

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X

JPHYTO 2018; 7(6): 460-463

November- December

Received: 09-11-2018

Accepted: 23-11-2018

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Teratogenic potential of *Urtica massaica* (Mildbr.) and *Croton megalocarpus* (Hutch) in mice

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ABSTRACT

Urtica massaica (Urticaceae) and *Croton megalocarpus* are used either as vegetables or as food additives and as medicine in traditional African societies. However, in spite of the widespread consumption of these plants as folklore remedies and for diet, there is a scarcity of scientific data on their teratogenicity. Hence this study sought to assess the teratogenic effects of these plant extracts in an animal model. The study was conducted using Swiss albino mice. The extracts of these plants were administered orally in mice which were then euthanized. The weights of the gravid uterus and pups, as well as the number of pups, were determined. The pups were examined for gross malformations. The data set was analysed using one-way analysis of variance and Tukey as the post-Anova test. $P < 0.05$ was the limit of significance. *U. massaica* and *C. megalocarpus* caused 40% and 20% fetal partial resorption respectively. The latter also caused microcephaly and polyhydramnios. Hence, *U. massaica* leaves and *C. megalocarpus* stem extracts exhibited teratogenic activity and should be used with care during pregnancy.

Keywords: Teratology, *Urtica*, *Croton*, Fetal resorption, Microcephaly, Polyhydramnios.

INTRODUCTION

Urtica massaica (Urticaceae) – stinging nettle, *mpupu* in Kiswahili and *Thabai/Hatha* in Kikuyu, is a perennial herb that grows in wet parts of Kenya highlands [1]. It grows up to 2m high and is covered with stinging hairs. The leaves are large and heart-shaped with a serrated margin and a pointed tip and the flowers are small and green in colour, arising from the leaf axis; while the fruits are small, green, and flattened, with compressed seeds like those of a tomato [2].

It is traditionally used as a vegetable in Kenya where it is boiled and eaten [1]. The leaf extracts are rich in proteins, vitamins, minerals and all the essential amino acids [3].

The roots and the leaves *U. massaica* have many traditional uses in African traditional medicine such as the treatment of stomach aches, malaria, bruises, injuries, fractures, venereal diseases, rheumatism, urethral leak, and hepatic diseases [1]. A similar plant, *U. dioica*, has been used as a medicinal plant since time immemorial. In ancient times, *U. dioica* leaves were used in ancient Greece for their diuretic and laxative properties [4]. The Native North Americans and in Ayurvedic medicine used it for gynaecological problems [4]. It has exhibited anti-inflammatory, analgesic, hypotensive, diuretic, antihyperglycemic, antiproliferative on prostate cells and prostate cancer, antioxidative, hepatoprotective, and broad-spectrum antiviral effects [4]. The anti-diabetic properties of *U. dioica* were comparable to that of the anti-diabetic sulfonylurea drug glibenclamide [5]. *U. dioica* lectin exhibited antifungal properties against several fungal species [6].

Croton megalocarpus (Euphorbiaceae), *Mukinduri* in Kikuyu and *Msenefu* in Kiswahili, is a spreading deciduous tree which grows up to 35m tall [7]. It is found in east and central Africa [8]. It is widespread in Kenya [7]. *C. megalocarpus* wood is used for firewood, timber and charcoal [7] while the seeds may be used as biofuel [9].

Macerated roots of *C. megalocarpus* are taken as a vermifuge and as treatment for whooping cough, pneumonia, stomach aches, fever, and malaria [8] and as a purgative [10]. The sap from young leaves and twigs is applied on wounds [8].

A blend extract of the leaves, root bark, and stem of *C. megalocarpus* was shown to exhibit significant antinociceptive effect and inhibited chronic, peripheral and central nociceptive activity [11]. The bark extract of *C. megalocarpus* showed significant antifungal properties against *A. flavus* in maize [12]. The plant extracts from *C. megalocarpus* have also exhibited significant trypanocidal potential against *Trypanosoma evansi* [13]. However, in spite of the widespread use of *U. massaica* and *C. megalocarpus* as folklore remedies and for diet, there is a scarcity of scientific data on their teratogenic effects. Hence, this study aimed at evaluating the teratogenic effects of the two plant extracts in mice.

MATERIALS AND METHODS

Plant Collection

Processed commercial *Urtica massaica* leaves powder was obtained from Professor Kamindu Gikonyo of the Department of Pharmacy and Complementary Medicine, Kenyatta University Nairobi Kenya. *Croton megalocarpus* stem bark was sourced from Gatundu in Kiambu County in June 2017 and identified and authenticated in University of Nairobi under voucher number VW2017/01.

Preparation of the extract

About 100g of *Urtica massaica* powder was weighed and methanol added to cover the powder. The mixture was stirred and then allowed to stand for 2 hours, after which it was decanted. More methanol was added, stirred, and allowed to stand for 24 hours before decanting. The supernatant was filtered, and the filtrate concentrated using a rotor evaporator to obtain the plant extract which was stored in a universal bottle in a cool, dry place. The procedure was repeated with *Croton megalocarpus*.

