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ORIGINAL ARTICLE

The Impact of *Nigella Sativa* Oil and *Aloe Vera* Gel on Rabbit Oxidative Status

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Abstract

OBJECTIVE: This study aimed to analyze the oxidative parameters of *Nigella sativa and Aloe Vera* gels in rabbits.
DESIGN: A randomized controlled experimental study.
ANIMALS: Fifteen rabbits weigh 1.5 ± 0.5 kg.
PROCEDURES: Rabbits were allocated to one of three groups of five animals each. The first group served as the control group, while rabbits in the second group were administered 400 mg/kg *N. sativa* oil (100 %). The third group was given an aqueous extract of 10 % w/v of 15 ml/l *Aloe Vera gel*. All treatments were administered daily for two successive weeks. Blood samples were collected from the ear vein of each rabbit to determine the antioxidant status on the 1st day, 1st week, and 2nd week after treatment.
RESULTS: The obtained results showed that administration of *N. sativa* oil and *Aloe Vera* gel improved antioxidant activity by increasing catalase, reducing glutathione, and decreasing malondialdehyde (MDA) levels in rabbits post-treatment.
CONCLUSION AND CLINICAL RELEVANCE: Our data highlight that using *N. sativa* and *Aloe Vera* gel are good supplements for rabbits without any adverse effects.

Keywords: *Aloe vera* gel, Antioxidant enzymes, *Nigella sativa* oil, Rabbits

1. Introduction

Rabbits play an important role in economic development and contribute to household food and nutritional security. Rabbit production is still a new enterprise and is mainly a smallholder system that has advantages over other livestock systems because of the small rabbit's body size, high rate of reproduction, adaptability to inexpensive housing, and useful by-products [1]. According to Brambilla et al. [2], two different stressful situations, physiological or pathological, can be considered from a prognostic point of view in the state of animal welfare. In the case of physiological stress, animals can develop an adaptive response that is expressed by activating endogenous antioxidant mechanisms, which can compensate for the imbalance in oxidative status. Conversely, under conditions of pathological stress, the adaptive response of the organism is inadequate and leads to the excessive production of free radicals, which results in oxidative stress. Excessive generation and/or inadequate removal of free radicals results in destructive and irreversible cell damage [3].

Recently, there have been initiatives to promote research into the utilization of traditional plant-based medicines. Researchers have reported that *Nigella sativa* seeds or their extracts have digestive and appetite stimulants, analgesics, anthelmintics, antimicrobial, antidiabetic, anticancer, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal-protective, and antioxidant properties [4]. *Aloe Vera* has a long history as a medicinal plant with diverse therapeutic applications. It has therapeutic effects such as immune stimulation, promotion of radiation damage repair, wound healing, anti-inflammatory effects, antiviral, antifungal, antibacterial, antidiabetic, and antineoplastic activities, stimulation of hematopoiesis, and antioxidant effects [5].

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This study was conducted to evaluate the oxidative indicators of *N. sativa* oil and *Aloe Vera* gel aqueous extract in rabbits.

2. Patients and methods

2.1. Materials

Oil emulsions of *N. sativa* L. seeds (100 %) and *Aloe Vera* leaves were purchased from a local market and stored in a dry atmosphere. EDTA was obtained from the El Nasr Company (Egypt). The reagent kits for the biochemical tests were purchased from Biomed Egypt Company, while the kits for oxidative stress were obtained from Biodiagnostic Company (Egypt).

2.2. Preparation of 10 % aloe vera gel

Fresh *Aloe* leaves were purchased from a local market for gel extraction. *Aloe* gel was manually extracted from the leaf by cutting it using a pocket knife. The latex on the leaf was removed, and the gel was collected in a beaker. A 10 % (w/v) concentrated infusion was prepared by placing 100 g of fresh gel in a glass bottle and one liter of hot boiled distilled water was poured onto it. The bottle was shaken for 5–7 min to ensure thorough mixing and then kept for 6–8 h at room temperature before use [6].

2.3. Experimental animals

Fifteen rabbits (1.5 ± 0.5 kg B.) were used in this study and allocated to three groups. The animals were housed in polypropylene cages, with each cage that contained five rabbits. They were supplied with food and clean drinking water that was renewed every day, and the animals were left for accommodation for 14 days before the experiment.

2.4. Experimental design

The animals were divided into three equal groups (five rabbits each). Group 1 (control group) was intraperitoneally injected with 0.2 ml of physiological saline for two weeks. In group 2, the rabbits received a daily oral dose of *N. sativa* oil (400 mg/kg) by gavage [7]. In group 3, rabbits were supplied daily in drinking water with *Aloe Vera* gel (10 %) extract at a dose of 500 mg/kg for 2 weeks [6].

