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IMPACT OF ALPINIA OFFICINARUM AND ZINC ON: 1- SOME BLOOD PARAMETERS, IMMUNITY AND ANTIOXIDANT STATUS IN CALIFORNIAN RABBIT BUCKS

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ABSTRACT

Thirty six Californian male rabbits, 4-5 months age with average body weight of 2980±30.3 gm, were randomly divided into six treatments and used for the present study. The objective of this study was to investigate the influence of the dry powder from the rhizomes of Alpinia galanga (AGR) and zinc sulphate ($ZnSO_4$) supplementation on blood parameters, immunity and oxidative status of Californian rabbit bucks. All rabbits were fed on the basal diet, the 1st group was served as control, nd2 and 5th groups and 3rd and 6th groups were fed basal diet supplemented with 1 kg and 2 kg/AGR/ton feed, respectively, while, 4th, 5th and 6th groups were received 200 mg Zn/L of drinking water for 60 days.

***Results indicated that** supplementation of AGR and Zn had the highest significant values of TP, Alb, AST and ALT, whereas the control group had the lowest value. Significant improved of IgG in the groups supplemented with AGR2, Zn, AGR1+Zn and AGR2+Zn. Groups of Zn, AGR1+Zn and AGR2+Zn recorded high significant value of IgM. Supplementation of AGR with or without Zn had significantly lower concentrations of TG, cholesterol and LDL compared to the control group. Significant increases were noticed in CAT, GSH, SOD, and TAC as compared with control one.*

***Conclusively,** it is concluded that rabbit's bucks blood parameters, immunity and oxidative status may be improved with alpinia galanga and zinc supplementation to rations of Californian rabbit bucks.*

Key words: Rabbits, blood parameters, *Alpinia galanga*, immunity, oxidative status.

INTRODUCTION

Medicinal plants and their derivatives are widely used in traditional societies around the world, and they are increasingly becoming popular in modernity as natural alternatives to synthetic chemicals Raviraja and Monisha (2015). *Alpinia officinarum* (AGR) is an important member of the *Zingiberaceae*

family (Saboo *et al*., 2014). Tannins, alkaloids, flavonoids, vitamins and saponins are the important ingredients of AGR (Haghighian *et al.,* 2015). Previous research has revealed that *Alpinia galanga* has a spectrum of pharmacological activities, including antibacterial, antifungal, antiviral, antiprotozoal, immunomodulatory, anti-oxidant (Sharma *et al.,* 2018), antidiabetic, hypolipidemic, and many other pharmacological effects (Kiuchi *et al.,* 2002;.Chouni and Paul, 2018). *Alpinia officinarum* could have been a great source of free radical scavengers in nature (Kim *et al*., 1997).

Trace elements, especially zinc, are important for health and immunity, even though they are generally required in trace quantities. They play a role in the evolution, reproduction, and growth. Trace elements function as cofactors for enzymes that are vital for animal immunity (Aksu *et al.,* 2012). Important enzymes that do use trace elements as cofactors comprise superoxide dismutase, glutathione reducatase, glutathione peroxidase, thioredoxinreeducates, ceruloplasmin, and catalase. These enzymes serve as antioxidants, avoiding oxidative stress by mitigating oxidants synthesized in response to specific stimuli. Moreover, trace minerals contribute to an animal's general wellbeing, increasing disease resistance. Trace elements are required for the normal functioning of a variety of enzymes and proteins that are engaged in a variety of physiological, biochemical, and metabolic processes that contribute in growth and production (Yatoo *et al.,* 2013). Trace elements, in principle, promote immunological response and productivity (Terpilowska and Siwicki, 2011).

Therefore, impact of alpinia officinarum and zinc on: 1- some blood parameters, immunity and antioxidant status in Californian rabbit bucks.

MATERIALS AND METHODS

Housing and management:

From December till February (winter season), the current study was carried out in a private rabbitry farm in Qalubia governorate, Egypt. The researchers aimed to study the impacts of *Alpinia galanga* and zinc sulphate ($ZnSO_4$) supplementation on blood parameters of Californian rabbit bucks. Thirty six Californian male rabbits, 4-5 months age with average body weight of 2980±30.3 gm, were randomly distributed into six treatments (6 individual each) were used. Rabbits were housed in wire galvanized batteries approximately 60x55x40 cm in a naturally ventilated container. Pellet feeders and automatic drinkers were fitted in the batteries. The basal experimental ration was formulated and pelleted to cover the nutrient requirements of rabbits according to NRC, (1977), as shown in Table 1.

Table 1. The composition and chemical analysis of the basal experimental diet

Ingredients	**%**	***Calculated analysis***			
Yellow corn	6.22	Crude protein, %			18.8
Soybean meal, 44%	22.33	Crude fiber, %			13.0
Wheat bran	23.33	Ether extract, %			3.0
Barley	15.00	Digestible energy (kcal/kg diet)			2680
Alfalfa hay	30.12	n-6 poly unsaturated FAs%			0.3
Ground limestone	1.00	n-3 poly unsaturated FAs%			1.03
		Zn, ingredients +Premix (50+50mg)			100
Dicalcium Phosphate	1.20	***Determined analysis (g/kg diet)***			
Common salt	0.50	Dry matter	897.1	Crude fiber	138.5
Vit. + min. premix*	0.30	Organic matter	801.4	Ether extract	26.2
Total	100.00	Crude protein	169.8	Nitrogen-free extract	575.0
				Ash	87.9

*Each 3 kg of premix contains: Vit. A: 12,000,000 IU; Vit. D_3: 3,000,000 IU; Vit. E: 10.0 mg; Vit. K_3: 3.0 mg; Vit. B_1: 200 mg: Vit. B_2: 5.0 mg Vit. B_6: 3.0 mg: Vit. B_{12}: 15.0 mg; Biotin: 50.0 mg; Folic acid: 1.0 mg; Nicotinic acid: 35.0 mg: Pantothenic acid: 10.0 mg; Mn: 80 g; Cu: 8.8 g; Zn: 50 g; Fe: 35 g; I: 1 g; Co: 0.15g and Se: 0.3g.

