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Influence of Carrot (*Daucus carota*) Leaves Aqueous Extract on Reproductive and Hematological Characteristics, Oxidative Stress Markers and Toxicity Indicators of Rabbit (*Oryctolagus cuniculus*) Does

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Abstract

The present study was conducted to evaluate the effects of aqueous extracts of carrot leaves (*Daucus carota*) on reproductive characteristics in rabbits (*Oryctolagus cuniculus*). For this purpose, a total of 40 nulliparous (females that have never been in gestation before) and sexually mature rabbits of 7 months old, whose average weight was 3.08 kg were used. The animals were divided into 5 groups (T0-, T0+, T1, T2 and T3) of 8 does each comparable in terms of body weight. The rabbits were placed individually in wire cages equipped with a feeding and a drinking trough. Throughout the trial period, the does received water *ad libitum* and calculated feed. In addition to feed, these animals received daily by gavage distilled water (T0-), vitamin C of 150 mg/kg body weight (T0+). The other three groups (T1, T2 and T3) received respectively 100 mg/kg, 200 mg/kg and 400 mg/kg of aqueous extracts of carrot leaves during the entire period of the test. After a month of testing, the does were presented to males at a ratio of two females to one male for mating. Fourteen days after mating the females were sacrificed for collection of some data.

The results showed that the groups of animals having received the extract were very receptive during mating compared to the animals in the control groups, those of T2 (200 mg) had a significant ($p < 0.05$) increase in progesterone concentration than the other groups. The gestation rate was significantly higher in does receiving 200 mg (T2) and 400 mg (T3) of aqueous extract of carrot leaves compared to the control groups. The level of GSH and that of MDA in the treated rabbits recorded relatively decreased values compared to the control groups. The concentrations of ALAT, ASAT and urea decreased in animals given the extract as compared to those given Vitamin C. The least value of platelets was obtained in animals that received 400 mg/kg body weight of the extract. In regards to these results, the aqueous extract of carrot leaves may be used as a natural source of antioxidants instead of synthetic products thereby improving the reproductive performances of rabbit does at the rate of 200 mg and 400 mg. This is because it is at these doses that most studied characteristics were improved though further research is required in order to conclude with certainty.

Keywords: antioxidants, carrot leaves, oxidative stress, rabbit does, reproduction, synthetic products

1. Introduction

Reproduction is a biological process which permits species to increase in number (Ragab et al., 2016). Reproduction allows, among other things, the perpetuation of species via the dynamics of its populations as stipulated by Chatellier and Dupraz (2019). According to Popli et al. (2022), and Zeng et al. (2021), gamete formation, fertilization and gestation are essential stages for species perpetuation in mammals and in rabbits particularly. The process of reproduction is of principal importance (López-Torres et al., 2020) for the perpetuation of animal species. A dysfunction in this process has deleterious effects in breeding (El-Ashram et al., 2020; Huang et al., 2023). These dysfunctions are many, they can be among others, some classes of infantilizing drugs (Long et al., 2019), hypogonadism (Anangwe et al., 2024), aging of organs, pollution (Canipari et al., 2020; Reiter et al., 2023), as well as environmental stress (Huang et al., 2023). These reproductive dysfunctions are attributed to oxidative stress that which results from both internal and external sources. These dangerous effects linked to reproductive disorders and dysfunctions generally lead to infertility or a drop in weight and number of young and/or in the number of litters leading to significant losses and economic shortfalls for breeders (Esslemont et al., 2001; Inchaisri et al., 2010). To fight against the negative impact of this oxidative stress, there is need for antioxidants which come from both natural and synthetic origins.

Breeders now turn towards the use of synthetic products to improve the success in reproduction in farm animals. This large variety of synthetic substances includes synthetic biochar which is used to boost growth performance, egg yield, resistance to pathogens and could also lead to reduction of methane production (Man et al., 2021). Dixit mushroom is used to refine the taste and texture of flesh, raises carcass weight in cattle and mammals like rabbits, for example (Abdel-Azeem et al., 2018; Ragab et al., 2016). Another product is Zeolite A, which is being sold under the name (Catalysis A ®), is used against worms and vitamin deficiencies in domestic livestock (Papaioannou et al., 2005). Using synthetic food supplements has shown very dangerous residual side effects on production quality (Tsiplakou et al., 2021), on the health of animal *in situ* (Placha et al., 2022), that of consumers (Inchaisri et al., 2010), and equally on the environment (Dasgupta et al., 2021; Gonçalves & Maximo, 2023). These synthetic substances have been shown to be less available and affordable by developing countries.

To face these disadvantages of synthetic products, scientists and breeders very often look into pharmacopoeia (a book that contains information about medicinal plants and drugs) plants with antioxidant (Attia et al., 2017), pro-ovulatory (Dal Bosco et al., 2011; Edwin et al., 2009), progestin and pro-fertile (Kamel et al., 2022) properties. These pharmacopoeia plants are more available, less expensive and less harmful to the environment.

