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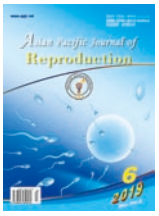

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Effects of *Crassocephalum bauchiense* (Hutch) leaf aqueous extract on toxicity indicators and reproductive characteristics in *Oryctolagus cuniculus* exposed to potassium dichromate

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ABSTRACT

Objective: To evaluate the effects of *Crassocephalum (C.) bauchiense* (Hutch) leaf aqueous extract on the biochemical markers of toxicity, oxidative stress indicators and reproductive parameters in rabbit does (*Oryctolagus cuniculus*) exposed to potassium dichromate.

Methods: A total of 36 nulliparous and sexually mature female rabbit does, aged 8 months and weighing 2.8-3.0 kg, were divided into 6 groups of 6 animals. After mating, group T0 received distilled water (1 mL/kg body weight), while groups T0-, VC100, AE100, AE200 and AE400 were treated with 40 mg/mL/kg body weight of potassium dichromate. In addition, group VC100 received 100 mg/mL/kg body weight of vitamin C, while groups AE100, AE200 and AE400 received aqueous extract of *C. bauchiense* leaves at 100, 200 and 400 mg/mL/kg body weight, by oral gavage for 27 days, respectively. On the 28th day *post-coïtum*, animals were sacrificed. Biochemical indicators such as creatinine, urea, alanine amino tranferase, aspartate amino transferase, total protein and total cholesterol were measured by using a spectrophotometer. Follicle stimulating hormone, luteinizing hormone and progesterone were measured by enzyme-linked immuno sorbent assay. Oxidative stress markers like malondialdehyde, catalase, superoxide dismutase and total peroxidase in ovary homogenates were measured by spectrophotometer.

Results: Serum concentrations of urea, creatinine, alanine aminotransferase, aspartate aminotransferase and total cholesterol were significantly higher ($P<0.05$), and total protein level was significantly lower in T0- group (receiving potassium dichromate only) compared to other groups. Administration of ethanolic extract of *C. bauchiense* significantly decreased serum concentrations of urea, creatinine, alanine aminotransferase, aspartate aminotransferase and total cholesterol, and significantly increased total protein level. However, ethanolic extract of *C. bauchiense* had no significant effect on the foetotoxic characteristics. The serum concentrations of follicle stimulating hormone and luteinizing hormone were significantly ($P<0.05$) lower in T0- group as compared to the control group while progesterone were comparable ($P>0.05$) among all groups; *C. bauchiense* leaf extracts significantly ($P<0.05$) increased the level of follicle stimulating hormone, but the increase in luteinizing hormone was not significant ($P>0.05$). Catalase and total peroxidase activities significantly decreased ($P<0.05$), and malondialdehyde significantly increased in T0- group compared to other groups. The administration of *C. bauchiense* leaf extracts significantly ($P<0.05$) increased the activities of catalase and peroxidase.

Conclusions: Potassium dichromate-induced oxidative stress involves in hepatotoxicity, nephrotoxicity and reprotoxicity. The aqueous extract of *C. bauchiense* leaves could mitigate these adverse effects *via* antioxidant properties.

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1. Introduction

Reproduction is an essential function that allows species continuity. It improves productivity and perpetuates animal species[1]. Its perturbation or dysfunction leads to negative consequences on animal productivity[2]. Many factors can be responsible for this perturbation namely stress (heat, feed, oxidative), aging, some drugs[3] and xenobiotics such as heavy metals. One of the main mechanisms by which these factors induce low reproduction is oxidative stress. It occurs when the generation of reactive oxygen species and other radical species exceed the scavenging capacity of antioxidants in an organism[4]. Oxidative stress is involved in endometriosis, ovarian cancer, polycystic ovary disease, intrauterine growth restriction, abortions, retardation of embryo growth and various other pathologies affecting the female reproductive process[5], through the induction of cell membrane damage, DNA damage and apoptosis[6].

