WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 10, 32-51. Research Article ISSN 2277– 7105

IN VIVO MUTAGENICITY AND REPRODUCTIVE TOXICITY STUDIES OF THE MIXTURE OF ROOT BARK POWDERS FROM *CALOTROPIS PROCERA* AND *ZANTHOXYLUM ZANTHOXYLOÏDES*

SOMDA Donzèo Gaétan*[1,2], OUEDRAOGO Geoffroy Gueswindé[2,3], ILBOUDO Sylvain[2,3], OUEDRAOGO Moussa[1], GUISSOU Innocent Pierre[4], OUEDRAOGO Noufou[2,3]

[1]Laboratoire du Développement du Médicament, École Doctoral de Santé, Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.

[2]Département de Médecine et Pharmacopée Traditionnelles et Pharmacie, Institut de Recherche en Sciences de la Santé, Centre National de la Recherche Scientifique et Technologique (MEPHATRA-PH/IRSS/CNRST), 03 BP 7047 Ouagadougou 03, Burkina Faso.

[3]International Research Laboratory, Environnement, Santé, Sociétés (IRL 3189, ESS) CNRST/CNRS/UCAD/UGB/USTTB, Burkina Faso.

[4]Université Saint Thomas d'Aquin, 06 BP 10212 Ouagadougou 06, Burkina Faso.

Article Received on
30 May 2022,

Revised on 20 June 2022,
Accepted on 10 July 2022

DOI: 10.20959/wjpr202210-24883

***Corresponding Author**
SOMDA Donzèo Gaétan
Laboratoire du Développement du Médicament, École Doctoral de Santé, Université Joseph KI-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.

ABSTRACT

Herbal therapies are used by about 88% of the world's population and 90% of the African population still depend on medicinal plants for their health and well-being. In Burkina Faso, traditional medicine remain the main source of primary health care for 70% of the population. However, some plant-based preparations have toxic effects that can lead to mortality or morbidity. This work aimed to evaluate the mutagenic potential on mouse erythrocytes and the effects on reproduction of the mixture of *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root bark powders, plants used in the traditional treatment of sickle cell disease and in the formulation of FACA®, in order to make its use safe for the management of sickle cell disease. The mutagenicity study was performed according to OECD guideline 474 on NMRI mice. The reproductive toxicity study of the mixture was

performed on Wistar rats according to OECD guideline 422. The results of the study showed that the mixture of *Calotropis procera* and *Zanthoxylum zanthoxyloides* root bark powders is not mutagenic in mice up to 2000 mg/kg bw. This mixture causes a dose-dependent stimulation of spermatogenesis in male rats. In female rats, this mixture causes a decrease in fertility at 500 and 1000 mg/kg bw. However, at 250 mg/kg bw it has no effect on reproductive capacity in rats. The use of the mixture of *Calotropis procera* and *Zanthoxylum zanthoxyloïdes* root powders at low doses does not have a mutagenic effect on mice erythrocytes and does not affect reproductive health in rats.

KEYWORDS: *Calotropis procera*; *Zanthoxylum zanthoxyloïdes*; FACA®; mutagenicity; reprotoxicity.

INTRODUCTION

Medicinal plants are important sources of bioactive compounds used by humans since ancient times to treat their diseases. Herbal therapies are used by about 88% of the world's population for their primary health care.[1] In Africa, plants have played an important role in maintaining human health and improving the quality of human life for several thousand years.[2] Nowadays, approximately 90% of the African population still relies on medicinal plants to maintain and manage their health and well-being.[3] In Burkina Faso, traditional medicine and pharmacopeia remain the primary source of health for 70% of the population.[4]

This strong adherence of the population to natural therapies is due to the fact that these therapies are less invasive and less expensive.[5] Yet several works have reported that some herbal preparations have toxic effects. Some of these preparations have been associated with toxic effects that can lead to mortality or morbidity, which requires careful monitoring.[3] *Calotropis procera* (*C. procera*) and *Zanthoxylum zanthoxyloïdes (Z. zanthoxyloïdes)* are two plants used in the traditional medecine for the management of many pathologies. The combination of these two plants is used traditionally for the treatment of sickle cell disease. The mixture of root bark powders from these plants is used in the formulation of FACA®. FACA® a phytomedicine developed by the Institut de Recherche en Sciences de la Santé of Burkina Faso for the management of sickle cell disease. This phytomedicine obtained its marketing authorization in 2010 and was included in the list of essential medicines in Burkina Faso in 2011.[6] Numerous studies have demonstrated various pharmacological properties of FACA® including antifalciformant, anti-inflammatory, analgesic, antipyretic and muscle relaxant properties.[7–9] Toxicological studies on acute oral toxicity in mice and rats[10,11] and

subchronic toxicity (90 days) in rats[10] have been conducted. However, there are no data on the mutagenic effects and reprotoxicity of FACA®. Therefore, the present study aimed to investigate the mutagenicity and the effects on reproduction of the mixture of *C. procera* and *Z. zanthoxyloïdes* root powders to better understand the safety profile of this combination for the management of sickle cell disease.

MATERIALS AND METHODS

Plant material

The plant material of the study was a mixture of *C. procera* and *Z. xanthoxyloïdes* root bark powders (FACA® powder) obtained from the Phytomedicine Production Unit (U-PHARMA) of the "Institut de Recherche en Sciences de la Santé (IRSS)", Ouagadougou, Burkina Faso.