Among these plants is the *Daucus carota* (L), commonly known as carrot, a plant of the Umbelliferae family and order Apiales (Crous et al., 2020). It is a biennial herb with thick, elongated taproots of orange color (Krähmer et al., 2016a). Its leaves and roots are rich in vitamins, minerals, and secondary metabolites essential for health (Dawid et al., 2015; Mandrich et al., 2023). Vitamins A, B, C and E and some phenolic compounds in the leaves and roots of carrots help to improve the immune system (Ahmad et al., 2019) and decrease the risk of certain cardiovascular, optical and degenerative diseases (Boadi et al., 2021). Equally, ß-carotenes contained in carrots are known as antioxidants and reducers of bad cholesterol (Bystrická et al., 2015; Boadi et al., 2021) through resisting oxidation (Koley et al., 2014). Some studies have proven that the consumption of the roots and leaves of this plant improves the functioning of some reproductive organs (Ahmad et al., 2019). These organs are responsible for producing the egg and sperm cells (gametes), and hormones. The hormones function in the maturation of the reproductive system, the development of sexual characteristics, and regulation of the normal physiology of the reproductive system. The phyto-chemistry of this plant particularly indicates that the leaves possess polyphenols, flavonoids and carotenoids that are classes of compounds recognized for their beneficial activities for health (Char, 2017).

The influence of a substance in an organism depends on the dose, form, mode of administration among other variables. Although a number of studies have been conducted on the contribution of carrot leaf properties to health, information is rare on its use in farm animals as a natural source of antioxidant in order to improve production performance.

It is in this context that this study was conducted globally to improve the production of domestic animals using medicinal plants like the aqueous extract of carrot leaves.

2. Materials and Methods

2.1 Animals

Forty nulliparous New Zealand breed female rabbits (*Oryctolagus cuniculus*) of seven months old, with an average weight of 3.01 kg, constitute the animal material for this study.

2.2 Housing

The animals were housed in a sheet metal building, plastered and open to 1/3 on one side. They were kept individually in wire cages (96 cm long, 40 cm wide, 25 cm high) that were made of galvanized metal. Each cage was provided with a feeder and a drinker (about 800 ml capacity). This building was previously disinfected using a virion solution (10 g per 15 litres of water) sprayed in the building and in all the cages before the introduction of the animals.

2.3 Feeding

Throughout the entire period of the test, the rabbits received *ad libitum* water and a calculated feed was also given, whose chemical characteristics are listed in Table 1.

Table 1. Composition and chemical characteristics of calculated feed fed to the animals

Ingredients	Quantities (%)
Maize	27.00
Wheat Brand	14.00
Palm kernel meal	18.00
Soya bean meal	5.00
Cotton seed meal	4.00
*CMAV*10%	5.00
Fish meal	3.00
Palm oil	2.00
Shell fish	1.50
Salt	0.50
Rice pulp	20.00
Total	100.00
Calculated bromatological characteristics of the ration	
Crude protein (% DM)	16.47
ME (kcal/kg DM)	2435.23
Crude fiber (% DM)	13.65
Calcium (% DM)	1.26
Phosphorus (% DM)	0.55
Soduim (% DM)	0.28
Lysine (% DM)	0.83
Methionine (% DM)	0.36

Note. ME: metabolizable energy; DM: dry matter.

2.4 Plant Material

Fresh carrot leaves (*Daucus carota*) after harvest were sorted from their stems and dried in the oven (65 °C) for 48 hours. They were then crushed into fine powder using an electric grinding mill. The powder (2 kg) was dissolved in 10 litres of distilled water for 48 hours hrs. The mixture was stirred once every 6 hours throughout the process, to facilitate the extraction of the bioactive molecules contained in the powder. The solution was filtered with the use of Whatman number 3 filter paper. The homogenous filtrate obtained was distributed in stainless steel plates and placed in an oven at a temperature of 50 °C for drying for 72 hours to obtain aqueous extracts.

2.5 Phytochemical Analysis of Aqueous Extracts of Carrot Leaves

The determination of the phytochemical components present in the extract was carried out and the results obtained are presented Table 2.

Table 2. Phytochemical composition of aqueous extract of carrot leaves

Phytochemical components	Results
Alkaloids	+
Phenols	+
Flavonoids	+
Sterols	+
Triterpenoids	+
Tannins	+
Saponins	+

Note. +: Presence.

2.6 Experimental Design

At the beginning of the trial, the animals were weighed to form 5 groups of 8 rabbit does each comparable on the basis of their live weight and placed in a completely randomized experimental design. The aqueous extract of carrot leaves was dissolved in distilled water. Each group received one of the doses of extracts daily according to its live weight using a 5 ml syringe as follows:

- T0-: negative control group received distilled water (3 ml/animal);
- T0+: positive control group received vitamin C (150 mg/kg body weight);
- The other three groups (T1, T2 and T3) respectively received 100, 200 and 400 mg of aqueous extract/kg body weight (BW).