When humans and animals are exposed to certain heavy metals like iron, copper, cadmium, chromium, mercury, nickel, vanadium, it will induce oxidative stress, toxic and carcinogenic effects, resulting in lipid peroxidation, depletion of protein and many other effects[7]. Chromium is a naturally occurring element found in rocks, animals, plants, soil and in volcanic dust and gases[8]. It comes in several different forms including trivalent chromium and hexavalent chromium. During the reduction process, chromium produces reactive oxygen species[9] and generates oxidative stress. Multiple studies of the developmental toxicity of hexavalent chromium in experimental animal models such as rats and mice have shown decreases in viability of the embryo and fetus (both pre- and post-implantation resorptions), decreases in foetal weights and crown-rump lengths, changes in placental weights (decrease or increase) and increases in frequencies of external and skeletal abnormalities[10,11].

The harmful action of these heavy metals can, however, be blocked by antioxidant substances which scavenge the free radicals and detoxify the organism[12]. Plants constitute the main source of natural antioxidant molecules, which have the capacity to eliminate or neutralize the deleterious reactive oxygen species[13]. Recently, interest has been increased considerably in finding naturally occurring antioxidants to replace synthetic antioxidant used in foods or medicinal products, which are being restricted due to their adverse reactions such as carcinogenicity[14]. Also, they are expensive, making them inaccessible for a greater part of the population in developing countries. Various herbs with antioxidant properties have been studied[15]. That is the effects on animal reproduction of aqueous extracts of these plants, for example, wing extracts (*Allium sativum*)[16], leaves of *Tribulus terrestris*[17], leaves of *Momordica charantia*[18], *Phoenix dactylifera* and *Nasturtium officinale*[19], essential oils of *Syzygium aromaticum*[20] and *Psidium guajava* leaves[21] were tested. Results obtained were interesting for some and less important for other researches. Studies are limited to some plants only, yet many other plants like *Crassocephalum (C.) bauchiense* can be potential candidates for reproduction improvement in breeding animals. The antioxidant activities of some species of the *Crassocephalum* genus are well described in previous studies[22,23].

C. bauchiense is a species of flowering plant in the Asteraceae family. Phytochemical tests have shown that it contains many antioxidant compounds such as phenols, flavonoids, alkaloids, tannins, triterpenes, and sterols[12]. Thus, it could protect animal reproduction from oxidative stress induced by heavy metals and other factors. That is why the current study was initiated, to contribute to the improvement of the reproduction in farm animals using medicinal plants.

2. Materials and methods

2.1. Experimental animal

Thirty-six adult fertile rabbit-does (New Zealand breed *Oryctolagus cuniculus*) of 8 months old, weighing 2.8-3.0 kg, reproduced at the Teaching and Research Farm of the University of Dschang, were used. The animals were maintained individually in wire netting galvanized metal cages (100 cm long, 45 cm wide and 25 cm high). They received water and feed *ad libitum*. The guidelines on Care and Use of Laboratory Animals were followed (NIH Publication, No. 85-23, revised 1985). The study protocol (No. 00183, ECDAS-UDs-DSAES dated 24-02-2016) was approved by the Department of Animal Science, the University of Dschang, Cameroon.

2.2. Plant material

Leaves of *C. bauchiense* were collected from Ndop (North West Region of Cameroon). The species had been identified and authenticated at the Cameroon National Herbarium in Yaounde where the voucher specimen was deposited, by referring to the sample number 7954/SRF/Cam.

2.2.1. Plant extracts obtainment

The leaves of *C. bauchiense* were dried at room temperature for 10 days and powdered to coarse particles using a grinding mill. A total of 250 g of powder was soaked in 1.5 L of distilled water and left at room temperature for 48 h. Filter paper Whatman number 3 was used to obtain a homogeneous solution which was evaporated in the oven at 50 ℃ to obtain aqueous extracts.

