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TERATOGENIC EFFECTS OF *CARISSA SPINARUM* STEM AND ROOT EXTRACTS IN MICE

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ABSTRACT

Carissa spinarum is used both as food and as medicine in traditional African societies. Its fruits and flowers are eaten as food; while its bark, roots, branches, and leaves are used to treat several ailments including gonorrhoea, chest pains, stomach pains, and coughs. *C. spinarum* has been shown to have anti-inflammatory, analgesic, antiherpetic, and anticancer properties. However, there is a scarcity of scientific data on its teratogenic effects. The aim of this study was to evaluate the teratogenic effects of the *C. spinarum* stem and root extracts in mice. The study was conducted using Swiss albino mice where the plant extracts were administered orally in pregnant Swiss albino mice from the 6th to the 15th day of pregnancy. On the 18th day, the mice were weighed and euthanized. The parameters recorded were as follows: maternal weights, weights of gravid uterus, and resorption. The data was tabulated as means and standard error of the means for each data set and one-way Anova/*Tukey* used for analysis. The difference in activity between the two extracts was compared using the *Student's* t-test. $P < 0.05$ was set as the significance limit. Up to 100% resorption was observed in both *C. spinarum* stem and root treated groups. From the observations of this study, it is concluded that C. *spinarum* stem and root extracts showed significant teratogenic activity. Therefore, care needs to be exercised during administration in pregnancy.

Key words: *Carissa spinarum*, *edulis*, teratogenicity, foetal resorption, anembryonic pregnancy, miscarriage.

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INTRODUCTION

Carissa spinarum, or *edulis (mtandamboo* in Kiswahili, *mukawa* in Kikuyu, and *lamuriak* in Maasai) is a dense evergreen spinous shrub of the family *Apocynaceae* (Maundu PM *et al*., 1999). The plant can be found in Kenya and throughout much of Africa and in Asia from Yemen to India (Maundu PM *et al*., 1999; World Agroforestry Centre, 2005). It rarely stands by itself but scrambles on other bushes (Maundu PM *et al*., 1999). It has glossy green, broadly ovate to elliptic leaves with a pointed apex; flowers that are reddish-pink on the outside but white on the inside; green round fruits that have a red/purple tinge and turn dark purple (almost black) when they ripen; and few, dark brown, often compressed/underdeveloped seeds (Maundu PM *et al*., 1999). All parts of the *C. spinarum* plant release a white non-toxic latex that has the appearance of milk (Mutshinyalo T and Malatji R, 2012). The flowers and fruits of the herb are edible and are consumed (World Agroforestry Centre, 2005). The unripe fruits have a tart taste and are hard while the ripe fruits are soft and delicious (Maundu PM *et al*., 1999). The unripe fruits are also fermented to make pink wine and vinegar (Mutshinyalo T and Malatji R, 2012). The herb is also used as folklore medicine for general fitness, gonorrhoea, chest pain relief, lower abdominal pain in pregnant women, polio symptoms, breast cancer, headaches and fever in children, and for strengthening bones (Maundu PM*et al*., 1999).

Extracts from the plant have been reported to exhibit antipyretic (Gitahi SM *et al*., 2015),

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antinociceptive (Gitahi SM *et al*., 2015), anti-inflammatory (Saidu IN *et al*., 2013) and hypoglycaemic (El-Fiky FK *et al*., 1996) activity. *C. spinarum* has also been shown to be antiplasmodic, anticonvulsant, hepatoprotective, anti-arthritic, antioxidant, anti-helminthic, wound healing, antimicrobial, diuretic, antiviral, and erythropoietic (Amreen F *et al*., 2013). The extract from the stem showed significant anticancer therapeutic potential against human leukaemia cells (Sehar I *et al*., 2011).

Twelve compounds have been isolated from the plant including a coumarin, two cardiac glycosides, and nine lignans (Wangteeraprasert R *et al*., 2012). The cardiac glycoside evomonoside was shown to be responsible for the anti-herpetic activity against HSVI and HSVII (Wangteeraprasert R *et al*., 2012). In addition, the lignans (-)-carinol, (-)-carissanol and (-)-nortrachelogenin exhibited anticancer properties against breast cancer and lung cancer cells (Wangteeraprasert R *et al*., 2012). All lignans showed moderate antioxidant activity (Wangteeraprasert R *et al*., 2012).