The does were weighed every seven days using an electronic scale (QUIGG®) with a capacity of 5 kg and a precision of 1 g. After 30 days of gavage, the does were placed in the cages of sexually mature, untreated males at a ratio of two females for one male (2♀:1♂). Mating was considered successful when the male, after mounting the doe, fell on its side with a dull cry. After mating, gavage continued for fourteen days after which the does were sacrificed to evaluate the effects of the extracts on some reproductive and hematological characteristics, oxidative stress markers and toxicity indicators.

2.7 Parameters Studied and Data Collection

2.7.1 Animal Weight

The weight of the breeding does was recorded every seven days. The weight gain of the does was obtained by taking the difference between the weight at the week considered and that of the previous week. The following formula was used:

$$BWG = W_n - W_{n-1} \tag{1}$$

where, BWG = Body Weight Gain; W_n = weight at the week considered; W_{n-1} = weight in the previous week.

Average Daily Gain (ADG): The average daily gain was obtained by dividing the weight gain during a period by the duration of that period.

$$ADG = \frac{\text{Weight gain (g)}}{\text{Duration (days)}} \tag{2}$$

2.7.2 Reproductive Characteristics

The rate of gestation was calculated as follows:

$$\text{Gestation rate} = \frac{\text{Number of pregnant females}}{\text{Number of females put to breeding}} \times 100 \tag{3}$$

2.7.3 Blood Collection

During the slaughter of the rabbits, blood was collected from the jugular vein in 10 ml tubes labeled with anticoagulant and without anticoagulant. For the tubes with anticoagulant, the blood was used for hematological analyses. As for the dry tubes without anticoagulant, the blood was left to rest for 24 hours and then centrifuged at 3000 rpm for 5 minutes to obtain the serum. The serum was then stored at -18 °C until use.

2.7.4 Evaluation of Hematological Characteristics

The evaluation of hematological characteristics like red blood cells, white blood cells, platelets, and other parameters were done using an automated hematology analyzer (Model KT 6180 S/N701 106101557).

2.7.5 Oxidative Stress Markers

Indicators of oxidative stress like malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidases (GPx) were measured according to the methods described respectively by Oyedemi et al. (2010), modified by Kodjio et al. (2016), Dimo et al. (2006), Sajeeth et al. (2011), and Habbu et al. (2008).

(1) Estimation of Catalase Activity

Catalase activity was estimated depending on the ability of H_2O_2 to decompose by the action of CAT to produce H_2O and O_2. The decrease in the absorbance in the UV region per time is corresponded to CAT activity. 2.0 ml of 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 mins by the decrease in absorbance at 240 nm.

(2) Estimation of Glutathione

Glutathione level was measured based on the reaction of GSH with 5,5-dithiobis-2-nitro-benzoic acid (DTNB) from (Sigma Aldrich, Germany) at pH 8.0. The optical density of yellow colour measured at 412 nm. 0.1 mL ofserum samples were added to 0.9 mL of (0.6 mmol DTNB) reagent and 10 µL of trichloroacetic acid (Labtech, Australia), mixtures were incubated at 25 °C for 5 min. After centrifugation, the absorbance of yellow colour measured and the results were calculated from a glutathione standard curve.

(3) Estimation of Lipid Peroxidation

Lipid peroxidation was estimated by reaction of thiobarbituric acid (TBA) (from Qulaikems, India) with Malondialdehyde (MDA). In the presence of an acid and heat (pH 2-3, 100 °C), MDA condenses with two molecules of TBA to produce a pink color complex which absorbs at 532 nm. 105 µl of orthophosphoric acid at 1% and 500µl of the precipitation mixture (1% TBA in a 1% acetic acid solution) was added to 100 µl homogenate. The mixture of each tube was homogenized and placed in a boiling water for 15 minutes. The tubes were cooled in an ice-bath and the mixture was centrifuged at 3500 rpm, for 10 mins. Absorbance was read at 532 nm against the control.

(4) Estimation of Superoxide Dismutase

Adrenaline is stable enough when pH is acidic. When pH increases, the rate of auto-oxidation of adrenaline increases. The dosage of SOD is 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. Micro tubes of serum were introduced into the spectrometer as well as1660 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.

2.7.6 Biochemical Analyses

(1) Markers of Hepatotoxicity

Blood was collected and placed into test tubes without anticoagulant, then centrifuged at 3000 rpm for 15 minutes. The serum was collected, aliquoted and stored at -20 °C for the measurements of Aspartate-Amino Transferase (ASAT) and Alanine Amino-Transferase (ALAT) by the enzymatic analysis method based on the protocol listed in the instructions for the commercial kit CHRONOLAB®.

(2) Nephrotoxicity Markers

Creatinine was measured by the colorimetric or kinetic method in serum according to the instructions for the commercial CHRONOLAB® kit; Urea was measured by the Urease-GLDH method from the commercial CHRONOLAB® kit.

2.7.7 Serum Progesterone Measurement

Serum progesterone was measured using the ELISA kit from Fortress Diagnostics (United Kingdom). The measurement was performed by the solid-phase enzyme-linked immunosorbent assay (ELISA) method as described in the kit instructions. The concentrations of progesterone were obtained by projecting the optical densities read with the ELISA counter onto the calibration curve.