Animals

Wistar rats of both sex weighting 262.75 ± 8.66 (for male) and 229.25 ± 10.63 (for female), were used for the reproductive toxicity study. Male and female NMRI micewith mean weights of 36.4 ± 1.14 (male) and 30.4 ± 1.34 (female) were used for the mutagenicity test. These animals were provided by the IRSS pet Shop and were raised in plastic cages at room temperature (23-25°C) with 40-60% humidity. They were fed with 29% protein-enriched wheat cake and tap water. These animals were subjected to 12 hours of light and 12 hours of darkness. The animals were used according to protocols already validated by the Institute of Research in Health Sciences (IRSS, Burkina Faso) and which meet the international standards set by the European Union on the protection of animals (CEC Council 86/609).

Mutagenicity study

The mutagenicity study of the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders was performed according to OECD guideline 474[12] on mice. Five groups of ten mice (five males and five females) were constituted, including three test groups and two control groups (positive and negative control). The different groups were homogeneous and the females were non-pregnant. Potassium bromate ($KBrO_3$) was used as a positive control. It was dissolved in 0.9% NaCl solution and the root bark powders in distilled water before administration. The volumes administered were proportional to the weight of the animals (1mL per 100g body weight).

Administration of the doses

The negative control was given distilled water orally and the positive control received 80 mg/kg body weight of potassium bromate ($KBrO_3$) intraperitoneally. The three test groups received orally respectively 500, 1000 or 2000 mg/kg body weight of aqueous suspension of the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders. All these substances were administered to the animals once a day for a week.

Slide preparation

At the end of the test period, peripheral blood was collected from the tail vein of each mouse 48 hours after the last administration. From this blood, we made blood smears on slides. These smears were dried for 20 min after which they were fixed and stained with Giemsa. These slides were analyzed with an olympus light microscope at X1000 magnification.

Analysis and processing of results

The percentage of micronucleated polychromatic erythrocytes (MPE) and the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) was determined for each mouse by counting one thousand (1000) erythrocytes. All slides were coded before microscopic analysis.

Reproductive toxicity study

The reproductive toxicity study of the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders was performed according to OECD guideline 422[13] on four groups of twenty rats (10 males and 10 females) including three test groups and one control group. To the control group, distilled water was administered per gavage and to the three test groups, we similarly administered respectively 250, 500 or 1000 mg/kg body weight of aqueous suspension of the mixture of *C. procera* and *Z. zanthoxyloïd*es root bark powders orally. The test substance was administered once daily to 5 rats of each test group for 14 days. At the end of the first two weeks, treated males were paired with untreated females of the same group and treated females with untreated males (one male to one female) for two weeks. Administration of the test substance was continued during the mating period and beyond until at least 28 days for males and 63 days for females. The dilution solvent was distilled water. The volumes administered were proportional to the weight of the animals (1 mL per 100g body weight).

Clinical examination

During the test period, the animals were clinically examined at least twice daily for signs of morbidity or mortality related to the test substance. All animals were weighed on the first day of test substance administration and at least once a week thereafter, as well as on the last day.

General Autopsy

At the end of the test period, all animals were fasted 12 hours prior to sacrifice.

In males, the testes, epididymis, seminal vesicle, and prostate were removed, washed with ice-cold saline, and weighed. The organs were examined macroscopically to assess changes in appearance, size, color, and shape. Then their were weighed so to calculate the relative weight of each organ.

In females, the ovaries and uterus, including the cervix, were also weighed immediately after dissection (to avoid dehydration). These organs were examined macroscopically and the relative weight of each organ was calculated.

Evaluation of the effect of the mixture on male fertility

At the end of the test period, all animals were sacrificed. Spermatozoa were collected by dissection of the epididymis in a 3% sodium chloride solution (this hypertonic solution causes the death of spermatozoa without causing their lysis. Thus, the spermatozoa are immobile and the counting is facilitated). The mixture was diluted to $1/100^{th}$. After homogenization of the mixture, the solution was deposited with a micropipette in order to fill by capillarity, without air bubbles, the chamber of the hematimeter (Malassez cells). After sedimentation (for a few minutes) the spermatozoa were counted under the microscope at a magnification of x400 on 5 large squares. The number of spermatozoa was calculated according to the following formula:

$$n = (n_1 \times fd)/(s \times p).$$

n = number of spermatozoa/mL of semen; n_1 = number of spermatozoa counted

fd = dilution factor; s = considered surface (in mm^2) and p = chamber depth

Evaluation of the effect of extracts on female fertility and development

Each litter was examined as soon as possible after parturition to determine the following variables: number of rats born, total number of weaned animals, number of stillbirths and

runts, and the presence of gross anomalies. Litters were weighed within 24 hours of parturition on days 4 and 13 post-partum. These values were used to calculate:

- Birth index (%) = (number of pups born alive/total number of pups born) X100,
- Viability index (%) = (Number of live pups at day 4 postpartum/number of live births) X 100,
- Weaning Index (%) = (Number of live pups at weaning/number of live pups born) X 100.

STATISTICAL ANALYSIS

Results were presented as mean ± standard deviation. Data were calculated separately for males and females using Microsoft Excel 2010. Statistical analysis of the results was performed by one-way analysis (ANOVA) using GraphPad Prism. 5 software (GraphPad software, San Diego, California, USA) followed by Dunett's multiple comparisons tests. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Results

Mutagenesis study

The results of the mutagenesis test are presented in Figures 1 and 2 and Table 1. From these results, the frequency of micronucleated erythrocytes and the PCE/NCE ratio were significantly increased in mice treated with potassium bromate ($KBrO_3$), used as a positive control, compared with untreated mice. However, in mice treated with the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders, no significant difference was observed in either the frequency of micronucleated erythrocytes or the PCE/NCE ratio compared to untreated mice.