MATERIALS AND METHODS

Collection and preparation of the plant materials

Carissa spinarum stems and roots were sourced from Nanyuki, Laikipia County. They were identified and authenticated in the University of Nairobi under voucher number VW2017/02. The stems and roots were each separately ground using an electric grinding mill into fine powder to be used in the extraction process. About 100g of each powder was weighed and methanol added to cover the powder. The mixture was stirred and allowed to stand for 2 hours. The mixture was then decanted, after which more methanol was added, stirred, and allowed to stand for 24 hours before decanting once again. The supernatant was filtered, and the filtrate concentrated using a rotor evaporator to obtain the plant extract. The plant extract was then stored in a universal bottle in a cool, dry place.

Experimental animals

Virgin female Swiss albino mice weighing about 20g each were obtained. The mice were sorted into four groups (n=5). Potent male mice were introduced for mating at a male-to-female ratio of 1:3. The mice were placed in cages maintained at room temperature for a week prior to the study for acclimatization. Water and standard commercial feed from Unga Limited were provided *ad libitum.* These experiments were approved by the departmental animal ethics committee and conducted in accordance to the guidelines for the care and use of laboratory animals (Wolfensohn S and Lloyd M, 2013).

Drugs and chemicals

Distilled water, methanol, chloroform, and phenytoin sodium were used.

Teratogenicity assay

Evaluation of the teratogenicity of the stem and root extracts of *C. spinarum* was conducted in line with the FDA single-generation developmental toxicity assessment guidelines (Hodgson E, 2004). The dosage 1000mg/kg was used for the plant extracts. Distilled water was used to dissolve the extracts while phenytoin sodium was used as the positive control.

The mice were mated, and pregnancy confirmed by checking for the vaginal plug and the protrusion of the mammary glands (Croy AB *et al*., 2013). Four groups of five mice each were used where group 1 received the vehicle (distilled water). Groups 2 and 3 received 1000mg of the C*arissa spinarum* root and stem extracts orally respectively while group 4 received phenytoin sodium orally. Pregnant mice were given the doses orally daily from the 6^{th} to 15^{th} day of pregnancy. On the 18^{th} day, the mice were weighed then euthanized using chloroform and a caesarean section performed to remove the gravid uterus. Weights of the gravid uterus were recorded. Any evidence of foetal resorption was also recorded. In rodents, a lost pregnancy is resorbed (Flores LE *et al.*, 2014).

Data analysis

The data was tabulated as means and standard error of the means for each data set and one-way Anova/*Tukey* used for analysis. The difference in activity between the two extracts was compared using the *Student's* t-test. $P < 0.05$ was set as the significance limit.

RESULTS

Table 1.Effect of *C. spinarum*stem and root extracts on the live weights of the pregnant mice

Treatment	Mean live weights of pregnant mice ± SEM
Vehicle	44.99 ± 2.03
C. spinarum stem extract	$24.91 \pm 0.31^{a**}$
C. spinarum root extract	$17.166 \pm 1.21^{a**}$
Phenytoin sodium	$24.81 \pm 0.73^{**}$

[a] indicates $p < 0.05$ against each other. ** indicates $p < 0.001$ against the vehicle

Table 2. Effect of *C. spinarum* stem and root extracts on the mean weight of gravid uterus

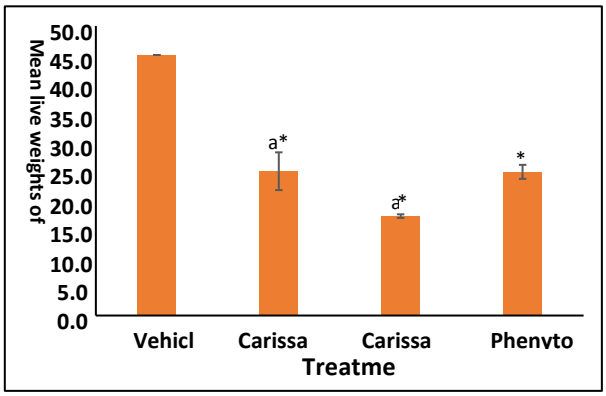
Treatment	Mean weights of the gravid uteruses ± SEM
Vehicle	13.588 ± 1.97269
C. spinarum stem extract	0.146 ± 0.01288[b**]
C. spinarum root extract	0.076 ± 0.00748[b**]
Phenytoin	0.082 ± 0.01319[**]

[b] indicates p=0.002 between the two treatments. [**] indicates p<0.001 against the vehicle.