2.7.8 Relative Weights of Organs

After dressing the animals, organs including the liver, heart, lungs, kidneys, spleen and uterus were removed and weighed using an electronic balance (QUIGG®) and their volume was measured by the liquid displacement method using distilled water in a graduated tube. The relative weights of the organs were determined according to the following formula:

$$\text{Relative weight of organs (\%)} = \frac{\text{Organ weight (g)}}{\text{Live weight at slaughter (g)}} \times 100 \quad (4)$$

For pregnant females, the uterus was incised and the embryos were counted to determine the number of embryos per female.

2.8 Statistical Analyses

The data obtained were subjected to one-way analysis of variance (ANOVA 1). When there were significant differences between the means, the Duncan test was applied to separate them. The Chi-square test was used to compare the data in percentages. The SPSS 20.0 statistical software was used and the significance threshold was set at 5%.

3. Results

3.1 Effects of Aqueous Extract of Carrot Leaves on the Average Weight of Does

Table 3 shows the effects of carrot leaves extract on the average weight of does. It appears that, animals that received 200mg of extracts (T2) presented a significantly ($p < 0.05$) higher average body weight during the first and second weeks of the ~~test~~ study compared to the other groups.

Table 3. Effects of carrot leaf aqueous extract on the live weight of animals

Period	Control		Treatments (*D. carota*/kg/bw)			p-value
	T0-	T0+	T1	T2	T3	
Week 1	2.65±0.23^{b}	2.75±0.34^{b}	2.76±0.31^{b}	3.26±0.26^{a}	2.79±0.28^{b}	0.060
Week 2	2.62±0.15^{b}	2.73±0.24^{b}	2.73±0.25^{b}	3.30±0.31^{a}	2.83±0.24^{b}	0.011
Week 3	2.64±0.16	2.81±0.24	2.64±0.16	2.76±0.23	2.85±0.16	0.430
Week 4	2.70±0.25	2.61±0.10	2.91±0.17	2.64±0.33	2.94±0.19	0.150
Week 5	2.62±0.25	2.80±0.28	2.80±0.27	2.79±0.17	2.82±0.17	0.730
Week 6	2.69±0.24	2.80±0.29	2.85±0.20	2.88±0.25	2.83±0.16	0.810

Note. a, b: lines with the same letter do not differ significantly ($p > 0.05$); p: Probability; T0-: distilled water; T0+: Vitamin C at a dose of 150 mg/kg bw; T1, T2 and T3: aqueous extract of *Daucus carota* at doses 100, 200 and 400 mg/kg bw respectively.

3.2 Effects of Aqueous Extract of Carrot Leaves on Some Reproductive Characteristics

3.2.1 Effects of Aqueous Extract of Carrot Leaves on Receptivity, Number of Embryos and Pregnancy Rate

Table 4 summarizes the effects of aqueous extract of carrot leaves on receptivity, number of embryos and pregnancy rate. Does treated with the carrot leaf extract were more receptive than those in control groups. A significant increase in the gestation rate and number of embryos was observed in animals that received 200 mg and 400 mg of the extract compared to the other groups.

Table 4. Effects of aqueous extract of carrot leaves on some reproductive characteristics

Reproductive characteristics	Control		Treatments (*D. carota*/kg/bw)			p-value
	T0+	T0-	T1	T2	T3	
Receptivity (minutes)	33.5±4.43^{a}	26±6.16^{b}	11.75±2.5^{c}	9.75±3.5^{c}	7±2.16^{c}	0.000
Number of embryos	3.0±3.46^{b}	3.0±3.46^{b}	6.5±1.0^{ab}	7.5±1.0^{a}	7.5±1.0^{a}	0.021
Pregnancy rate (%)	75±20^{ab}	50±23^{b}	75± 20^{ab}	100±0.00^{a}	100±0.00^{a}	0.03

Note. a, b, c: Means with the same letters on the same line are not significantly different ($p > 0.05$); p: Probability; T0-: distilled water; T0+: Vitamin C at a dose of 150 mg/kg bw; T1, T2 and T3: aqueous extract of *Daucus carota* at doses 100, 200 and 400 mg/kg bw respectively.

3.2.2 Effects of Aqueous Extract of Carrot Leaves on Serum Progesterone Concentration

Figure 1 illustrates the effects of aqueous extract of carrot leaves on serum progesterone concentration. The serum progesterone concentration observed in animals in T2 was ignificantly ($p < 0.05$) higher compared to other groups.