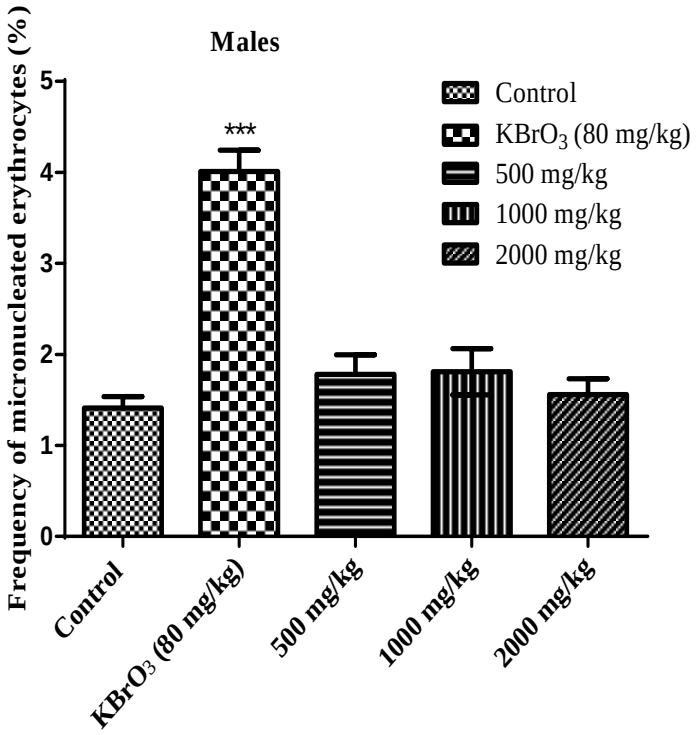

Figure 1: Frequency of micronucleated erythrocytes in the peripheral blood of male mice treated with the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders. $KBrO_3$: positive control (80 mg/kg); *** P<0.001

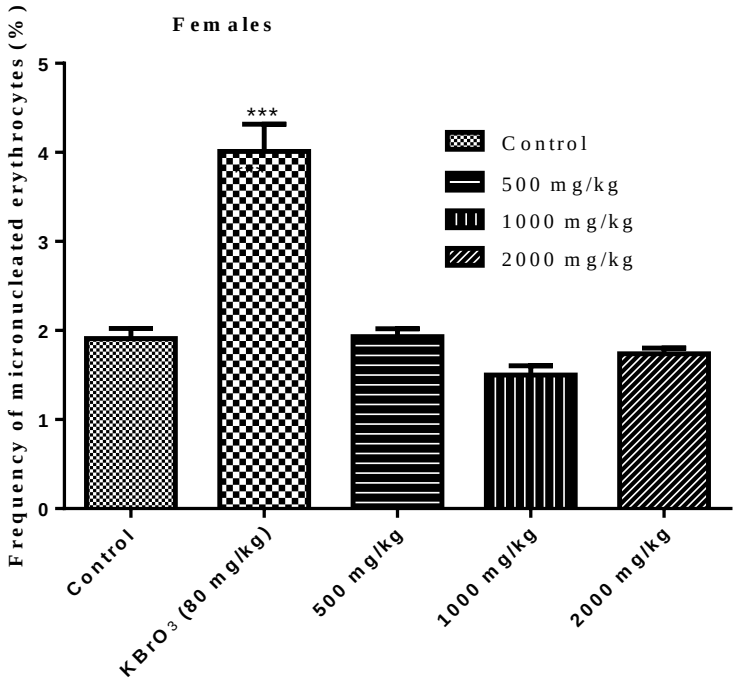

Figure 2: Frequency of micronucleated erythrocytes in the peripheral blood of female micetreated with the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders. $KBrO_3$: positive control (80 mg/kg); *** P<0.001

Table 1: Number of polychromatic erythrocytes per 1000 erythrocytes from control and mixture-treated rats with *C. procera* and *Z. zanthoxyloïdes* root bark powders.

	Sex	control	KBrO4	500 mg/kg	1000 mg/kg	2000 mg/kg
PCE	M	12.88 ± 6.61	46.38 ± 17.37***	18.90 ± 10.15	19.75 ± 3.66	16.60 ± 6.91
	F	20.00 ± 8.69	21.13 ± 8.84	14.40 ± 5.93	19.40 ± 5.68	17.90 ± 5.86
PCE/NCE (%)	M	1.31 ± 0.68	4.89 ± 1.89***	1.93 ± 1.05	2.02 ± 0.38	1.69 ± 0.71
	F	2.05 ± 0.9	2.16 ± 0.92	1.46 ± 0.61	1.98 ± 0.59	1.83 ± 0.61

Reproductive toxicity study in rats

In males rats

Body Weight variation

Figure 3 shows the body weight variation of male rats during the treatment period. Analysis of the results in this table shows a significant decrease in weight gain in rats treated with 500 and 1000 mg/kg bw during the 3rd and 4th week of treatment.