Table 3. Effect of *C. spinarum* stem and root extracts on foetalresorption

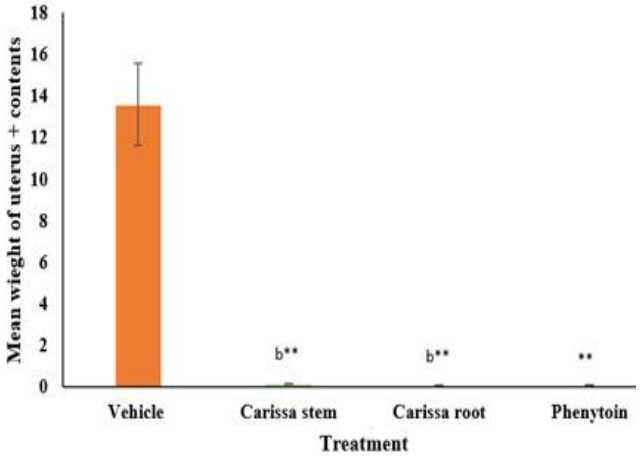
Treatment	Partial foetalresorption (%)	Absolute foetalresorption(%)
Vehicle	0%	0%
C. spinarum stem extract	20%	80%
C. spinarum root extract	0%	100%
Phenytoin	0%	100%

Fig 1. Effect of the various treatments on the mean live weight of pregnant mice



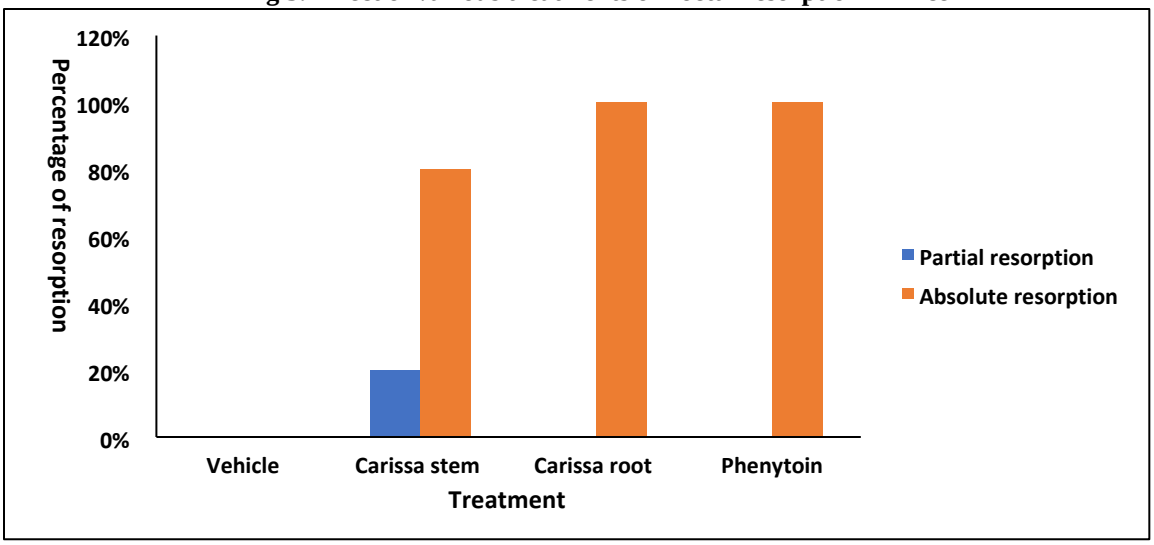
[a]indicates p< 0.05 against each other and [**]indicates p < 0.001 against the vehicle. The error bars represent the standard errors of the means of each group (n_1= n_2= n_3= n_4 =5).

Fig 2. Effect of various treatments on the mean weight of the gravid uterus.



[b]indicates p=0.002 between the two treatments and [**] indicates p<0.001 against the vehicle. The error bars represent the standard errors of the means of each group (n_1= n_2= n_3= n_4 =5).

Fig 3. Effect of various treatments on foetal resorption in mice



DISCUSSION

In the study, the live weights of the pregnant mice treated with *Carissa spinarum* stem extracts and *Carissa spinarum* root extracts significantly ($p<0.001$) reduced in the pregnant mice relative to the mice treated with vehicle. This effect was comparable to that of phenytoin (Figure 1, Table 1).

Low maternal weight during pregnancy usually results from loss of appetite, stress, disease, malnutrition, or as an effect of drugs or supplements. It poses serious risk to both the mother and the foetus (Alpers DH, 2008). Female subjects with low weight during pregnancy have an increased risk of developing toxaemia of pregnancy as well as giving birth to underweight babies (Alpers DH, 2008). These babies in turn have higher risks of morbidity and mortality as compared to new-borns of normal weight (Komlos J, 1998).