2.2.2. Preparation of C. bauchiense leaf extracts solution

Solutions of the aqueous extract of *C. bauchiense* leaf were prepared at different experimental doses by dissolving 100, 200 and 400 mg of *C. bauchiense* leaf extract in 900, 800 and 600 mL of distilled water. The above doses chosen were based on those of other studies on the plant exctract and median lethal dose of extract of *C. bauchiense* leaves from Mouekeu *et al*[12].

2.3. Preparation of potassium dichromate and vitamin C solutions

This chemical was obtained from Sigma Aldrich, Germany. The solution of potassium dichromate used was prepared by dissolving 40 mg of potassium dichromate crystals, in 60 mL of distilled water. Then the solution was homogenized using a magnetic agitator with trade mark of LABINCO (Model L-71, Netherlands). Soluble tablets of vitamin C (Vitamin C Cevite) containing 500 mg per tablet were bought from the local drugstore. Vitamin C tablets were produced by Shalina, Nariman Point, Mumbai, India. Vitamin C solution was prepared by dissolving 100 mg of vitamin C in 900 mL of distilled water.

2.4. Experimental design and trial conduct

After successful mating of 36 fertile rabbit does with some untreated sexually mature males, with sex ratio 1:2 (1 male for 2 females), they were randomly divided into 6 groups [T0 (control), T0-, VC100 (reference treatment for antioxidant groups), AE100, AE200 and AE400] of 6 rabbit does each, comparable in terms of body weight (bw). For 27 days *post-coïtum*, females in T0 group received distilled water (1 mL/kg bw), while those of other groups received 40 mg/mL/kg bw of potassium dichromate (40 mg/mL/kg bw was obtained from our previous study on the toxicity of varying doses of potassium dichromate[24]). In addition, females in VC100 group were given 100 mg/mL/kg bw of vitamin C and those of groups AE100, AE200 and AE400 respectively received 100, 200 and 400 mg/mL/kg bw of aqueous extract of *C. bauchiense* leaves by oral gavage, done daily between 6:30 and 7:30 am. At 28th day *post-coïtum*, animals were sacrificed for tests.

2.5. Foetotoxicity characteristics of rabbit does

The uterus was opened and the number of fetuses was counted. The viability rate was calculated as: the number of viable fetuses/ total number of fetuses ×100.

The number of corpora lutea was counted on the ovaries. The uterus was steeped in a solution of sodium hydroxide 2% for 10 min to count the number of implantation sites in order to determine the number of resorptions by the method described by Lim *et al*[24]. The number of corpora lutea was compared to the number of implantation sites which permitted to determine the number of pre-implantation resorptions using the following formula[25].

Pre-implantation resorptions = number of corpora lutea – number of implantation sites

Post-implantation resorptions were early when only placenta tissues were visible and late when placenta tissues and embryonic tissues were observed[26]. The following formula was used:

Post-implantation resorptions = number of implantation sites – number of life fetuses

2.6. Biochemical and hormonal analyses

Blood samples (5 mL) were obtained between 6:30 and 7:30 am by cardiac puncture and stored at 4 ℃. Serum was collected 12 h later for the estimation of biochemical parameters and reproductive hormone levels. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, total cholesterol and total proteins in serum were measured using a spectrophotometer. The biochemical measurements were performed according to the details given in the instructions of the commercial Chronolab kits (Barcelona, Spain).

Follicle stimulating hormone (FSH), luteinizing hormone (LH) and progesterone were determined in serum by AccuDiag™ELISA kits from OMEGA DIAGNOSTICS LTD (Scotland, England). FSH and LH concentrations were obtained from linear regression equations. For progesterone, concentrations were obtained by projecting optical density of samples read on ELISA plate reader on calibration curves of progesterone constructed from standard concentrations provided by kits. Limits of detection of assay were approximately 1.5 mIU/mL for FSH, 1.0 mIU/mL for LH and 0.3 ng/mL for progesterone. Intra-assay coefficients of variance were 5.59 % for FSH, 6.95% for LHS and 3.79% for progesterone, while coefficients of variance were less than or equal to 10% for the three hormones mentioned.

