

Oral Acute Toxicity Study and Effect of Methanolic Seed Extract of Wonderful Kola (*Buchholzia Coriacea* R.W Buchholz) on Implantation in Wistar Albino Rats

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ABSTRACT

Background: *Buchholzia coriacea* (wonderful kola), belonging to the family of Capparidaceae, is an evergreen shrub, that is geographically distributed in Cameroon, the Central African Republic, Gabon, Angola, Nigeria, and Ghana, among others. It is used in traditional medicine for the treatment of various ailments and for various purposes. This study was conducted to determine the LD50, phytochemical constituents, and evaluate the effect of a methanolic seed extract of *Buchholzia coriacea* on implantation in female Wistar albino rats. **Methodology:** The phytochemical screening was done according to the standard method, to detect phytochemicals present in the methanolic seed extract of *Buchholzia coriace*. A total of 36 adult female Wistar albino rats were used for this study. Twelve rats were used for the acute toxicity study, whereas for the implantation study, 24 mature female rats were divided into four groups (control, low, medium, and high dose groups). Group I was designated as a control, and the other three groups as test groups. Two hundred and fifty (250), 500, and 1000 mg/kg of methanolic seed extract of *Buchholzia coriacea* were administered to the test groups for 10 days, after which the uteri were removed and implantation sites were counted. **Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, and saponins. Acute toxicity study revealed that *Buchholzia coriacea* has LD50 > 5000 mg/kg.. Administration of the methanolic seed extract of *Buchholzia coriacea* did not significantly (p 0.05) alter the number of implantation sites across the test groups when compared with the control. **Conclusion:** The results showed that the administration of a methanolic seed extract of *Buchholzia coriacea* had no significant effect on implantation.

Key words: Acute, *Buchholzia coriacea*, implantation, Phytochemicals and toxicity

INTRODUCTION

Acute toxicity test estimates the adverse effects that occur after the exposure of an organism to a single dose of a substance through a particular route (oral, dermal or inhalation). The effect of any substance is related to its absorption and distribution within tissues^[1]. The LD50 test

(median lethal dose) was initiated by Trevan in 1927^[2] to evaluate the dose of a substance that will result in 50% mortality in given specie. It estimates the probable hazards of substances in an organism. Though the main target point is death, non-lethal effects may arise as signs of toxicity. These signs

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depend on the amount and nature of the substance administered. These signs of toxicity may include, body weakness, erected hair, loss of appetite, diarrhoea and bulging of the eyes^[4]. Anderson^[5] defined phytochemicals as plant-derived chemicals which are beneficial to human health and disease prevention. Plants have basic nutritional importance by their content of protein, carbohydrates, fats and oils, minerals, vitamins, and water responsible for growth and development in man and animals. In addition to vitamins and pro-vitamins in fruits and vegetables, the presence of bioactive plant components often called phytochemicals has been considered of crucial nutritional importance in the prevention of chronic diseases such as cancer, cardiovascular disease and diabetes^[6]. It has been discovered that regular consumption of fruits, vegetables, herbs and spices has associated health benefits, but their mechanism has become clear only in recent years. These plants contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals^[7].

Implantation is the term used to describe the process of attachment and invasion of the uterine endometrium by the blastocyst (conceptus) in placental animals^[8]. In humans, this process begins at the end of week 1, with most successful human pregnancies the conceptus implants 8 to 10 days after ovulation, and early pregnancy loss increases with later implantation^[8]. The implantation process continues through the second week of development. The initial phase of the implantation process is "adplantation". This first phase requires the newly hatched blastocyst to loosely adhere to the endometrial epithelium, often "rolling" to the eventual site of implantation where it firmly adheres.

This process requires both the blastocyst adhesion interaction with the endometrium during the receptive window and subsequent development of the placenta allowing maternal support for embryonic and foetal development. During the "window of implantation", uterine endometrium transforms into a receptive status to accept the

embryo, thereby establishing successful embryo implantation^[9]. Wonderful kola or *Buchholzia coriacea* (R.W. Bucholz) belongs to the family of *Capparaceae*. It is an evergreen shrub, which is distributed in Cameroon, Central African Republic, Gabon, Congo, Angola, Nigeria and Ghana, among others^[7,11]. It was named after R.W. Bucholz who collected the plants in the late 19th century^[12].

