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FULL PAPER

Acute oral toxicity study of wound healing drink from *Ocimum tenuiflorum* on adult female Sprague-Dawley rats

[1]Rohini, J., [1*]Rabeta, M.S. and [2]Wan Ezumi, M.F

[1]*Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*
[2]*Biomedicine Programme, School of Health Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia*

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Abstract

In this study, the extract of *Ocimum tenuiflorum* was formulated to promote wound healing and no acute toxicity study has been reported on this type of formulation. Acute oral toxicity results of the rats after administration of 5000 mg/kg body weight (BW) formulation showed no toxic effects and behavioural changes of the rats after 14 days of observation. On day 14, the rats were anaesthetized to collect the vital organs for histopathological examination. The microscopy study of the visceral organs of wound healing powder group shows no sign of toxicity in comparison to untreated rats organs. In conclusion, the result of the acute oral toxicity test of wound healing drink showed no sign of toxicity, as corroborated by a histopathological study.

1. Introduction

Recent years have seen food researchers exploring various types of agricultural sources in the search for new food/beverage that could yield physiological benefits to consumers. Plant from *Lamiaceae* family and genus *Ocimum* are the most famous for its medicinal properties (Triveni *et al*., 2013). *Ocimum tenuiflorum* is a herbal plant that is available in most South Asian countries (Hannan *et al.*, 2006). This plant commonly called Holy Basil or Tulsi in most countries (Kalabharathi *et al*., 2011) while in Malaysia it is called as "ruku". In Ayurvedic herb, it is listed in Malaysian Herbal Monograph and Globinmed proved lots of medicinal benefits including wound healing (Institute for Medical Research (IMR), 2015; IMR, 2019).

The most popular finding reveals that *O. tenuiflorum* plays a role in antibacterial (Joshi *et al*., 2011), antidiabetic (Mousavi *et al*., 2016), antioxidants (Rabeta and Lai, 2013), anticancer (Lam *et al*., 2018), hepatoprotective (Lahon and Das, 2011), anti-inflammatory (Kumar *et al*., 2015), wound healing (Rohini *et al*., 2019). Besides the therapeutic benefits of herbal plants, the toxicity and safety profile of plants are also important (Gatne *et al*., 2015). Hence, plants need to undergo toxicity assessment before being used as medicine. Several researchers have conducted raw and crude extract toxicity studies to evaluate the toxicity of the formulated extract before its application to the healthcare field (Chandrasekaran *et al*., 2013; Gautam and Goel, 2014; Raina *et al*., 2015).

Therefore, this study focused on acute toxicity since this is the first study to assess the short-term effects of the wound healing drink from *O. tenuiflorum*. The drink itself was recently developed and therefore, the acute toxicity study was adequate to provide crucial basic information on the safe acute doses, the potential acute effects and target organs of toxicity prior to the repeated dose toxicity study. Following this experiment, the subacute, subchronic and finally chronic toxicity studies will also be conducted when the appropriate doses of this herbal drink of *O. tenuiflorum* are optimised from this current study.

2. Materials and methods

2.1 Plant material collection and extraction

First, the plant was collected from the botanical garden, Perak and authenticated (11400), and the initial weight was approximately 1 kg and all the leaves were washed with running tap water. Aqueous extract of the dried leaves powder was prepared according to Shetty *et al*. (2008) with some modification. The obtained extract

*Corresponding author.
Email: *rabeta@usm.my*

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was stored in an air-tight container in freezer -20°C until use.

2.2 Formulation preparation

The formulation of wound healing drink was prepared using complexation method via solvent evaporation technique according to Shetty *et al.* (2008). Lecithin powder was prepared for the formulation of the aqueous leaves extract. Amphiphilic properties of lecithin have shown increased insolubility and ease the permeation across the phospholipid bilayer of the epithelium (Zhou *et al*., 2017). The extract and lecithin were prepared by physical mixture using 50% ethanol until complete dissolution. The solutions beakers were closed and allowed to mix until equilibrium was reached for about 3 hours with slight agitation at 90 rpm in an orbital shaker.