2.7. Oxidative stress markers

Activities of catalase (CAT), total peroxidase, superoxide dismutase (SOD), and level of malondialdehyde (MDA) in the ovary were measured by spectrophotometer.

2.7.1. Estimation of CAT activity

CAT activity was estimated according to Aebi[27] depending on the ability of H_2O_2 to be decomposed by the action of CAT to produce H_2O and O_2. The decrease in the absorbance in the ultraviolet region per time was corresponded to CAT activity. A total of 2.0 mL of the substrate (10 pmol/mL of H_2O_2 in 50 mmol/L sodium-potassium phosphate buffer, pH 7.0) was incubated with 100 μL serum. The decomposition of H_2O_2 was followed directly for 2 min by the decrease in absorbance at 240 nm.

2.7.2. Estimation of total peroxidase

Total peroxidase level was measured according to the method of Moron *et al*[28] based on the reaction of glutathione with 5, 5-dithiobis-2-nitro-benzoic acid (Sigma Aldrich, Germany) at pH 8.0. The optical density of yellow color was measured at 412 nm. A total of 0.1 mL of serum samples were added to 0.9 mL of 0.6 mmol 5, 5-dithiobis-2-nitro-benzoic acid reagent and 10 μL of trichloroacetic acid (Labtech, Australia), mixtures were incubated at 25 ℃ for 5 min. After centrifugation the absorbance of yellow color was measured, the results were calculated from a glutathione standard curve.

2.7.3. Estimation of SOD

Adrenaline was stable enough when pH was acidic. When pH increased, the rate of auto-oxidation of adrenaline increased. The dosage of SOD was thus based on the capacity of SOD to inhibit or slow down auto-oxidation of adrenaline to adreno-chromium in a milieu of a base. The method proposed by Misra and Fridovich[29] was used in this study. Micro tubes of serum were introduced into the spectrometer as well as 1 660 μL of carbonate buffer solution (pH=10) and 200 μL of adrenaline (0.3 mM). The absorbance of adreno-chromium formed was read at 480 nm 30 and 90 s after the initiation of the reaction.

2.7.4. Estimation of MDA

Lipid peroxidation (LPO) was estimated by the reaction of thiobarbituric acid from (Qulaikems, India) with MDA according to Botsoglou *et al*[30]. In the presence of an acid and heat (pH 2-3, 100 ℃), MDA condensed with two molecules of thiobarbituric acid to produce a pink color complex which was absorbed at 532 nm. A total of 105 μL of orthophosphoric acid at 1% and 500 μL of the precipitation mixture (1% thiobarbituric acid in a 1% acetic acid solution) were added to 100 μL homogenate. The mixture of each tube was homogenized and placed in boiling water for 15 min. The tubes were cooled in an ice-bath and the mixture was centrifuged at 3 500 rpm for 10 min. Absorbance was read at 532 nm against the control.

2.8. Statistical analysis

The data about the effects of different treatments on parameters were analyzed by one-way analysis of variance. The Duncan test was performed to analyze difference between treatment groups. The results were expressed as mean ± standard deviation (mean ± SD). The limit of significance was set at 5% and the software SPSS 20.0 was used for the analysis.

3. Results

3.1. Effects of C. bauchiense on biochemical markers of nephrotoxicity and hepatotoxicity

Table 1 showed the effects of aqueous extract of *C. bauchiense* on nephrotoxicity and hepatotoxicity biochemical markers in rabbit does exposed to potassium dichromate. The serum levels of creatinine and urea were significantly (P<0.05) higher in potassium dichromate-treated animals (Group T0-) compared to the control group (Group T0) treated with distilled water only. Administration of aqueous extract of *C. bauchiense* significantly decreased the levels of creatinine and urea compared with animals in Group T0-.