The seeds gave the plant its common name of 'wonderful kola' because of its usage in traditional medicine. The parts commonly eaten are the seeds which are either cooked or eaten raw^[13], the seeds are covered in a purple aril which is chewed in Ivory Coast and has a sharp pungent taste^[14]. Wonderful kola has numerous medicinal uses: The bark can be made into a pulp for inhalation or ground to relieve headache, sinusitis, nasal congestion, smallpox or skin itching. The pulped bark is applied to the chest to treat chest pains and also boils. In Liberia, the seeds are used on skin eruption and internally for worms. In Ivory Coast, the crushed-up seeds, are pasted over the stomach for difficult childbirth^[15]. It is considered an anthelmintic agent or substance^[16]. Its leaves and seeds are reported scientifically to have good anthelmintic agent or substance^[16], antibacterial^[11], antimicrobial^[11], hypoglycemic^[18], antimalarial^[19], abortifacient and cytotoxicity effects^[20].

It is also used as a cough medicine and in the treatment of ulcers. *Buchholzia coriacea* has been used in the treatment of hypertension by drinking the fluid squeezed out of the leaves with pea leaves and small salt. In Ivory coast the twig bark decoction of the plant *Buchholzia coriacea* is used for the treatment of rheumatism and kidney pain, it is also used for the treatment of infections of the eye and the treatment of pain in the back (fruit pulp massaged in). For the treatment of earache, the seeds are pounded in a little bit of water and the resulting liquid is dropped into the ear^[21]. The seeds or kernels of the plant *Buchholzia coriacea* are edible and have a spicy taste and they can be used as a condiment^[22].

METHODOLOGY

This research was conducted in the department of human anatomy, University of Maiduguri, Borno State. Borno state is located within latitudes 10 N and 14 N and longitude 11 30 E and 14 45 E. The state which has an area of 61,435sq km shares borders with the republic of Niger to the north^[23].

Ethical clearance

Ethical clearance was obtained from the Department of human anatomy ethical committee (UM/HA/PGR16.20-O8803).

Phytochemical screening was done according to the standard methods to detect phytochemicals present in methanolic seed extracts of *buchholzia coriacea*^[24,25,26&27].

The acute oral toxicity study was carried out in accordance with the protocol described by Lorke,^[28] (Table 1). Nine rats were allocated into three groups of three rats each in phase I. The groups received MSEBC at 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. Three rats were distributed into three groups (n=1) and received 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of MSEBC respectively. All the rats were monitored for two weeks for any sign of toxicity (lacrimation, salivation, tremor and diarrhoea) and/or mortality.

The implantation activity was determined according to the method of Olagbende-Dada^[29]. Twenty-four mature female Wistar albino rats were divided into four groups (6 rats per group) A, B, C and D. Group A was used as control and the other three groups as a test group. Female rats at the proestrus phase of the oestrus cycle were caged with males with proven fertility in a ratio of 2:1. The female rats were examined the following morning for evidence of copulation. A vaginal smear was examined for thick clumps of spermatozoa. The day on which spermatozoa were found in the smear was considered the first day of pregnancy. Two-Fifty (250)mg/kg of body weight, 500mg/kg of body weight and 1000mg/kg of body

weight of the extract was administered orally to rats of group B, C and D respectively for 10 days from day 1 to day 10 of pregnancy, the same volume of vehicle was given to control group. On day 11, all groups of rats had laparotomies under ether anaesthesia to determine the number of implantation sites in the horns of the uteri. This was done using Salewski technique^[30]. Uteri were subjected to Salewski staining for the detection of implantation sites and the stained implantation sites were counted.

The results were expressed as Mean \pm SEM. One-way analysis of variance (ANOVA) was used to compare the mean differences between control and treated groups. $P \leq 0.05$ was considered statistically significant^[28].

RESULTS

Acute Toxicity Study

The Administration of methanolic seed extract of *buccholzia coriacea* did not result in mortality in Wistar albino rats at 10,100,1000,1600,2900 and 5000mg/kg after 24hrs of treatment (Table 1). Also, the rat's behaviour and activities were normal. This suggests that the extract is relatively safe and the lethal dose (LD_{50}) is greater than 5000mg/kg.

Administration of methanolic seed extract of *buccholzia coriacea* causes a non-significant increase in the number of implantation sites in the low dose group (7.17 \pm 1.72) and medium dose group (6.83 \pm 0.98) compared to the control group (5.33 \pm 1.03) and a non-significant decrease (3.13 \pm 2.40) in high dose group.