2.3 In vivo acute oral toxicity study

Oral toxicity study was done according to the Organization for Economic Co-operation and Development (OECD) guidelines 425. Since *O. tenuiflorum* have been studied for toxicity and showed no toxicity on rats (Parasuraman *et al*., 2015), the limit test was chosen.

2.4 Animal preparation

Adult female Sprague-Dawley (SD) rats age 8-10 weeks old, weighing around 180-200 g were used in this experiment. Ethical approval for this study was obtained from The Universiti Sains Malaysia (USM) Institutional Animal Care and Use Committee (USM IACUC/USM JKPPH) with reference number USM/IACUC/2018/ (111) (915). Food and water intake were not measured. Moreover, water containers will be cleaned and freshwater will be replaced daily.

2.5 Animal treatment

A total number of three rats were used in the study. A rat was treated with 5000 mg/kg body weight (BW) (0.5 mL) at one time and observed for any signs of toxicity and mortality for the first 4 hours (based on Organization for Economic Cooperation and Development OECD 425). Female nulliparous and non-pregnant rats were used. Animals were observed for 14 days and sacrificed on the 14th day to collect the vital organ for pathological examination.

2.6 Histopathological study

On the 14th day of toxicity study, the rats were sacrificed, and the vital organs (lungs, heart, liver and kidney) were isolated. Rats were anaesthetized using ketamine and xylazine intraperitoneally 50 mg/kg and 5 mg/kg BW, respectively prior to sacrifice. All organs were posthumously excised, blotted and weighed for microscopic evaluation. Tissues were fixed in a fixative (i.e., 60% absolute ethanol, 30% formaldehyde and 10% glacial acetic acid) and embedded in paraffin. Samples were subsequently sectioned at 4 µm and subsequently stained with haematoxylin/eosin.

3. Results and discussion

3.1 In vivo acute oral toxicity study

Figure 1 shows a microscopic view of the histopathological assessment of the visceral organs collected from rats. It shows no alteration in the form of the nucleus, cells and cytoplasm when compared with untreated (control) rats. This finding suggested that the given formulation did not affect the internal organs of the rats and had no toxicity.

Toxicity is defined as poisonous effects or side effects that create interaction between cells and toxicants. Toxicity effects may differ depending on the species and metabolism of the compounds (Angelina *et al*., 2014). The toxicity effects might occur earlier without the binding of toxicants to the visceral organs (Syahmi *et al*., 2010). Thus, a toxicity study needs to be carried out in consideration of human health, because toxicity may have adverse effects on the public.