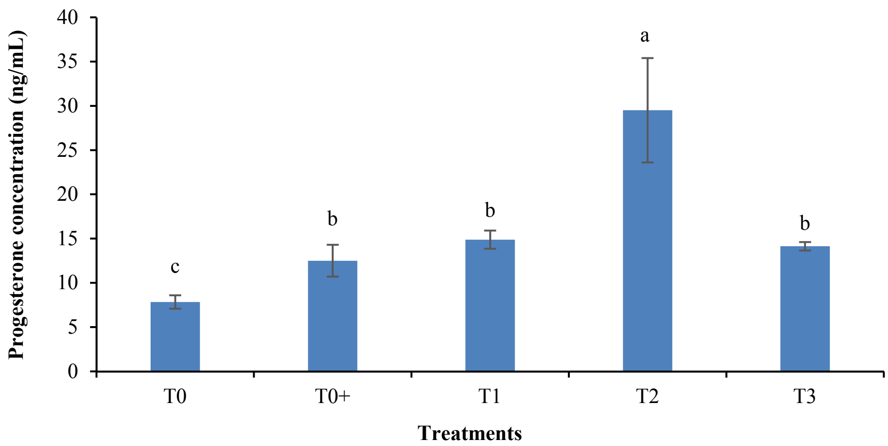

Figure 1. Effects of aqueous extract of carrot leaves on serum progesterone concentration

Note. a, b, c: bars with the same letters on the same line are not significantly different (p > 0.05); T0-: distilled water; T0+: Vitamin C at a dose of 150 mg/kg bw; T1, T2 and T3: aqueous extract of *Daucus carota* at doses 100, 200 and 400 mg/kg bw respectively.

3.2.3 Effects of Aqueous Extract of Carrot Leaves on the Relative Weight of Reproductive Organs

The effects of aqueous extract on the relative weight of reproductive organs are presented on Table 5. It is found that, aqueous extract of carrot leaves did not significantly affect the relative weight of reproductive organs.

Table 5. Effects of aqueous extract of carrot leaves on the relative weight of reproductive organs

Organs relative weights (%)	Control		Treatments (*D. carota* kg/bw)			p-value
	T0+	T0-	T1	T2	T3	
Right ovary	0.02±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.01	0.126
Left ovary	0.03±0.01	0.01±0.00	0.01±0.01	0.01±0.00	0.01±0.00	0.112
Oviduct	0.04±0.05	0.03±0	0.06±0.05	0.04±002	0.02±0.01	0.739
Uterus	0.49±0.1	0.68±0.16	0.27±0.1	0.39±0.4	0.35±0.05	0.105
Vagina	0.11±0.01	0.11±0.01	0.11±0.00	0.11±0.01	0.11±0.00	0.845

Note. p: Probability; T0-: distilled water; T0+: Vitamin C at a dose of 150 mg/kg bw; T1, T2 and T3: aqueous extract of *Daucus carota* at doses 100, 200 and 400 mg/kg bw respectively.

3.3 Effects of Aqueous Extract of Carrot Leaves on Oxidative Stress Markers

Table 6 shows that the animals of T0+ recorded a significantly ($p < 0.05$) higher GSH level–compared to the animals of the other groups. The levels of MDA were comparable among all the groups except in T3 where a significant increase was noticed.

Table 6. Effects of aqueous extract of carrot leaves on oxidative stress markers

Oxidative stress indicators	Control		Treatments (*D. carota*/kg/bw)			p-value
	T0+	T0-	T1	T2	T3	
CAT (U/g TP)	1.35±0.32	1.41±0.36	1.18±0.23	1.34±0.36	1.27±0.29	0.873
GSH (M/g TP)	35.18±3.27^{a}	29.41±4.52^{b}	31.8±4.03^{ab}	28.58±1.91^{b}	26.89±3.58^{b}	0.042
MDA (µM)	0.93±0.15^{b}	0.83±0.09^{b}	0.97±0.26^{b}	0.75±0.07^{b}	1.32±0.26^{a}	0.007
SOD (U/min/g TP)	0.39±0.11	0.23±0.11	0.21±0.08	0.31±0.13	0.22±0.1	0.155

Note. a, b: Means with the same letters on the same line are not significantly different (p > 0.05); p: Probability; T0-: distilled water; T0+: Vitamin C at a dose of 150 mg/kg bw; T1, T2 and T3: aqueous extract of *Daucus carota* at doses 100, 200 and 400 mg/kg bw respectively. TP: total proteins; CAT: Catalase; GSH: Reduced Glutathion; MDA: Malondialdehyde; SOD: Superoxide dismutase.

3.4 Effects of Aqueous Extract of Carrot Leaves on Hematological Characteristics

The effects of aqueous extract of carrot leaves on hematological characteristics are demonstrated on Table 7. PLT and PLCC recorded the lowest values in T3 as compared to the other groups. Despite the absence of significant difference, HCT, HGB, RBC and WBC reported the highest values in T3 with respect to the other groups.