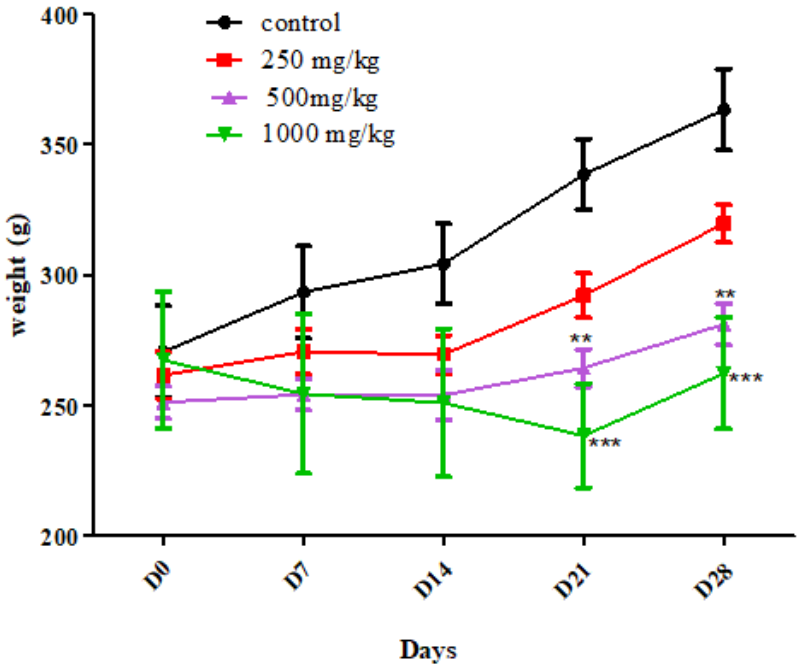

Figure 3: body weight evolution in males rats

Relative weight of sexual organs

The results of the study of the effect of the mixture of *C. procera* and Z. *zanthoxyloides* root bark powders on the male rats sex organs are presented in Table 2. Administration of this mixture resulted in a significant increase in relative testicular weight in rats treated at 500 and 1000 mg/kg body weight.

Table 2: Relative weights of male reproductives organs of control rats and those treated with the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders.

Dose	Organs			
	Seminal vesicle	**Prostate**	**Epididymis**	**Testicles**
Control	0.573 ± 0.076	0.181 ± 0.039	0.216 ± 0.083	0.424 ± 0.109
250 mg/kg	0.588 ± 0.137	0.183 ± 0.025	0.272 ± 0.111	0.502 ± 0.084
500 mg/kg	0.530 ± 0.134	0.172 ± 0.015	0.277 ± 0.046	0.613* ± 0.076
1000 mg/kg	0.426 ± 0.069	0.129 ± 0.021	0.268 ± 0.049	0.654** ± 0.083

n = 5; results are presented as mean ± SD; *: p < 0.05; **: P < 0.01

Effects of the mixture on fertility

The mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders caused a dose-dependent increase in sperm count in all treated rats compared to control (Figure 4). However, statistical analysis of the results shows that the difference between the sperm counts of treated and control rats was not statistically significant.

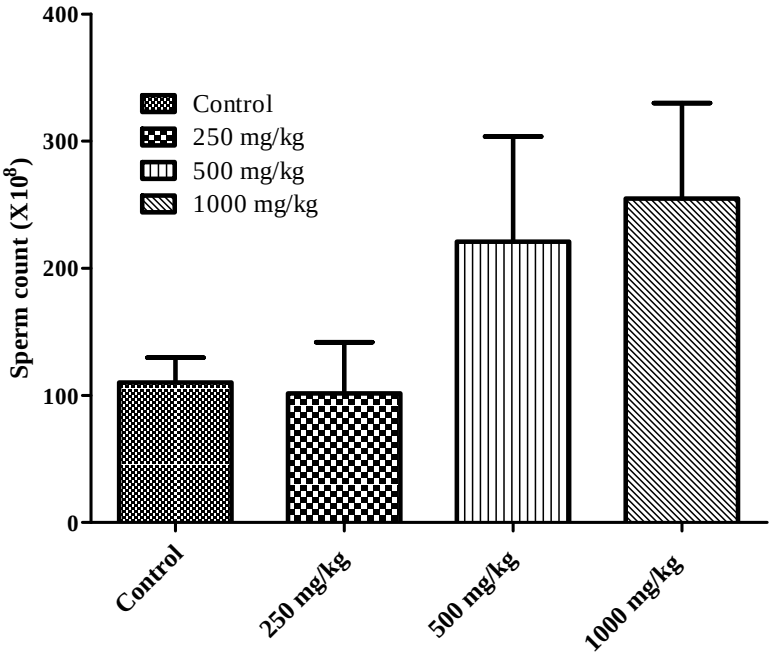

Figure 4: Variation in sperm count in control rats and those treated with the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders

In females rats

Body weight variation

Figure 5 shows the body weight evolution of female control rats and those treated with mixtures of *C. procera* and *Z. zanthoxyloïd*es root powders. The analysis of these results shows a decrease in body weight in all treated rats during the first two weeks. However, this

decrease in weight was significant only in females treated with 1000 mg/kg bw. From the second week onwards, all rats gained weight. However, there was a significant decrease in weight gain throughout the test period in rats treated with 500 mg/kg and 1000 mg/kg during the 5th week of treatment.

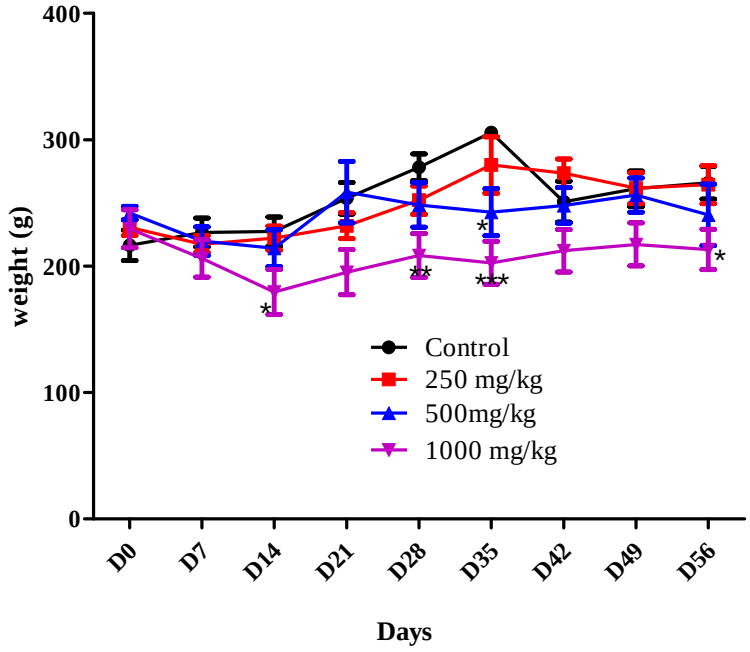

Figure 5: Body weight of the control and treated females rats

Relative weight of reproductive organs

Table 3 shows the relative weights of the uteri and ovaries of control and treated females rates. Statistical analysis of these results revealed no significant difference between the relative weights of ovaries and uteri of treated rats compared to controls.

