onset of an adverse effect. If a dose causes 10% or more reduction in body weight then it is considered to be a toxic dose. In present investigation all the animals in treated groups did not show any decrease in body weight indicating no signs of toxicity. There was no significant change in the consumption of food and water. Mortality is an important criterion in assessing the acute toxicity (LD_{50}) of a drug. There was no mortality recorded even at the maximum dose of the extract.

Effect of aqueous extract of *Eugenia uniflora* leaves in weight of organs like liver and kidney after the administration of different doses is presented below,

Table 2: Effect of aqueous extract of *Eugenia uniflora* leaves on organ weight

Groups	Dose	Liver (g)	Kidney (g)
Group I	Control	7.69 ± 0.04	2.22 ± 0.04
Group II	100 mg/kg b.wt	7.50 ± 0.08	2.70 ± 0.02
Group III	200 mg/kg b.wt	7.37 ± 0.05	2.76 ± 0.06
Group IV	300 mg/kg b.wt	7.20 ± 0.11	2.15 ± 0.08
Group V	400 mg/kg b.wt	7.34 ± 0.13	2.77 ± 0.03
Group VI	500 mg/kg b.wt	7.14 ± 0.02	2.73 ± 0.09

Values are expressed as mean ± SD of six animals, Units: Grams

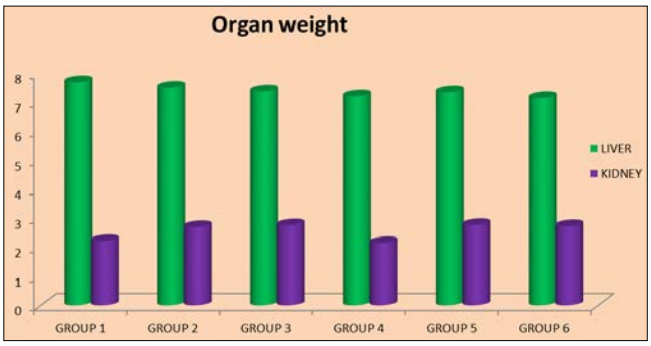

Fig 2: Effect of aqueous extract of *Eugenia uniflora* leaves on organ weight

Organ weight is the most sensitive drug toxicity indicator to evaluate the effect of drug toxicity. A significant change in organ weight in treated and control animals occur often precede morphological changes. Number of factors has reported that may influence the organ weight of an animal including age, strain of animal, sex, environmental and experimental conditions. In present investigation there was no treatment related toxicity signs in weight of liver and kidney of rats during the treatment with different doses of aqueous extract of *Eugenia uniflora*.

Conclusion

Natural product remedies are popular and gaining acceptability among people in prevention of diseases. However we do not have sufficient scientific data on their safety and toxicological profile. This assumes importance since the natural products are more often used under self-medication without a medical supervision. The aqueous extract of *Eugenia uniflora* leaves contain pharmacologically active compounds which have documented high antioxidant and other beneficial pharmacologic activities. From the physiological parameters examined the extract showed non toxic nature even at the highest dosage. However, more studies should be conducted for further understanding.

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