Table 7. The effects of aqueous extract of carrot leaves on hematological characteristics

	Control		Treatments (*D. carota*/kg/bw)			p-value
	T0+	T0-	T1	T2	T3	
GRAN#	0.42±0.09	0.53±0.25	0.53±0.2	0.66±0.51	0.47±0.22	0.833
GRAN%	21.45±6.64	18.56±6.09	15.35±11.03	18.13±14.5	15.67±12.98	0.927
HCT	33.6±5.48	26.67±17.4	33.22±4.36	26.97±15.41	37.47±3.34	0.59
HGB	12.47±1.17	9.57±6.07	11.72±1.44	9.52±5.28	13.25±1.18	0.53
LYM#	1.3±0.61	1.8±0.7	1.52±0.41	3.06±0.92	2.77±2.07	0.221
LYM%	59.62±15.55	59.86±14.86	65.17±17.66	62.23±19.18	69.4±19.55	0.929
MCH	24.07±3.34	25.4±3.82	21.97±1.29	22.97±2.47	22.17±0.69	0.354
MCHC	37.5±3.34	39.3±7.15	35.27±0.29	36.6±3.23	35.3±0.48	0.558
MCV	64.17±3.8	62.65±1.33	62.5±3.66	63.2±3.28	62.92±1.82	0.934
MID#	0.37±0.12	0.63±0.35	0.52±0.22	0.66±0.23	0.45±0.17	0.418
MID%	18.92±9.04	21.56±8.86	19.47±7.25	19.63±4.68	14.92±6.6	0.816
MPV	5.27±0.65	6±1.00	5.72±0.92	5.8±1.16	5.55±0.72	0.833
PLCC	$4.25±1.5^{ab}$	$5.75±1.5^{a}$	$6.00±1.41^{a}$	$5.5±1.73^{a}$	$2.5±1.00^{b}$	0.021
PLCR	5.15±3.79	5.22±3.39	7.87±6.00	5.97±6.14	6.36±4.09	0.929
PCT	0.07±0.07	0.06±0.02	0.06±0,06	0.07±0.05	0.01±0.01	0.503
PDW	6.05±0.71	6.62±1.48	6.00± 0.23	6.22±0.71	6.05±0.5	0.822
PLT	$151±14.76^{a}$	$88.5±9.14^{c}$	$102.82±13.51^{bc}$	$118.75±11.58^{b}$	$57.00±19.37^{d}$	0.000
RBC	5.26±0.95	4.24±2.76	5.33±0.69	4.33±2.5	5.96±0.37	0.613
RDWCV	16.92±2.98	16.55±2.23	16.4±0.67	17.4±2.84	15.85±1.16	0.885
RDWSD	36.07±0.35	35.2±2.21	35.37±1.44	35.72±0.35	35.37±1.55	0.903
WBC	2.1±0.66	2.25±0.86	2.45±0.7	2.67±1.78	3.70±2.10	0.509

Note. a, b, c, d: Means with the same letters on the same line are not significantly different ($p > 0.05$); P: Probability; T0-: distilled water; T0+: Vitamin C at a dose of 150 mg/kg bw; T1, T2 and T3: aqueous extract of *Daucus carota* at doses 100, 200 and 400 mg/kg bw respectively. WBC: White blood cells; RBC: Red blood cells; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelets; LYM: Lymphocytes; GRAN: Granulocytes; RDWCV: Coefficient of variation in the red blood cell distribution index; RDWSD: Standard deviation in the red blood cell distribution index; PCT: platelettocrit; MPV: mean platelet volume; PDW: platelet distribution index.

3.5 Effects of Carrot Leaves Aqueous Extract on Nephrotoxicity and Hepatotoxicity Indicators

Table 8 presents the effects of carrot leaves aqueous extract on nephrotoxicity and hepatotoxicity indicators. It follows that, the concentrations of ALAT, ASAT and urea were significantly higher in animals given vitamin C (T0+) in comparison with the other groups. Cholesterol levels were comparable ($p > 0.05$) among animals that received extracts and control groups except in T3 where it was significantly ($p < 0.05$) higher.

Table 8. Effects of carrot leaves aqueous extract on nephrotoxicity and hepatotoxicity indicators

	Control		Treatments (*D. carota*/kg/bw)			p-value
	T0+	T0-	T1	T2	T3	
ALAT (U/I)	114.14±6.79^{a}	6.98±2.36^{cd}	5.67±2.15^{d}	15.71±5.25^{c}	48.45±10.69^{b}	0.000
Albumine (g/dL)	2.35±0.48^{b}	2.39±0.38^{b}	3.13±0.13^{a}	2.69±0.2^{ab}	2.8±0.33^{ab}	0.027
ASAT (U/I)	618.73±55.22^{a}	151.24±33.79^{c}	85.11±10.89^{d}	74.2±24.9^{d}	354.21±45.73^{b}	0.000
Cholesterol (mg/dL)	61.01±11.67^{bc}	43.93±15.82^{c}	67.45±15.18^{b}	50.74±13.72^{bc}	98.51±13.12^{a}	0.001
Creatinine (mg/dL)	1.31±1.78	4.05±3.35	3.5±1.11	2.9±0.70	3.39±0.71	0.311
Globuline (g/dL)	1.7±0.81	1.92±0.57	1.26±0.75	1.19±1.25	1.65±1.23	0.799
Total protein (g/dL)	4.05±1.04	4.31±0.45	4.39±0.67	3.88±1.41	4.46±1.35	0.926
Urea (mg/dL)	55±8.12^{a}	40.25±7.84^{b}	39.00±3.55^{b}	24.75±4.5^{c}	44.5±4.43^{b}	0.000

Note. a, b, c, d: Means with the same letters on the same line are not significantly different ($p > 0.05$); P: Probability; T0-: distilled water; T0+: Vitamin C at a dose of 150 mg/kg bw; T1, T2 and T3: aqueous extract of *Daucus carota* at doses 100, 200 and 400 mg/kg bw respectively. ASAT: Aspartate aminotransferase; ALAT: Alanine aminotransferase.