Table 3: Relative ovary and uterus weights of control and *C. procera* and *Z. zanthoxyloïdes* root bark powder treated rats.

Doses	Organs	
	ovary	uterus
Control	0.025 ± 0.008	0.266 ± 0.213
250 mg/kg	0.063 ± 0.078	0.243 ± 0.093
500 mg/kg	0.023 ± 0.005	0.205 ± 0.052
1000 mg/kg	0.030 ± 0.010	0.228 ± 0.079

n = 5; results are presented as mean ± SD

Effect of the mixture on females rats fertility

The results of the effect of the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders on fertility parameters in female rats are presented in Tables 4 and 5. According to these results the lowest pregnancy, viability, and weaning rates were obtained with the female rats treated at 500 and 1000 mg/kg body weight.

Table 4: Fertility index of control females rats and those treated with the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders (treated females mated with untreated males).

Dose	Gestation index	Birth index	Viability index	Weaning index
Control	80	89.63 ± 21.99	83.23 ± 18.58	77.86 ± 24.92
250mg/kg	60	97.22 ± 4.81	100.0 ± 0.00	75.00 ± 35.36
500 mg/kg	40	50.00 ± 70.71	50 ± 70.71	-
1000 mg/kg	-	-	-	-

n = 5; results are presented as mean ± SD

Table 5: Fertility index of females control rats and those untreated but mated with treated males rats.

Dose	Gestation index	Birth index	Viability index	Weaning index
Control	80	89.63 ± 21.99	83.23 ± 18.58	77.86 ± 24.92
250mg/kg	60	92.87 ± 10.10	100.0 ± 0.00	100.0 ± 0.00
500 mg/kg	80	95.83 ± 8.34	80.00 ± 40.00	80.00 ± 40.00
1000 mg/kg	80	100.00 ± 0.00	87.50 ± 17.68	83.34 ± 23.57

n = 5; results are presented as mean ± SD

Body weight variation of pups

Tables 6 and 7 present the weight evolution of the pups from the control and treated female rats with the mixture. The analysis of these results shows that there is no significant difference in body weight between the treated and control rats.

Table 6: Body weight variation of the pups (treated females mated with untreated males).

Doses	Dates		
	D0	**D4**	**D13**
Control	5.35 ± 0.54	6.74 ± 1.99	17.36 ± 2.39
250 mg/kg	5.63 ± 0.84	7.31 ± 2.05	13.88 ± 2.95
500 mg/kg	6.35 ± 1.26	9.07 ± 0.00	-
1000mg/kg	-	-	-

n = 5; results are presented as mean ± SD

Table 7: Body weight variation of the pups (untreated females mated with treated males).

Doses	Dates		
	D0	D4	D13
Control	5.35 ± 0.54	6.74 ± 1.99	17.36 ± 2.39
250 mg/kg	6.16 ± 1.23	9.37 ± 2.61	17.63 ± 5.18
500 mg/kg	6.46 ± 1.33	9.20 ± 3.53	21.02 ± 7.84
1000mg/kg	5.10 ± 1.20	8.02 ±1.82	15.86 ± 0.19

n = 5; results are presented as mean ± SD

DISCUSSION

Despite the wide range of beneficial actions of plants, some plants can be toxic to the body.[14] Plants used as food ingredients or in traditional medicine may not only be toxic but also mutagenic or even carcinogenic.[15] Many plants contain substances that are toxic to the reproductive system and can cause disruption of the maternal hormonal balance or interfere with the development and maintenance of pregnancy.[16,17]

The objective of this work was to determine the mutagenic potential in mice and the reproductive effects in rats of the mixture of C. procera and Z. zanthoxyloid root powders.

Genomic instabilities are induced by metabolic processes, as well as by exogenous factors including diet, lifestyle and environmental stresses. Reactive oxygen species (ROS), highly reactive and unstable oxidative molecules produced during normal cellular metabolism, can cause oxidative stress when their cellular levels exceed the level of cellular antioxidants. This oxidative stress eventually damages cellular macromolecules such as membrane lipids, proteins and nucleic acids. DNA is one of the main targets of ROS, which induce DNA mutations that lead to cancers and age-related disorders. Genotoxicity can also be the consequence of long-term exposure to very low levels of chemicals or be hereditary in nature.

Genotoxic effects of chemicals can be monitored using a wide range of in vitro and in vivo biomarker assays.[18] The most commonly used methods for assessing levels of DNA damage involve scoring chromosomal aberrations, micronuclei, and/or sister chromatid exchanges in proliferating cell populations. Chromosomal damage is manifested by the formation of micronuclei in polychromatic bone marrow erythrocytes and peripheral blood erythrocytes.[19,20] The mouse micronucleus test is the most widely used and best validated in vivo genotoxicity test for assessing the genotoxic potential of chemicals and/or radiation.[21] It detects clastogenic/aneugenic activities of compounds causing chromosomal breaks leading

to an increase in the frequency of micronuclei; suggesting mutagenic effects at the chromosomal level.[22,23] Genotoxicity is indicated in this assay by the increased frequency of micronuclei in polychromatic erythrocytes (MnPCE) in treated animal groups compared compared to control groups.[24]

The results of this study show a significant increase in the frequency of micronucleated erythrocytes in the peripheral blood of the mice treated with $KbrO_3$, used as a positive control compared to the negative control. In contrast, no significant difference in the number of micronuclei in peripheral blood was observed in mice treated with the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders compared to the controls.