Table 1. Result of acute toxicity study

| | Dose (mg/kg) | Observations | Number/percentage mortality |
|-----------------------|--------------|--|-----------------------------|
| The first phase (n=3) | | | |
| I | 10 | Rats' behaviour and activities were normal | 0 (0%) |
| II | 100 | Rats' behaviour and activities were normal | 0 (0%) |
| III | 1000 | Rats' behaviour and activities were normal | 0 (0%) |
| Second phase (n=1) | | | |
| I | 1600 | Rats' behaviour and activities were normal | 0 (0%) |
| II | 2900 | Rats' behaviour and activities were normal | 0 (0%) |
| III | 5000 | Rats' behaviour and activities were normal | 0 (0%) |

N= number of Rats per group

Table 2: Effect of Methanolic Seed Extracts of *Bulchozia coriacea* on Implantation

| Group | Control | Low dose | Medium dose | High dose |
|-----------------------------|-----------|-----------|-------------|-----------|
| Dose(mg/kg) | 0 | 250 | 500 | 1000 |
| (No. of implantation sites) | 5.33±1.03 | 7.17±1.72 | 6.83±0.98 | 3.13±2.40 |

Values expressed as mean ± Standard deviation $P < 0,05$

DISCUSSION

Phytochemical screening has revealed many bioactive as well as toxic agents of plants that can affect the oestrous cycle, conception and reproduction. Numerous chemical agents contained in plant extracts can cause hormonal imbalances^[29]. Phytoconstituents interact with the reproductive system in many ways. Alkaloids have been shown to decrease plasma concentrations of hormones that encourage fertility^[30]. Saponins have been reported to reduce fertility in animals upon continuous administration^[31]. Also, saponins are known to improve oocyte quality by decreasing the proportion of abnormal oocytes which may be present in the animal^[32]. The results from the phytochemical screening of the methanolic seed extract of *buccholzia coriacea* observed in this study revealed the presence of alkaloids, tannins, saponins, carbohydrates, and flavonoids. This is per studies conducted by^[12,30,31], where they investigated the phytochemical constituents of the methanolic seed extracts of *buccholzia coriacea* (wonderful kola). Both researchers in their separate studies detected the presence of some of these phytochemicals such as saponins, alkaloids and flavonoids. Also, the result of the present study agrees with a study conducted by Umeokoli^[38]

where he reported the absence of anthraquinone.

Administration of methanolic seed extract of *buccholzia coriacea* did not result in mortality in Wistar albino rats at 1600, 2900 and 5000mg/kg after 24hrs of treatment. Also, signs of toxicity such as loss of appetite, weakness, piloerection, diarrhoea, lethargy, stretching of the abdomen and one-sided movement were not observed. This suggests that the extract is relatively safe and the lethal dose (LD_{50}) is greater than 5000mg/kg. The relative safety observed may have been responsible for the widespread use of the plant both as food and for the treatment of various ailments. This finding is in agreement with the results obtained by Theophine *et al.*^[29] who showed that *buccholzia coriacea* seed extract has an oral lethality dose greater than 5000mg/kg body weight. The lack of associated lethality with this high dose indicates a high safety profile of the extract of *buccholzia coriacea* seeds as reported by Lorke^[28]. Also, the present study agrees with that conducted by Adisa *et al.*^[18] who observed that no death was recorded after the toxicity study and therefore concluded that LD_{50} of *buccholzia coriacea* is greater than 30,000mg/kg body weight.

Administration of methanolic seed extract of *buccholzia coriacea* suggests no significant difference in the number of implantation sites in the treated groups compared to the control. The recent findings agree with a study conducted by Oludare

and Iranloye^[31], where there was no significant difference in the number of implantation in low salt-fed rats on days 6 and 8 of pregnancy. However, contrary to the present study, the high salt-fed rats showed a significant decrease in the number of implantation on days 6 and 8. Also, the present study disagrees with a study carried out by Oyesola *et al.*^[32] where they observed a significant decrease in the number of implantation sites in pirimiphos-methyl treated rats sacrificed on day 6 in groups B, C and D when compared with group A (control). A similar pattern was observed on day 8 in groups B, C and D when compared with control. The implantation process is a critical event in the embryo's life and a central step to the establishment of placentation and pregnancy^[33]. It is initiated on day 5 and completed by day 7 of the pregnancy^[33].

CONCLUSION

Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, carbohydrates, and saponins. Conclusively, the oral acute administration of methanolic seed extract of *bucchozia coriacea* at 5000mg/kg in rats produced no adverse effects during the study period. Hence, the LD50 of methanolic seed extract of *buchholzia coriacea* in the present study is greater than 5000 mg/kg. The result of the present study suggests that *bucchozia coriacea* is a nontoxic extract with no significant effect on implantation.

Recommendations

Administrations of *buchholzia coriacea* at a higher dose than the doses used in this study should be tried, other routes of administration should be tried. Also, the effects of the extract on oestrous cycle in female albino rats should be investigated

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