3.6 Effects of Aqueous Extract of Carrot Leaves on the Relative Weight of Organs and Carcass

Table 9 indicates that the weight of the liver significantly ($p < 0.05$) increased respectively for animals in batches (T0-, T1 and T2) in comparison with the positive control group (T0+) and T3.

Table 9. Effects of aqueous extract of carrot leaves on the relative weight of organs and carcass

Relative organs weight (%)	Control		Treatments (*D. carota*/kg/bw)			p-value
	T0+	T0-	T1	T2	T3	
Heart	0.33±0.04	0.34±0.08	0.35±0.08	0.25±0.05	0.34±0.06	0.278
Liver	2.42±0.12^{b}	2.8±0.11^{a}	2.81±0.13^{a}	2.93±0.11^{a}	2.5±0.21^{b}	0.001
Lungs	0.78±0.16	0.63±0.16	0.6±0.11	0.5±0.08	0.76±0.18	0.092
Spleen	0.12±0.05	0.09±0.03	0.08±0.05	0.07±0.03	0.11±0.04	0.436
Carcass	55.41±3.33	53.51±1.75	52.69±5.11	54.29±8.26	51.52±4.62	0.847

a, b, c, d: Means with the same letters on the same line are not significantly different ($p > 0.05$); p: Probability; T0-: distilled water; T0+: Vitamin C at a dose of 150 mg/kg bw; T1, T2 and T3: aqueous extract of *Daucus carota* at doses 100, 200 and 400 mg/kg bw respectively.

4. **Discussion**

Using synthetic feed supplements ~~has~~ have shown very harmful side effects on the quality of production (Tsiplakou et al., 2021). Using plant-based feed supplements is fast becoming essential as an alternative in supplementing the needs, among others, in vitamin C (Hemilä & Chalker, 2023), in order to improve the quality, quantity as well as the cost of production in rabbit farming.

The results of this work showed that animals that received 200 mg of aqueous extracts (T2) of carrot leaves had a significantly higher average body weight ($p < 0.05$) during the first and second weeks of the trial compared to other groups. These results are in line with those reported by Djuissi et al. (2021) in adult primipa rabbits which received avocado powder at doses of 0.5%, 1%, and 1.5%; Chongsi et al. (2023) in rabbits treated with 0.6% and 1.2% of spirulina powder. These equally agree with the findings of Sorelle et al. (2020) in rabbits treated with the aqueous extract of spirulina at the concentration of 5, 10 and 20 mg/kg as well as those of Dorice et al. (2023) in rabbits given aqueous extract of guava leaves at the concentration of 10, 20 and 30 mg/kg.

Animals that received doses of carrot leaf extract had higher receptivity and recorded a significantly ($p < 0.05$) higher number of embryos than animals in control groups (T0- and T0+). Similar reports were made by Kumar (2022); when they showed that papaya pulp powder supplementation significantly raised the number of embryos implanted in the uterus as well as the rabbits receptivity (Edwin et al., 2009; García-Fernández et al., 2024).

Likewise, the serum progesterone concentration observed in animals given aqueous extract as well as the vitamin C group was higher than those given distilled water only. However, the highest concentration of progesterone was however noticed in rabbits administered 200 mg/kg bw of the extract and this was significantly higher compared to other groups. The significant increment in receptivity and thus fertility could be justified by the improvement in the concentrations of some sex hormones by the secondary metabolites found in the carrot leave aqueous extract. Indeed, ß-carotene, vitamins C and E, present in carrot leaves are particularly involved in the biosynthesis of steroid hormones like progesterone that are also included in ovulation and fertilization processes among the other reproductive characteristics (García-Fernández et al., 2024; Popli et al., 2022).

The level of glutathione reported a greater relative decline compared to the control group rabbits and the MDA level was significantly higher in the rabbits treated with 400 mg of carrot leaves aqueous extract, and with the lowest value in those that received 200 mg/kg bw of the extract. This significant decrease in glutathione levels can be explained by a reducing effect with respect to the vitamin C contained in the carrot leaves extract. Indeed, vitamin C and many other bio-compounds are commonly used to fight against oxidative stress. The evaluation of animal biochemical parameters permits the monitoring of the level of dietary intake. Likewise, biochemical profile of blood is extremely essential in animal health management (Quintavalla et al., 2001), it is a common instrument for early diagnosis, or adjustment of nutritional and metabolic disorders before the onset of more serious symptoms (Miranda et al., 2008).