Administration of the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders to mice caused no change in the frequency of micronucleated erythrocytes compared to controls. These results show that at the doses used in this test, the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders exhibit no mutagenic effect in mice peripheral blood cells. These results corroborate those of Dayana and Manasa[24] who have shown that the ethanolic extract of the root bark of *C. procera* exhibits antigenotoxic activity towards 7,12-dimethylbenz[a] anthracene (DMBA)-induced genotoxicity in Wistar rats. Other authors have shown that root and stem extracts of *C. procera* have antitumor and antiproliferative activities in vivo and in vitro against cancer cells.[25,26] In contrast, some authors have shown that *Z. zanthoxyloïdes* is genotoxic and cytotoxic to human leukocytes. The genotoxicity and cytotoxicity of this plant would lead to DNA damage. In this study the lack of genotoxicity of the mixture of *C. procera* and *Z. zanthozyloïdes* root bark powders could be justify by the presence of different compounds with antioxidant properties such as flavonoids (rutin and quercetin), alkaloids, coumarins, triterpenes, tannins, and phenols in extracts of *Z. zanthozyloïdes*[11,27], anthocyanins, sterols, alkaloids, coumarins, triterpenes, tannins, and phenols in different parts of *C. procera* and *Z. zanthoxyloïdes*.[28–30] According to Nunes et al.[31], plants rich in antioxidants such as anthocyanidins, flavonoids, vitamin C, vitamin E, carotenoids, and other polyphenolic compounds have protective effects against reactive oxygen species (ROS)-induced oxidative DNA damage.

Many plants contain substances that are toxic to the reproductive system and can cause disruptions in hormonal balance or interfere with the development and maintenance of pregnancy. These disturbances can affect the viability of embryos and fetuses, and cause teratogenic or abortifacient effects. In males, some substances can interfere with androgen

production, leading to changes in spermatogenesis or have spermicidal activity.[16,17] In females, the estrous cycle and its different stages are mainly regulated by the synthesis of ovarian estrogens, which in turn are controlled by the secretion of pituitary gonadotropins and hypothalamic release factors.[32] Among the substances that can interfere with androgen production are flavonoids known for their antisperm activity. They have antiandrogenic activity and thus affect male fertility. Coumarins are also well-known toxicants that have anti-fertility activity in mature female rats.[16]

Exposure of rats to a mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders resulted in a dose-dependent but statistically insignificant increase in sperm count in all treated rats compared to controls. In addition, a significant increase in relative testicular weight was observed in rats treated with 500 and 1000 mg/kg body weight. However, no significant differences in epididymides, prostate, and seminal vesicle weights were observed. This increase in testicular weight could be explained by an hypertrophy of these glands, caused by the hyperactivity induced by the mixture at high dose. On the other hand, no abnormalities of the reproductive organs were observed at the macroscopic examination. These results indicate that the mixture of *C. procera* and *Z. zanthoxyloïd*es root bark powders stimulate spermatogenesis and does not affect male reproductive organs in rats at the doses used in this study. These results are corroborated by the similarity of fertility indices between control female rats and those mated with treated male rats.

Testosterone is essential for normal spermatogenesis and the maintenance of the structural morphology and normal physiology of the seminiferous tubules. It maintains sperm viability and contributes to germ cell attachment in the seminiferous tubules. Intratesticular testosterone levels decrease can lead to germ cells detachment from the seminiferous epithelium and trigger germ/testicular cell apoptosis and degeneration. This also results in low total sperm count, increased sperm abnormalities, histopathologically defective or absent spermatogenesis, and testicular and epididymal changes.[33] Therefore, it would be possible that the mixture of C. *procera* and *Z. zanthozyloïdes* root bark powders improve serum testerone levels in rats.

Previous work on *C. procera* has shown that latex and ethanolic leaf extract significantly reduce serum testosterone, LH, and FSH levels in male rats. These extracts are also reported to cause seminiferous tubule atrophy, necrosis, and germ cell degeneration.[5,34] According to Sharma and Jacob[35], intermuscular administration of aqueous and ethanolic extracts of *C.*

procera flowers induces functional infertility and has potent antispermatogenic activity in albino mice. The difference between these results with those of our study is partly explained by the fact that the parts of the plant used are different, but also, could be related to the synergy of action of the secondary metabolites due to the mixture of the two plants in our study.

In the female rats, administration of *C. procera* and *Z. zanthozyloïdes* root bark powders resulted in decreased pregnancy, birth, viability, and weaning rates in female rats treated with 500 mg/kg bw. No gestation was observed in females treated with 1000 mg/kg bw. However, there were no significant differences in the relative weights of the ovaries and uteri of treated rats compared with controls. In addition, no significant differences were observed in the body weight variation of pups from treated females compared to controls. At 250 mg/kg bw, the results were similar to those of the controls. These results indicate that the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders disrupt the reproductive system in female rats at high doses. However, at the dose of 250 mg/kg, this mixture does not show any significant toxic effect on reproductive health in female rats.