Experimental animals

Virgin female Swiss albino mice weighing about 20g were obtained and sorted into groups of five for the teratogenicity assays. The mice were mixed with potent male mice for mating at a ratio of 1 male to 3 females. The mice were placed in cages maintained at room temperature for a period of seven days prior to the study to allow for acclimatization. Water and standard commercial feed from Unga Limited were provided *ad libitum*. The experiments were conducted *in vivo*, in accordance to the guidelines for the care and use of laboratory animals [14].

Drugs and chemicals

The standard drugs and chemicals used in the study included distilled water, methanol, chloroform and phenytoin sodium.

Teratogenicity assay

Evaluation of the teratogenicity of the plant extracts of *U. massaica* was conducted in accordance with the Food and Drug Administration (FDA) guidelines for the single-generation study on assessment of developmental toxicity [15]. Pregnancy was confirmed by checking for the vaginal plug and the protrusion of the mammary glands [16]. The dosage 1000 mg/kg of plant extract was dissolved in distilled water (vehicle) and phenytoin sodium was used in the study. The mice were divided into four (n = 5). Group one was given the oral dose of 1000 mg/kg of *U. massaica* extract daily from the sixth to the fifteenth day of pregnancy. On the eighteenth day of pregnancy, the mice were weighed then euthanized using chloroform and a caesarean section performed to remove the uterus and its contents. The procedure was

repeated with groups two, three and four where they received 1000mg/kg of *C. megalocarpus* extract; phenytoin, and the vehicle respectively. The parameters recorded were: the weight of the uterus and its contents, the weight of the foetuses only, the number of pups, weight of the pups, length of limbs, head and body. The pups were also checked for any gross malformations. Abnormalities of the pregnancy such as foetal resorption were also recorded.

Data analysis

The data for each set of experiments was tabulated in spreadsheets and expressed as means and their corresponding standard errors (S.E.M). It was then analysed using one-way analysis of variance and Tukey as the post-Anova test. The limit of significance was set at $p < 0.05$.

RESULTS

The plant extracts did not exhibit significant ($p > 0.05$) differences in the various weight and body length measurements, unlike phenytoin which exhibited a very significant ($p < 0.001$) reduction in the weight of the uterus and its contents.

Table 1: The effect of *Urtica massaica* and *Croton megalocarpus* extracts on fetal resorption

Treatment	Percentage fetal resorption
Vehicle	0 %
<i>Urtica massaica</i>	40 %
<i>Croton megalocarpus</i>	20 %
Phenytoin	100%

(* $p < 0.05$)

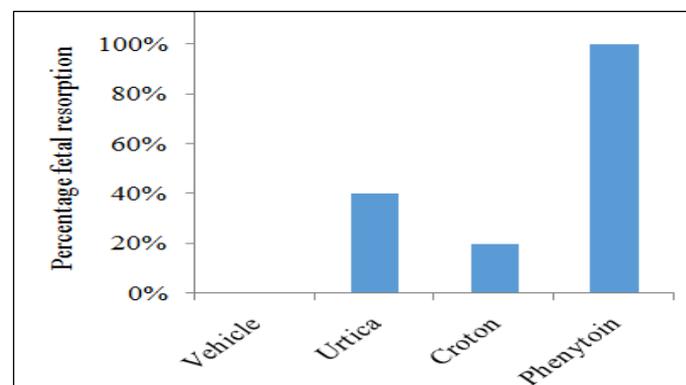


Figure 1: the effect of *Urtica massaica* and *Croton megalocarpus* extracts on fetal resorption in mice

Table 2: Effect of *Urtica massaica* and *Croton megalocarpus* extracts on mean fetal head length in mice

Treatment	Mean head length ± SEM
Vehicle	10.48 ± 0.13
<i>Urtica massaica</i>	10.19 ± 0.14
<i>Croton megalocarpus</i>	9.57 ± 0.21*
Phenytoin	0 ± 0

(* $p < 0.05$)

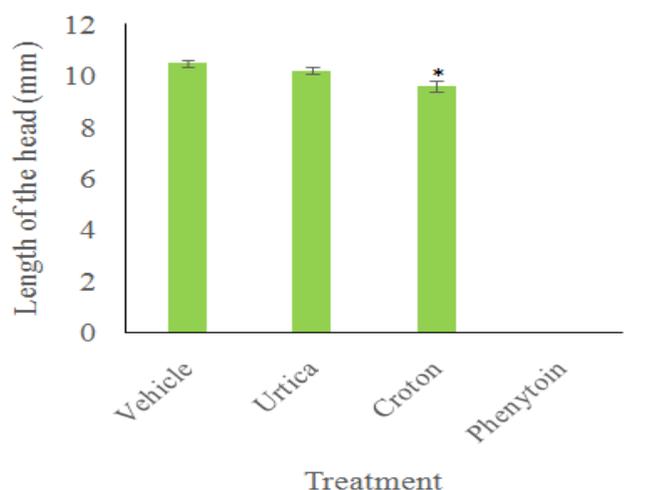


Figure 2: Effect of *Urtica massaica* and *Croton megalocarpus* extracts on fetal head length