2.5. Blood sample collection

At the end of the 1st day, 1st week, and 2nd week post treatment, five rats were randomly selected from each group, and blood samples were collected individually from the ear vein in tubes without anticoagulant for serum separation by centrifugation at 3000 rpm for 10 min for oxidant/antioxidant analysis.

2.6. Antioxidant biochemical analysis

GSH levels were measured according to the method described by Beutler [8]. The catalase (CAT) was evaluated according to the method described by Aebi [9]. Finally, MDA was estimated according to Satih [10].

2.7. Statistical analysis

The data were subjected to statistical analysis using a statistical software program (SPSS for Windows, version 23, USA). Data were analyzed using repeated-measures ANOVA. Statistical significance was set at P less than 0.05. Data are expressed as mean ± standard deviation [11].

3. Results

3.1. Evaluation of serum antioxidant enzymes

As shown in Table 1, *N. sativa* and *Aloe Vera* gel groups after one day and one week post-treatment evoked a significant increase ($P < 0.05$) in catalase. However, there was no significant difference after two weeks of therapy compared with the control group. Furthermore, there was a significant increase

Table 1. Effects of Nigella sativa oil (400 mg/kg) and Aloe Vera gel (15 mg/l water) on catalase, reduced glutathione (GSH), and malondialdehyde (MDA) of rabbits (mean ± SD).

Groups	Catalase (mg/dl)			Glutathione (U/ml)			Malondialdehyde (nmol/ml)		
	1st day	1st week	2nd week	1st day	1st week	2nd week	1st day	1st week	2nd week
Control	4.17 ± 0.45[b]	4.03 ± 0.50[b]	4.04 ± 0.28	0.78 ± 0.03[b]	0.76 ± 0.04[b]	0.75 ± 0.02[b]	1.77 ± 0.10[a]	1.73 ± 0.27	1.74 ± 0.54
Nigella sativa (100 %)	6.04 ± 1.45[a]	6.33 ± 1.84[a]	4.89 ± 1.76	0.80 ± 0.02[b]	1.14 ± 0.42[b]	1.56 ± 0.46[a]	1.50 ± 0.14[b]	1.60 ± 0.29	1.66 ± 0.23
Aloe Vera gel (10 %)	5.32 ± 0.45[a]	5.50 ± .79[a]	5.10 ± 0.33	1.40 ± 0.29[a]	1.60 ± 0.37[a]	1.78 ± 0.34[a]	1.60 ± 0.09[b]	1.66 ± 0.29	1.71 ± 0.26

Different letters in the same column are significant at $P < 0.05$.

($P < 0.05$) in reduced glutathione (GSH) after one day and one week in the group treated with Aloe Vera gel and nonsignificant change in *N. sativa* and control groups. In addition, there was a significant increase ($P < 0.05$) in reduced GSH in *N. sativa* and *Aloe Vera* gel-treated groups compared with that in the control group after two weeks.

In contrast, there was a significant reduction ($P < 0.05$) in the level of serum MDA in *N. sativa* and *Aloe Vera* gel groups during the first day of treatment in comparison with the control group. However, the *N. sativa* and *Aloe Vera* gel groups showed nonsignificant difference after 1 and 2 weeks of therapy compared with the control group, as shown in Table 1.

4. Discussion

In recent years, considerable attention has been given to the preservation of livestock welfare to ensure the optimal growth conditions of the animals and to protect them from multifactorial diseases, which can have a heavy impact on zootechnical productivity. The intensive breeding of rabbits in recent decades has resulted in many problems related to the appearance of certain enteric and metabolic infections that have caused high mortality in animals, thus heavily affecting the productivity of farms. These pathologies have multifactorial etiologies and occur at specific delicate stages of rabbit production due to predisposing factors such as imbalances in the diet, hygiene deficiencies, environmental and climatic factors, overcrowding, and stress. In addition to zootechnical parameters, evaluating the oxidative plasma status of farm animals is important for monitoring animal welfare [12].

Reactive species are produced in biological systems owing to redox reactions. An imbalance in prooxidant and antioxidant homeostasis leads to the production of toxic reactive oxygen and nitrogen species. Inactivation of metabolic enzymes, oxidation of biomolecules, and cellular damage are prominent characteristics of reactive species. Similarly, oxidative stress has been associated with more than 100 pathologies, such as atherosclerosis, diabetes, cardiovascular diseases, pancreatic and liver diseases, aging, and cancer. The toxicity of reactive species is balanced by integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants [13].