The serum levels of ALT, AST and total cholesterol increased significantly (P<0.05), while total proteins decreased significantly in Group T0- compared with Group T0. *C. bauchiense* leaf extracts significantly decreased ALT and AST (at all *C. bauchiense* doses) and total cholesterol (at dose 100 mg of *C. bauchiense*/kg bw), and significantly increased total proteins compared to Group T0-. The highest dose (400 mg/kg bw) of *C. bauchiense* showed better results than the lower doses for creatinine, ALT, AST and total proteins.

No significant difference in hepatotoxicity and nephrotoxicity biochemical markers was found between the group VC100 and the *C. bauchienses* treated groups (Group AE100, AE200 and AE400).

3.2. Effects of C. bauchiense on reproductive parameters

3.2.1. Foetotoxicity characteristics of rabbit does

The effects of aqueous extract of *C. bauchiense* on foetotoxicity characteristics were summarised in Table 2. The numbers of fetuses, corpora lutea, placentas, implantation sites, viable fetuses and pre-implantation resorptions were higher in AE200 group compared to other groups, but no significant difference was noticed. The placenta weight increased insignificantly in *C. bauchienses* treated groups compared to Group T0 and Group T0-, but the number of total resorptions in AE100 and AE400 groups showed the contrary results. There were no significant differences in all fetotoxic parameters between vitamin C group and *C. bauchienses* treated groups.

3.2.2. Reproductive hormones

The effects of *C. bauchiense* leaf aqueous extract on serum level of reproductive hormones were shown in Table 3. Potassium dichromate-treated group (Group T0-) showed significant decrease in serum FSH levels compared to the control group (Group T0) and VC100 group (P<0.05). In AE200 and AE400 groups, *C. bauchiense* leaf aqueous extract significantly increased FSH level (P<0.05), which were comparable to the control group (Group T0) and VC100 groups (P>0.05).

Table 1. Effects of aqueous extract of *Crassocephalum bauchiense* on hepatotoxicity and nephrotoxicity biochemical markers in rabbit does exposed to potassium dichromate.

Biochemical markers	T0	T0-	VC100	AE100	AE200	AE400
ALT (IU/mL)	18.69±4.12^{b}	27.09±5.63^{a}	15.19±2.15^{bc}	16.48±2.05^{bc}	14.78±3.11^{bc}	12.44±2.07^{c}
AST (IU/mL)	11.08±1.04^{b}	19.95±5.21^{a}	13.25±1.63^{b}	14.29±2.42^{b}	13.56±1.44^{b}	11.20±1.76^{b}
Total proteins (g/dL)	4.13±0.71^{b}	2.99±0.51^{c}	4.93±0.46^{a}	4.45±0.58^{ab}	4.35±0.76^{ab}	5.02±0.43^{a}
Total cholesterol (mg/dL)	27.56±8.32^{c}	42.59±4.34^{a}	36.79±4.43^{ab}	32.12±4.05^{bc}	34.20±5.84^{ab}	37.06±6.98^{ab}
Creatinine (mg/dL)	1.16±0.26^{c}	2.47±0.37^{a}	1.58±0.48^{bc}	1.91±0.31^{b}	1.61±0.29^{bc}	1.44±0.20^{bc}
Urea (mg/dL)	33.72±2.20^{b}	46.91±6.78^{a}	39.64±5.26^{ab}	40.89±4.95^{ab}	36.17±7.17^{b}	37.54±4.91^{b}

a, b, c: values affected with the same letter in the same line do not differ significantly (P>0.05). Data are expressed as mean ± SD. n=6 in each group. Group T0 (the control group): receiving distilled water; Group T0-: receiving potassium dichromate only; Group VC100: receiving potassium dichromate (40 mg/mL/kg bw) + vitamin C at dose of 100 mg/kg bw; Group AE100, 200 and 400 groups: receiving potassium dichromate (40 mg/mL/kg bw) + aqueous extract of *Crassocephalum bauchiense* at doses of 100, 200 and 400 mg/kg bw, respectively. ALT: alanine aminotransferase, AST: aspartate aminotransferase.