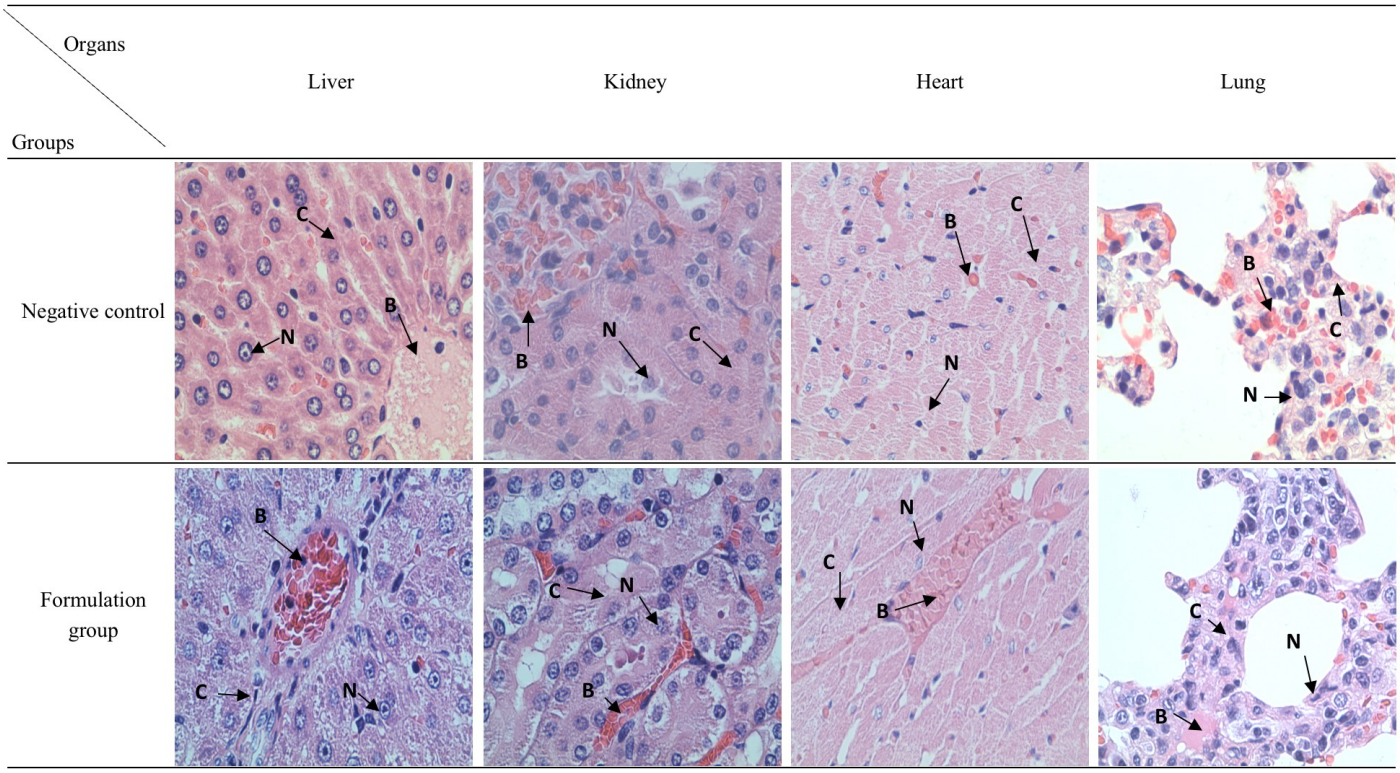
In the current study, a prepared formulation with lecithin complexation and the extract was not physically and histopathologically toxic on rats at concentrations of up to 5,000 mg/kg BW when administered orally. The ethanolic leaf extract of *O. tenuiflorum* at concentrations reaching 2,000 mg/kg BW is safe to be used in animals, as confirmed by a limit test study (Gautam and Goel, 2014).

Table 1 shows the physical and behavioural observation of rats after oral administration of wound healing drink. The mentioned parameters were absent during the study period, thereby indicating that all test rats showed no sign of toxicity. This evidence supports the claim that the chemical properties of the leaf extract

Table 1. Physical and behavioral observation of SD rats

Physical and behavioural observation	Presence (+); Absence (-)
Changes in skin, fur, eyes and mucous membrane	-
Changes in respiratory	-
Tremors	-
Convulsions	-
Salivation	-
Diarrhoea	-
Lethargy	-
Sleep	-

Figure 1. Microscopic view of stained organs of rats (B: blood vessels; C: cytoplasm; N: nucleus)



There were no indications of toxicity in all visceral organs examined. Their cellular arrangements were normal without major morphological changes (in terms of shapes and sizes) and looked comparable with those of the control groups. However, it is important to note that the differences in the staining colour of the slides were due to the technical imperfection including staining -batch variations.

did not change when formulated with lecithin powder. The inert properties of the formulation caused no toxicity at concentrations reaching 5,000 mg/kg BW in rats when prepared with lecithin. This phenomenon could be due to the physical properties of the powder, e.g. being a non-toxic vehicle and biodegradable (Saraf, 2010).

4. Conclusion

In conclusion, the formulation of wound healing drink prepared with aqueous *O. tenuiflorum* leaf extract and lecithin at a complexation was not physically and histopathologically toxic on Sprague-Dawley rats when administered orally. Thus, the formulation is safe to be used up to the tested concentration limit of 5,000 mg/kg BW. The present study recommends that further research should be performed prior to clinical trials to determine the effects of long-term dietary intervention with wound healing drink.

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