The significant increment in serum proteins like albumin in does receiving the aqueous extract of carrot leaves observed in this study may be due to the Carotenoids B which might have assisted by reducing the damage of liver, and increasing nutrient absorption. Albumin represents the essential protein that serves as a source of amino acids for tissue synthesis during the period of growth of animals (Filipowicz et al., 2008). The results of this study agree with those of Ankit et al. (2017), who showed that supplementing diet with garlic powders at 0.25 and 0.5% increased the total protein content in broilers, and Abdel-Ghaney (2017), who also observed that supplementing diet with thyme powders significantly increased the total protein, globulin and albumin content in broilers. This result may be justified by the production of immunoglobulins due to the immune response of broilers due to the contents of thyme administered to the broilers. The same could have taken place with Carotenoids B contained in the carrot leaves aqueous extract given to rabbits in the present study.

The markers of nephrotoxicity (Urea and creatinine) and hepatotoxicity (ALAT and ASAT), volume and weight of some organs made it possible to understand the metabolism of this extract at the level of the purifying organs. The activities of ALAT and ASAT were significantly higher in the Vitamin C group compared to the other groups. These results is in agreement with those obtained by Rehman et al. (2011) who reported lower activities of serum transaminases in poultry with aqueous extracts of medicinal plants (garlic, aloe vera, ginger). Equally, Tugiyanti et al. (2019) observed that the supplementation of avocado seed powder given to female quails showed that a supplement of approximately 6% resulted in the lowest ALAT levels. These observations of lower values compared to the positive control groups are consistent with those of Malekizadeh et al. (2012) who reported a significant decrease in serum ASAT and ALAT levels in Hyline leghorns (W-36) that received ginger rhizome powder at a rate of 3% in the rations for 9 weeks. The values of these characteristics in this work with respect to vitamin C groups stipulate that the doses of carrot leaf extract were not toxic and possibly regulated the liver activity of the rabbits.

According to Zounongo (2013), an increase in the serum transaminase level reflects hepatic cytolysis. A decrease in the concentration of serum transaminases (ALAT and ASAT) could be related to the hepatoprotective activity of carrot leaf aqueous extract, also indicating normal functioning of liver tissues (Orhue et al., 2015). The hepatoprotective properties of carrot leaf extract observed in the study could be related to its high content of antioxidant compounds such as flavonoids and alkaloids.

Urea concentration was reduced in rabbits receiving carrot leaf extract and those given distilled water only compared to those administered vitamin C. Urea is the end product of protein metabolism and a rise in uremia would reflect poor usage of proteins and amino acids (Abdel-Fattah et al., 2008). This decline in serum urea level could be linked to the production of ammonia in the caecum of rabbits, or to microbial activity which is similar to that of the rumen in ruminants (EL-Latif et al., 2019). Serum urea level is an index that reflects the state of metabolism of protein, kidney function and nutrition of the body. The results of this study are in agreement with that of Okali (2019) who recorded that supplementation feed with garlic and thyme powders at 3% separately and 1.5% in combination led to significantly reduced urea concentration in all groups of supplemented diets in goats.

The increase in live weight recorded in rabbits treated with aqueous extract of carrot leaves may be due to the availability of nutrients necessary for the constructive reactions of the body. Compounds like tannins, alkaloids, saponins, flavonoids and terpenoids contained in the extract could have destroyed pathogenic microorganisms in the digestive tract through their antifungal and bactericidal activities, thereby promoting the development of beneficial microorganisms and thus might have made digestion and absorption easier leading to efficient usage of nutrients (Idris et al., 2009). These findings corroborate the reports of Foldesiova et al. (2015) in rabbits receiving *Curcuma longa* powder in feed. However, they are in contradiction to the reports of Ashour et al. (2014) in growing rabbits after receiving *Yacca schidigera* extract. This contradictory result could be explained by the difference in the composition of the plants being used. This result is also similar to that of Abdel-Hakim (2009) registered in quails in Egypt. The variability in weight might be justified either by age or by the orientation of some breeds towards precocity in the production of meat (Alkan et al., 2010).

5. Conclusion

From these observations in rabbit does, it may be concluded that aqueous extract of carrot leaves may be used as a natural source of antioxidants to fight against oxidative stress which comes from internal and external origins, hindering animal production. Faced with the disadvantages of synthetic antioxidants, carrot leaves aqueous extracts may be used instead of synthetic products like the commercial vitamin C which was used as positive control in this study. This is because results were improved with aqueous extracts as well as vitamin C implying that carrot leaves are not toxic. The doses of 200 mg and 400 mg/kg bw may be used since most studied characteristics were improved or remained comparable with those that received no antioxidant source like the negative control group.

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Authors Contributions

Dr. CMMM, Dr. VBN, Mr. AA and Dr. FTL were responsible for study design and revising. Mr. AA, Dr. CMMN, Dr. DNAB and GNAJ were responsible for data collection. Dr. CMMM and Dr. MTMA drafted the manuscript and Prof. KA revised it. All authors read and approved the final manuscript and contributed equally to the study.

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Data Sharing Statement

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