Table 2. Effects of aqueous extract of *Crassocephalum bauchiense* on some foetotoxicity characteristics in rabbit does exposed to potassium dichromate.

Characteristicsof foetotoxicity	T0	T0-	VC100	AE100	AE200	AE400
Number of fetuses	7.00±0.82	6.25±1.71	5.67±1.53	7.00±0.82	7.67±2.08	6.00±1.00
Number of corpora lutea	7.75±1.70	7.50±0.58	6.00±1.73	7.50±0.5	8.67±1.53	6.00±1.00
Number of placenta	7.75±1.71	7.50±0.58	5.67±1.53	7.00±0.82	8.00±2.00	6.00±1.00
Weight of placenta	4.87±1.44	4.69±1.58	6.06±0.74	5.44±0.22	5.80±1.45	6.29±0.97
Number of implantation sites	7.75±1.71	7.50±0.58	5.67±1.53	7.00±0.82	8.00±2.00	6.00±1.00
Number of total resorptions	0.75±0.50	1.25±0.89	0.33±0.17	0.50±0.25	1.00±0.44	0.00±0.00
Number of pre-implantation resorptions	0.00±0.00	0.00±0.00	0.33±0.17	0.50±0.25	0.67±0.27	0.00±0.00
Number of post-implantation resorptions	0.75±0.50	1.25±0.89	0.00±0.00	0.00±0.00	0.33±0.17	0.00±0.00
Number of viable fetuses	7.00±0.82	6.25±1.71	5.67±1.53	7.00±0.82	7.67±2.08	6.00±1.00
Number of dead fetuses	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Data are expressed as mean ± SD. *n*=6 in each group.

Table 3. Effects of aqueous and ethanolic extracts of *Crassocephalum bauchiense* on serum reproductive hormones in rabbit does exposed to potassium dichromate.

Reproductive hormones	T0	T0-	VC100	AE100	AE200	AE400
FSH (mIU/mL)	10.67 ± 2.07^{a}	4.80 ± 1.3^{c}	9.67 ± 2.08^{a}	6.75 ± 2.06^{b}	10.00 ± 1.73^{a}	9.75 ± 1.26^{a}
LH (mIU/mL)	5.78 ± 0.65^{a}	3.24 ± 0.33^{b}	5.53 ± 0.42^{a}	4.00 ± 0.82^{b}	4.12 ± 0.18^{b}	3.96 ± 0.28^{b}
Progesterone (ng/mL)	51.88 ± 1.22^{a}	50.14 ± 1.09^{a}	51.05 ± 3.15^{a}	49.23 ± 3.88^{a}	48.32 ± 3.06^{a}	51.21 ± 1.94^{a}

a, b, c: values affected with the same letter in the same line do not differ significantly (P>0.05). Data are expressed as mean ± SD. *n*=6 in each group. FSH: follicle stimulating hormone; LH: luteinizing hormone.

Table 4. Effects of aqueous extract of *Crassocephalum bauchiense* on oxidative stress markers in rabbit does exposed to potassium dichromate.

Oxidative stress markers	T0	T0-	VC100	AE100	AE200	AE400
CAT (µM/min/g of tissue)	10.02 ± 1.65^{a}	5.43 ± 0.55^{c}	8.45 ± 1.29^{ab}	7.07 ± 0.96^{b}	7.13 ± 0.92^{b}	7.43 ± 0.74^{b}
MDA (µM/g of tissue)	0.08 ± 0.02^{b}	0.24 ± 0.05^{a}	0.11 ± 0.02^{b}	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}	0.06 ± 0.01^{b}
SOD (µM/min/g of tissue protein)	0.38 ± 0.06^{b}	0.61 ± 0.12^{a}	0.36 ± 0.05^{b}	0.40 ± 0.03^{b}	0.39 ± 0.04^{b}	0.36 ± 0.03^{b}
Total peroxidases (µM/g of tissue)	36.05 ± 3.42^{cd}	24.15 ± 4.09^{e}	31.94 ± 4.82^{d}	48.02 ± 6.49^{a}	41.56 ± 5.14^{bc}	45.11 ± 3.82^{ab}

a, b, c, d, e: values affected with the same letter in the same line do not differ significantly (P>0.05). Data are expressed as mean ± SD. *n*=6 in each group. CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde.