Several authors have reported the toxic effects of *C. procera* on reproduction in females. Ethanolic and aqueous extracts of *C. procera* roots caused a significative modification of the estrous cycle in 60 and 80% of rats treated at doses of 25, 50, and 100 mg/kg. The ethanolic extract was reported to have anti-implantation activity due to its estrogenic activity. Estrogenic substances inhibit pregnancy by suppressing the level of follicle-stimulating hormone FSH and luteinizing hormone LH which in turn prevent implantation.[36] Alkaloid extract from the leaves of *C. procera* causes in treated imagos a blockage of ovarian development in previtellogenesis in females.[37] Faye[38] showed that a ration containing 10% of the whole plant of *C. procera* caused an abortion rate of 88.8% in mice. Also,latex of *C. procera* has abortifacient effects in sheep.[39] The reproductive disturbances observed in female rats treated at high doses (500 and 1000 mg/kg bw) in this study could be related to the presence of estrogenic substances that inhibit FSH or LH secretion. It would also be possible that this mixture has a direct action on the reproductive organs (uterus and/or ovaries); at the origin of the decrease or blockage of the reproductive capacity in these rats. However, the dose of 250 mg/kg bw, does not significant toxic effect in rats. Thus, further studies are required to better elucidate the mechanism of action of this mixture on the reproductive system in females.

CONCLUSION

The results of this study show that the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders is not mutagenic in mice peripheral blood cells up to 2000 mg/kg bw. This mixture causes a dose-dependent stimulation of spermatogenesis in males. In females, the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders causes a decrease in fertility at 500 and 1000 mg/kg bw. However, the administration of this mixture at the dose of 250 mg/kg bw is without effect on the reproductive capacity of the rat.

Given these results, it can be concluded that the use of the mixture of *C. procera* and *Z. zanthoxyloïdes* root bark powders is relatively tolerated at doses below 250 mg/kg in rats. However, further studies are necessary to elucidate the reprotoxicity observed at higher doses in animals.

ACKNOWLEDGEMENTS

The authors would like to thank the Director of the Institute of Research in Health Sciences, Dr. Sylvin OUEDRAOGO and the production team of U-pharma who offered us the plant material for this study.

REFERENCES

1. Saha P, Talukdar AD, Nath R, Sarker SD, Nahar L, Sahu J. and Choudhury MD. Role of Natural Phenolics in Hepatoprotection: A Mechanistic Review and Analysis of Regulatory Network of Associated Genes. Front Pharmacol, 2019; 10: 509.
2. Dermane A, Kpegba K, Metowogo K, Joppa MK, Eklu-Gadegbeku K, Aklikokou AK, and GBEASSOR M. Evaluation of the anti-sickling activity of *Newbouldia laevis* P. Beauv extracts. Int J Biol Chem Sci., 2019; 12(6): 2808.
3. Elvis-Offiah UB, Bafor EE, Eze GI, Igbinumwen O, Viegelmann C, Edrada-Ebel R. In vivo investigation of female reproductive functions and parameters in nonpregnant mice models and mass spectrometric analysis of the methanol leaf extract of *Emilia Coccinea* (Sims) G Dons. Physiol Rep., 2016; 23: e13047.
4. Zerbo P, Millogo Rasolodimby J, Nacoulma Ouedraogo O, Van Damme P. Plantes médicinales et pratiques médicales au Burkina Faso : cas des Sanan. BOIS FORETS Trop, 2011; 307(307): 41.
5. Ahmed OM, Fahim HI, Boules MW, Ahmed HY. Cardiac and testicular toxicity effects of the latex and ethanolic leaf extract of Calotropis procera on male albino rats in comparison to abamectin. SpringerPlus., 2016; 5(1): 1644.

6. Ouedraogo S, Traoré S, Ouédraogo JCW, Koala M, Belemnaba L, Ouédraogo N, Kini F, Ouédraogo S, and Guissou IP. Quality control and standardization of FACA® syrup. Int J Drug Deliv, 2018; 9(4): 101.
7. Nikiema JB, Ouattara B, Semde R, Djierro K, Compaore M, Guissou IP, and Kasilo OMJ. Promotion de la Médecine Traditionnelle du Burkina Faso : Essai de développement d'un médicament antidrépanocytaire, le FACA®. African traditional medecine day, 2010; 14.
8. Guissou IP, Sawadogo M, Sawadogo A, Ouattara A. Etude de l'efficacité antidrepanocitaire de gelules faca chez les enfants en milieu hospitalier de Ouagadougou (CHN-YO). Pharm. Méd. trad. afro, 1995; 29-36.
9. Ouedraogo S, Traore A, Lompo M, Some N, Sana B, Guissou I. Vasodilator effect of Zanthoxylum zanthoxyloïdes, *Calotropis procera* and FACA, a mixture of these two plants. Int J Biol Chem Sci., 2012; 5(4): 1351-7.
10. Somda GD, Ouedraogo N, Geoffroy G, Ilboudo S, Ouédraogo M, Ouédraogo S, Lamien-Sanou A, KINI BF and Guissou IP. Acute and sub-chronic toxicity study of the mixture of *Calotropis procera* (Ait). R. Br. (Apocynaceae) and *Zanthozylum zanthozyloïdes* Lam. (Rutaceae) roots bark powder. Int. J. Biol. Chem. Sci., 2022; 16(1): 184-200.
11. Ouedraogo GG. Etude de la toxicité d'extraits de poudre d'écorces de racines de *Calotropis procera* (ait.) R.br. (Apocynaceae), composante du FACA®, phytomédicament utilisé au Burkina Faso. Thèse de Doctorat unique. Burkina Faso: Université de Ouagadougou, 2015; 211.
12. OECD. OECD guideline for the testing of chemicals. Mammalian erythrocyte micronucleus test, 2016.
13. OECD. OECD guideline for testing of chemicals. Combined repeated dose Toxicity Study with the reproduction/developmental toxicity screening test, 2015.
14. Franke SIR, Prá D, Giulian R, Dias JF, Yoneama ML, da Silva J, Erdtmann B, Henriques JAP. Influence of orange juice in the levels and in the genotoxicity of iron and copper. Food Chem Toxicol, 2006; 44(3): 425-35.
15. Verschaeve L. Genotoxicity and Antigenotoxicity Studies of Traditional Medicinal Plants: How Informative and Accurate are the Results? Nat Prod Commun, 2015; 10(8): 1934578X1501000.
16. de Cássia da Silveira e Sá R, Leite MN, de Moura Reporedo M, Nóbrega de Almeida R. Evaluation of long-term exposure to Mikania glomerata (Sprengel) extract on male

Wistar rats' reproductive organs, sperm production and testosterone level. Contraception, 2003; 67(4): 327-31.