Antioxidant therapies and defenses protect biological sites by removing or quenching free radicals. Medicinal plants can not only protect against oxidative damage but also play a vital role in health maintenance and prevention of chronic degenerative diseases. Therefore, greater importance is now being attached to the use of locally available medicines to reduce reliance on expensive imported drugs [14]. *N. sativa* belonging to the family *Ranunculaceae* had widespread pharmacological activities, such as carminative, antidiabetic, stimulant, analgesic, antipyretic, anti-inflammatory, antidiarrheal, antibacterial, antioxidant, and protective effects against liver damage [15]. The main compounds in *N. sativa* are thymoquinone, p-cymene, carvacrol, 4-terpineol, and t-anethole. Thymoquinone and its derivatives (dithymoquinone, thymolhydroquinone, and thymol) are putative pharmacologically active ingredients of *N. sativa* that could improve growth performance, rabbit health, and antioxidant state [16]. In addition, Aloe Vera gel contains a large number of bioactive compounds such as flavonoids, terpenoids, lectins, fatty acids, anthraquinones, mono- and polysaccharides, tannins, sterols, enzymes, salicylic acid, minerals, and vitamins, which can be used to alleviate symptoms or prevent oxidative stress-related diseases.

Lipid peroxidation is one of the most predominant and recognized consequences of intensified generation of free radicals, and oxidative stress can be evaluated based on the concentration of MDA, the main product of lipid peroxidation. The enzymatic system plays the largest role in the antioxidant activity of the body, including the following enzymes: total superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [17]. Reduced GSH is an important component of the intracellular protective mechanism against oxidative stress [18].

The results of this study indicate that *N. sativa* oil significantly increased catalase and GSH levels. This result is in agreement with Kanter et al. [19] who reported an increase in antioxidant enzymes, such as catalase, superoxide dismutase, reduced GSH, and GPX, in the liver and kidney of rats treated with *N. sativa*. Similar findings were reported by Ali [20] who studied the protective effect of *N. sativa* on the kidney and found an increase in catalase and GSH levels in the renal tissue supernatant. Moreover, researchers recorded an increase in the activities of certain antioxidant enzymes, such as pancreatic tissue SOD, GSH, and catalase, and serum NO levels in *N. sativa*-treated rats [20–24].

In addition, this work mirrored a significant increase in catalase and GSH in the *Aloe Vera* gel-treated group, which is in accordance with the results reported by Can et al. [25] and Rajasekaran et al. [26] who reported that catalase activity showed

a significant increase in its level and also an increase in superoxide dismutase, reduced GSH, and GPX in the liver and kidney of diabetic rats treated with *Aloe Vera* gel extract. In addition, Anilakumar et al. [27] reported a significant elevation in catalase and GSH activity in the livers of rats treated with *Aloe Vera* gel.

MDA is another marker of oxidative damage and ROS generation. In this study, MDA levels were significantly decreased compared with those in the control group, which is consistent with the findings of Meral et al. [28] and Pourghassem et al. [29] who stated that there was a lower level of MDA in *N. sativa*-treated rabbits, similar to the results reported by Kanter [30] in rats. In addition, Can et al. [25] stated that the fresh extract of *Aloe Vera* caused a decrease in MDA formation in the liver. In addition, El-Gindy et al. [31] found that all *N. sativa* treatments significantly increased the observed blood total antioxidant capacity and significantly decreased the malondialdehyde levels compared with the basal diet group.

Finally, the antioxidant effect of *N. sativa* seems to be due to its oil, thymoquinone (TQ), flavonoids, and antioxidant vitamins such as ascorbic acid. It has been shown that *N. sativa* oil and TQ inhibit nonenzymatic lipid peroxidation in liposomes, and both, especially TQ, act as scavengers of various reactive oxygen species [32]. In addition, the *Aloe Vera* gel contains polysaccharides, phenolic compounds, and aloesin derivatives, which could be related to the effectiveness of aloe as an antioxidant [33].

5. Conclusion

It can be concluded that *N. sativa* and *Aloe Vera* gel can be used as supplements in rabbits to antagonize oxidative stress.

Authors contribution

Amer and Eltaysh designed the experiments. Nessma Alam conducted the experimental protocol and Eman S. El-Ashry wrote the paper and took responsibility for correspondence to the journal. Youssef El-Saedy revised the statistical analysis, and Magdy S. Amer revised the paper accordingly. All authors have approved the final version of the paper for publication.

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Ethical compliance

This study was conducted according to the standards of the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University, Egypt.

Data access statement

Research data supporting this publication are available from the MVMJ located at http://mvmjresearchcommons.org/home.

Conflict of interest

The authors declared no potential conflicts of interest with respect to this article.

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