The serum level of LH decreased significantly (P<0.05) in Group T0- compared to the control group (T0). Administration of aqueous extract of *C. bauchiense* or vitamin C increased LH level although the significant difference was recorded only in VC100 group. The serum level of progesterone was comparable (P>0.05) among all treatment groups.

3.3. Effects of C. bauchiense on oxidative stress markers

As shown in Table 4, potassium dichromate-treated group (Group T0-) showed significant decrease in CAT levels compared to the control group (Group T0) (P<0.05), and in AE100, AE200 and AE400 groups, *C. bauchiense* leaf aqueous extract significantly increased CAT level (P<0.05). But CAT level in AE100, AE200 and AE400 groups was still significantly lower than that of the T0 control group (P>0.05). Treatment with vitamin C completely restored the activity of CAT.

The level of MDA and the activity of SOD significantly increased in potassium dichromate-treated group (Group T0-) compared to those of the control group (Group T0). Administration of *C. bauchiense* (at all doses) or vitamin C (100 mg/kg bw) significantly decreased MDA and SOD to normal levels (P<0.05). In addition, the activity of total peroxidases decreased significantly in animals treated only with potassium dichromate, compared to those of the control group. The administration of *C. bauchiense* aqueous leaf extract or vitamin C significantly (P<0.05) increased this enzyme up to normal limit.

4. Discussion

The exposure of animals to heavy metals can lead to adverse effects on their health in general and on their reproduction particularly. Kidney and liver are very important organs in the evaluation of the toxic potential of a substance[31]. They are associated with the metabolism and excretion of toxic substances[32] such as heavy metals. The biochemical analyses in this study showed an increase in concentrations of enzymes ALT, AST, creatinine, urea and total cholesterol levels, with a decline in total protein levels. This indicated general and systemic toxic effect of heavy metals on the rabbit does as reported by Jahnabi *et al*[33] in rats treated with potassium dichromate. Increase in cholesterol level in this study might be due to less utilization of these nutrients at tissue level. The increase in creatinine and urea could be due to the dysfunction of glomerules, which are the structures responsible for renal filtration. That of urea could also be explained by the increase in proteins catabolism, due to the high synthesis of the enzyme arginase which intervenes in the urea production[34]. High level of serum AST indicates liver damage, such as that due to viral hepatitis as well as cardiac infarction and muscle injury. Serum alanine aminotransferase catalyzes the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, serum alanine aminotransferase is more specific to the liver, and is thus a better parameter for detecting liver injury[35]. These results demonstrate that chromium toxicity was associated with hepatotoxicity and nephrotoxicity. This may be due to the impairment in their synthesis or poor liver function.

Similar results were observed by Zhu *et al*[36]. Interestingly, the results of the present work indicated that co-administration of *C. bauchiense* extracts and vitamin C to dichromate-treated group restored the concentrations of the above parameters within normal limits. This can be explained by the anti-oxidative and hepato-protective activities of the *C. bauchiense* extract that scavenged the reactive oxygen species induced by potassium dichromate due to anti-oxidants molecules (phenols, flavonoids, terpenoids) it contains. Similar observations were made by Mohamed and Saber[37] in rats exposed to potassium dichromate and treated with aqueous extract of Damsissa (*Ambrosia maritima*).