A study by the Cincinnati Children's Medical Centre showed that up to 25% of pre-term births may be attributed to abnormalities in the intervals between pregnancies, the maternal body mass index prior to pregnancy, and the magnitude of weight gain in pregnancy (Cincinnati Children's Organisation, 2016). Mothers who were underweight at the beginning of the pregnancy, as well as those who were of normal weight at the beginning of the pregnancy but had poor weight gain during pregnancy, were found to be amongst those in the highest risk group (Cincinnati Children's Organisation, 2016). In addition, poor maternal weight gain during the second and third trimesters has been linked to intrauterine growth restriction (Strauss RS and Dietz WH, 1999). Low pre-pregnancy weight and low maternal weight gain during pregnancy have been associated with a higher incidence of miscarriage (Derbyshire E, 2007).

Phenytoin, an antiepileptic drug, has been linked to malnutrition. Antiepileptic drugs are known to interfere with the metabolism of folate, making patients taking phenytoin at risk of developing folate deficiency and developing complications during pregnancy (Brewer JM and Waltman PA, 2003). A study on the long-term use of phenytoin and its effects on whole blood and red cell folate showed that while the short-term use of phenytoin significantly lowers the whole blood and red cell folate values, the long-term use of phenytoin does not cause a clinically-significant state of folate deficiency unless some other contributing factors are present (Weber TH *et al*., 1977).

Severe folate deficiency leads to megaloblastic anaemia (Alpers DH, 2008). In pregnant women, it has been linked to higher incidences of spontaneous abortions, stillbirth, placental abruption, toxaemia, premature rupture of the membranes, pre-term delivery, orofacial clefts, Down's syndrome, congenital heart defects, foetal growth restriction and limb defects (Alpers DH, 2008). In addition, phenytoin is known to interfere with vitamin D and calcium levels by increasing the metabolism of vitamin D thereby leading to a decrease in vitamin D levels which in turn alters the calcium homeostasis and leads to osteomalacia and osteoporosis (Brewer JM and Waltman PA, 2003). *Carissa spinarum* stem and root extracts exhibited maternal weight reduction that was comparable to the effect by phenytoin. Hence, it can be hypothesized that *C. spinarum* stem and root extracts may have caused malnutrition in the mice that could have resulted in lower maternal weight.

Resorption was observed in both *C. spinarum* stem and root groups. (Figure 3, Table 3). The resorption led to a significantly ($p<0.001$) lower weight of the gravid uteruses (Figure 2, Table 2). This effect is also comparable to that of phenytoin.

Resorption may be attributed to blighted ova. A blighted ovum, also known as an anembryonic pregnancy, is a pregnancy in which the embryo never develops or develops and is resorbed. Blighted ova may result from chromosomal abnormalities or possible exposure to teratogens (Sahar S *et al*., 2013). In humans, blighted ova are the most common reasons for spontaneous abortion (miscarriage) during the first three months of pregnancy (Asiyeh M *et al*., 2017). Blighted ova have also been linked to the development of hydatidiform moles (Bernd KW *et al*., 1981). A hydatidiform mole, also known as a molar pregnancy, is a tumour that develops in the uterus as a result of a non-viable pregnancy and it can be benign or malignant (Flaws B, 2005). Complications that arise from hydatidiform moles include intrauterine infection, septicaemia, haemorrhage, toxaemia of pregnancy, and development of choriocarcinoma or metastatic disease (Flaws B, 2005).

The resorption in the *C. spinarum* stem and root groups and in the phenytoin group may also be related to the mechanisms previously discussed in relation to the maternal weights of the mice. Mice from the three groups had significantly ($p<0.001$) lower maternal weights than those from the group that was administered the vehicle. Low maternal weight gain during pregnancy has been associated with a higher incidence of miscarriage (Derbyshire E, 2007). In rodents, a lost pregnancy is resorbed (Flores LE *et al.*, 2014).

From the study, it is evident that the methanol extracts from the plants had a substantial teratogenic effect. However, cold extraction method was used which is in variance to the hot method used in traditional African societies where it is prepared by boiling in bone soup. It is possible that boiling deactivates the toxic phytochemicals present in the plant extract, a hypothesis that requires to be investigated. In addition, it may be that the dose that was given is higher than what is taken traditionally.

CONCLUSIONS AND RECOMMENDATIONS

Carissa spinarum stem and root extracts caused resorption and hence show significant teratogenic activity. In addition, *C. spinarum* root extracts exhibit more toxicity than *C. spinarum* stem extracts. Therefore, caution should be advised when administering the plant extracts in pregnant women and breastfeeding mothers.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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