Table 3: Effect of *Urtica massaica* and *Croton megalocarpus* on the relative amniotic fluid volume

Treatment	Relative amniotic fluid volume ± SEM
Vehicle	1.2 ± 0.2
<i>Urtica massaica</i>	1 ± 0
<i>Croton megalocarpus</i>	3.6 ± 0.24 **
Phenytoin	0 ± 0

(**p < 0.001)

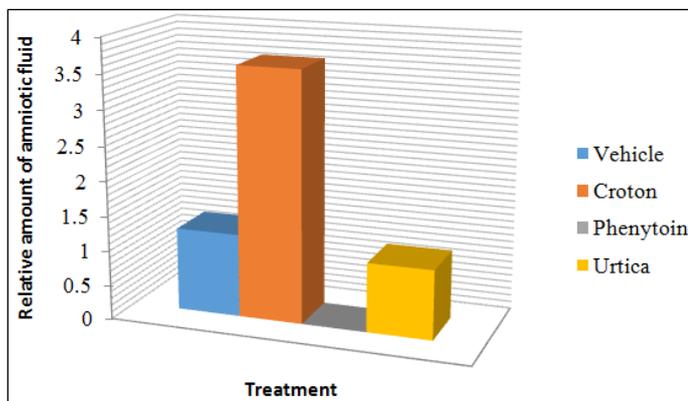


Figure 3: Effect of *Urtica massaica* and *Croton megalocarpus* on fetal amniotic fluid volume

DISCUSSION

In the study, the live weights of the mice treated with extracts had no significant difference from the ones treated with the vehicle. However, *U. massaica* and *C. megalocarpus* caused 40% and 20% partial resorption of intrauterine contents respectively (Fig 1; Table 1). In this regard, the placenta continued to grow but without foetuses. This effect lowered the overall mean weight of the uterus and its contents.

Resorption may be attributed to blighted ova, which may result from chromosomal abnormalities or possible exposure to teratogens [17]. In humans, blighted ova are the most common aetiology for spontaneous abortion during the first three months of pregnancy [18]. Blighted ova have been linked to the development of hydatidiform moles [19] also known as a molar pregnancy or a benign tumour that develops in the uterus as a result of a non-viable pregnancy [20]. The moles can also be classified into complete or partial moles based on the presence or absence of foetal formation within the neoplasia [21]. Complications that arise from hydatidiform moles include intrauterine infection,

septicaemia, haemorrhage, toxemia of pregnancy, and development of choriocarcinoma or metastatic disease [20].

The average weight of the pups from the groups treated with *U. massaica* and *C. megalocarpus* was not significantly different from that of the pups from the group treated with the vehicle. In addition, the body length and the length of the limbs of the pups from the groups treated with *U. massaica* and *C. megalocarpus* was not significantly different from that of the pups from the group treated with the vehicle. However, there was a significant reduction in foetal head size between the pups from the *C. megalocarpus* group and the pups from the vehicle-treated group (Fig. 2). The pups from the *C. megalocarpus* group had significantly smaller heads relative to the pups from the vehicle group (p=0.002) which corresponded to 8.6% head size reduction or microcephaly. Therefore, *C. megalocarpus* consumption in pregnancy may result in a higher risk of microcephaly in the offspring. In addition, the *C. megalocarpus* extract exhibited significant (p < 0.05) increase in amniotic fluid volume (polyhydramnios) relative to the vehicle. The condition usually is associated with maternal diabetes and malformations such as neural tube defects, obstruction of the foetal alimentary canal and hydrops [22]. The placenta, umbilical cord, and the foetal skin and membranes are involved in the dynamics of amniotic fluid and any abnormalities in the mechanisms involved in the production or removal of amniotic fluid lead to polyhydramnios [23]. It may be hypothesized that *C. megalocarpus* stem extracts may have interfered with any one or more of these mechanisms. However, methanol extraction is a cold method of extraction. Perhaps the cooking involved in preparation of these plants before consumption may have an effect of detoxifying them and hence reducing their teratogenicity. Therefore, it may be prudent to investigate the effect of plants extracted using hot solvent method.

CONCLUSION

High doses of the methanol extract of *U. massaica* leaves and *C. megalocarpus* stem bark extracts show teratogenic activity. Therefore, consumption of the plant extracts should be treated with utmost care during gestation.

Acknowledgements

I would like to thank Dr. Steve Runo, the Chairman of the Biochemistry and Biotechnology Department, Kenyatta University, for providing me with materials and equipment to carry out the research; Mr James Adino, Mr Kennedy Kilel, Mr Daniel Gitonga, Ms Josephine Wokabi, and Mr. Enoc Wambugu for academic and technical support.

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HOW TO CITE THIS ARTICLE

Wabai YW, Mwonjoria JKM, Njagi ENM. Teratogenic Potential of *Urtica massaica* (Mildbr.) and *Croton megalocarpus* (Hutch) in mice. *J Phytopharmacol* 2018; 7(6): 460-463.