The hypothalamic-pituitary-gonadal axis plays a critical role in the control of reproduction. Potassium dichromate exposure led to a decrease in FSH and LH. This might be due to the decrease in protein level. In fact, FSH and LH are hormones synthesized from proteins captured from the blood. Thus, the reduction in total proteins in blood could have led to decreases in FSH and LH concentrations in heavy metal-treated rabbit does. The abnormal levels of sex hormones recorded in this study are in accordance with those registered in female rats exposed to potassium dichromate by Assasa *et al*[38] suggesting a disruption of steroidogenic function. The increased concentrations of those hormones in females co-exposed to the heavy metal and aqueous extract of *C. bauchiense* could be attributed to the increase in proteins levels in those groups. The concentration of progesterone in the current study was comparable among treatments. This is not surprising since all the females were pregnant and then the progesterone had reached a certain level in order to maintain gestation successfully.

The current work showed that the numbers of fetuses, corpora lutea, placentas, implantation sites, viable fetuses and pre-implantation resorptions were higher in females treated with 200 mg/kg bw of *C. bauchiense* extract compared to other treatments, but no significant difference was noticed. The placenta weight increased insignificantly in females receiving aqueous extract of *C. bauchiense* referring to females receiving distilled water and those receiving potassium dichromate only. This can be due to the dose and duration with respect to other studies. These results are slightly towards the reports of Elsaieed and Nada[10] who exposed rats to 0.50 ppm of hexavalent chromium and De Flora *et al*[11] who administered 250 and 500 ppm of chromium to mice; all in drinking water. Effects found in their studies on female reproductive toxicity in mice and rats include: lengthening of the estrous cycle in both rats and mice, decreased mating and fertility indices; decreased numbers of corpus luteum, implantation sites and live fœtuses/litter; and increased frequencies of pre- and post-implantation resorptions.

The antioxidant enzyme CAT acts as a defense against free radicals. It is responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water[39]. Results of this work showed that treatment with potassium dichromate-induced oxidative stress was notified by a significant (P<0.05) decrease in CAT activity as compared to control values. Also, a significant increase in MDA was observed in potassium dichromate-treated does compared to control ones. The changes in CAT, SOD, peroxidase and MDA might be in response to increased oxidative stress and LPO. According to Halliwell *et al*[40], when a condition of oxidative stress strongly establishes, the defense capacities against reactive oxygen species become insufficient; in turn, reactive oxygen species also affects the antioxidant defense mechanisms, reduces the intracellular concentration of CAT and increases LPO indicated by the increase in MDA. The changes in these oxidative stress biomarkers have been reported to be an indicator of tissue's ability to cope with oxidative stress[41]. The results obtained for the oxidative stress biomarkers CAT, SOD and peroxidase reflect those reported by Mohamed and Saber[37]. The observed increase in MDA is a good evidence of the existence of oxidative stress. Results of the study revealed that co-administration of *C. bauchiense* leaf aqueous extract with heavy metal in rabbits does returned the CAT, SOD and peroxidase activities and MDA level at the control values. The observed normalization trend of CAT and MDA following *C. bauchiense* leaf aqueous extract administration could possibly be due to the scavenging effect of this extract. The high potential of phenolic compounds to scavenge radicals might be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups. Previous studies[12] and the present study showed that *C. bauchiense* contained phenolic, terpenoids (thymol, carvacrol), flavonoids (diosmetin, luteolin, and apigenin), tannins and alkaloids. According to Singh *et al*[42], the antioxidant activity of plant extracts could not only be attributed to the major compounds, but also minor compounds might play a significant role in the antioxidant activity, and synergistic effects were reported. Therefore, the possible mechanisms of protective activity of *C. bauchiense* leaf aqueous extract could arise from the free radical scavenging effects, preventing lipid peroxidation and improving the antioxidant system of the body.

In conclusion, the present study proves that potassium dichromate-induced considerable changes in biochemical markers of nephrotoxicity, hepatotoxicity, markers of oxidative stress and reproductive hormones, thus hexavalent chromium compounds may be one of the environmental factors that generate oxidative stress in female rabbits. Administration of *C. bauchiense* extract has reversed effects on the kidney and liver injuries as well as on oxidative stress markers. However, this extract did not significantly improve foetal characteristics.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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