17. Santos-Filho SD, Fonseca A de S da, Bernardo-Filho M. The male reproductive system and the effect of an extract of a medicinal plant (Hypericum perforatum) on the labeling process of blood constituents with technetium-99m. Braz Arch Biol Technol, 2007; 50(spe): 97-104.
18. Cavas T, Konen S. Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (Carassius auratus) exposed to a glyphosate formulation using the micronucleus test and the comet assay. Mutagenesis, 2007; 22(4): 263-8.
19. Sommer S, Buraczewska I, Kruszewski M. Micronucleus Assay: The State of Art, and Future Directions. Int J Mol Sci., 2020; 21(4): 1534.
20. Styles JA, Clark H, Festing MFW, Rew DA. Automation of mouse micronucleus genotoxicity assay by laser scanning cytometry. Cytometry, 2001; 44(2): 153-5.
21. Çelik A, Öğenler O, Çömelekoğlu Ü. The evaluation of micronucleus frequency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate. Mutagenesis, 2005; 20(6): 411-5.
22. Kwasniewska J, Bara AW. Plant Cytogenetics in the Micronuclei Investigation—The Past, Current Status, and Perspectives. Int J Mol Sci., 2022; 23(3): 1306.
23. Mavournin KH, Blakey DH, Cimino MC, Salamone MF, Heddle JA. The in vivo micronucleus assay in mammahan bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Research, 1990; 239: 29-80.
24. Dayana K, Manasa M. Evaluation of antigenotoxic activity of ethanolic extract of Calotropis procera root in 7,12-dimethylbenz[a]anthracene induced genotoxicity in Wistar rats. Natl J Physiol Pharm Pharmacol, 2018; 8(9): 1.
25. Mathur R, Gupta SK, Mathur SR, Velpandian T. Anti-tumor studies with extracts of Calotropis procera (Ait.) R.Br. root employing Hep2 cells and their possible mechanism of action. Indian J exp Biol., 2009; 47: 343-348.
26. Choedon T. Anticancer and cytotoxic properties of the latex of Calotropis procera in a transgenic mouse model of hepatocellular carcinoma. World J Gastroenterol, 2006; 12(16): 2517.
27. KOURI F. Investigation phytochimique d'une brosse à dents africaine *Zanthoxylum zanthoxyloïdes* (Lam.) Zepernick et Timler (Syn. Fagara zanthoxyloides L.) (Rutaceae). Thèse de doctorat. Université de Lausane, 2004; 211.

28. Srivastava D, Singh P. In – vitro fungitoxic evaluation and gcms analysis of. World J Pharm Res., 2015; 4(3): 1123-1135.
29. Barkat Ali Khan. Phytochemical analysis and acute toxicity tests of two medicinal plant extracts. J Med Plants Res [Internet], 2012; 6(19).
30. Essi LE Massara CC,Tiahou GG, Monde A, SESS ED. Le latex de *Calotropis procera*: effets sur des marqueurs biochimiques de fonctions organiques chez le rat ofa. Pharm. Méd. Trad. Afr., 2006; XIV: 81-95.
31. Da Silva NR, Silva KVF, Da Silva SM, Richter MF, Abin-Carriquiry JA, Martinez MM, De Barros AFF and Da Silva J. 2012. Genotoxic and Antigenotoxic Activity of Acerola (*Malpighia glabra* L.) Extract in Relation to the Geographic Origin. Phytother. Res., 2013; 27: 1495–1501.
32. Circosta C, Sanogo R, Occhiuto F. Effects of Calotropis procera on oestrous cycle and on oestrogenic functionality in rats. Il Farm, 2001; 56(5-7): 373-8.
33. Lonare M, Kumar M, Raut S, More A, Doltade S, Badgujar P, Telang A. Evaluation of ameliorative effect of curcumin on imidacloprid-induced male reproductive toxicity in wistar rats: Curcumin Ameliorates the Imidacloprid-Induced Reproductive Toxicity. Environ Toxicol, 2016; 31(10): 1250-63.
34. Akinloye AK, Abatan MO, Alaka OO, Oke BO. Histomorphometric and histopathological studies on the effectof *Calotropis procera* (giant milkweed) on the male reproductive organs of wistar rats. African Journal of Biomedical. Research, 2002; 5: 57‑61.
35. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in lants under stressful conditions. J Bot., 2012; 1-26.
36. Samy RP, Rajendran P, Li F, Anandi NM, Stiles BG, Ignacimuthu S, Sethi G, Chow VTK. Identification of a Novel Calotropis procera Protein That Can Suppress Tumor Growth in Breast Cancer through the Suppression of NF-κB. PLoS ONE, 2012; 7(12): e48514.
37. Abbassi K, Kadiri ZA, Ghaout S. Activité biologique des feuilles de Calotropis procera (Ait. R. Br) sur le criquet pèlerin (Schistocerca gregaria, Forsk, 2004; 1775: 14.
38. Faye B. Contribution à l'étude de la toxicité de Calotropis procera. Effet d'une alimentation à base de Calotropis procera sur la mortalité embryonnaire et néonatale chez la souris de laboratoire, 4.

39. El Badwi SMA, Bakhie AO. Toxicity of Calotropis procera latex in pregnant and non-pregnant goats. Scientific Research and Essays, 2010; 5(17): 2404-2408.