Materials plant: A dried AGR was purchased in Alexandria at a local market. Supplements of AGR powder were added to feeds as 1 or 2 kg/ton feed for groups (2 and 5) and (3 and 6), respectively, based on a dose 50 or 100 mg/kg body weight for each buck/day/2 month; according to **Sarieh *et al.*, (2014)**.

Chemical: Zinc sulfate is an inorganic compound and used as a dietary supplement to treat zinc deficiency, formula: $ZnSO_4$, molar mass: 161.47g/mol, soluble in water, were purchased from El-Gomhoria Company for chemical, Drugs and Medical Instruments, Alex, Egypt.

Kits: Biochemical analysis kits were purchased from Biodiagnostic Company for Pharmaceutical and Chemicals in Dokki, Egypt. For biochemical analysis, Spectrophotometers GNW-Model: SM-721, Absorbance Microplate Readers, and other laboratory equipment assistance were employed.

Chemical analysis:

Analysis of *Alpinia galanga* rhizomes were done in Alexandria, Egypt's City of Scientific Research and Technological Applications. The GCMS-QP 2010 system (Shimadzu, Japan) was utilized to determine the components of the Khella extract using mass spectrometry and gas chromatography (GC-MS). The sample was injected through a Rtx-5MS column at a rate of 0.9 mLmin-1 at 260°C using helium as a carrier (30 m 0.25 mm, 0.25 m thick). The oven temperature was set

to 61°C and the split injection mode was set at 50:1. At a 70 e Vionisation potential, the ion source temperature was 230°C, whereas the interface temperature was 250°C. Based on their rate, the extract contents were identified using the NIST11library (Gaithersburg, USA).

Experimental design was as follows:

Group 1: basal diet and served as control (C)
Group 2: basal diet + 1 kg/AGR/ton feed **(AGR**1)
Group 3: basal diet + 2 kg/AGR/ton feed **(AGR2)**
Group4: basal diet +200 mg Zn/L drinking water (**Zn**)
Group 5: basal diet+1 kg/AGR/ton feed **AGR1**+200 mg Zn/L water **(AGR1+Zn)**
Group 6: basal diet+2 kg/AGR/ton feed **AGR2** +200 mg Zn/L water **(AGR2+Zn**)
**Rabbits fed *ad libitum* basal diet containing 50 mg zinc/kg diet/premix source +50 mg/kg diet/ ingredient diet.

Blood samples:

At the end of the experiment, before accessing feed and water, blood samples were withdrawn from marginal ear veins under vacuuming in clean tubes with heparin in the morning (8.00:9.00 am) for each treatment group. Blood plasma was obtained by centrifuging the blood for 20 minutes at 3500 rpm and then storing it at –20°C for later analysis.

Blood biochemical constituents:

Total plasma protein (TP), and albumin (Alb) were measured by the methods described by Doumas et al., (1981); globulin) Glo) was calculated. Cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined according to (Reitman and Frankel, 1957); triglycerides (TG) was assessed according to (Fasati and Prencipe, 1982), however, high-density lipoprotein (HDL), low-density lipoprotein (LDL) were calculated using the formula:

LDL-c, (mg/dl =Total cholesterol–{HDL-c+(TG/5)}) , which explained by William *et al*., (1972).

Immune response and antioxidant status determination:

Different types of immunoglobulins in plasma (IgG and IgM) were determined using commercial ELISA kits. The antioxidant effectiveness was determined by measuring lipid peroxidation, enzymatic antioxidant status in plasma, catalase (CAT), reduced glutathione (GSH) and glutathione peroxidase (GPx) (Sanja *et al.*, 2015). According to Ippoushi *et al*., (2005), total antioxidant capacity (TAC) and malonaldehyde (MDA) were measured.

Sheep red blood cells (SRBC)-induced delayed type hypersensitivity reaction (DTH):

Footpad edoema was used as an indicator for delayed type hypersensitivity response to assess the influence of extracts on antigen-specific cellular immunity. To challenge with SRBC, on day 0, all of the groups were immunised by injection of 1 ml of SRBC cell suspension into the right hind foot pad, which was equivalent to 5 109 SRBC per ml, on day 15, all groups received a challenge, which comprised of injecting by 0.5 ml of SRBC cell suspension subcutaneously into the left hind foot pad, and evaluating the thickness of the left hind foot pad with a plethysmometer after 24, 48, and 72 hours.

The difference in Cm between the thickness of the left hind foot before and after the challenge was used to estimate DTH (Lagrange *et al.,* 1974).

Statistical analysis:

All data were subjected to analysis of variance as described in SAS Program (SAS, 2002*).*

The significant means differences among groups were separated by Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSION

Analysis of *Alpinia officinarum*:

Alpinia officinarum had considerably greater total phenols (12.34 mg/g DM), while carotenoids, total flavonoids, tannins, and saponins were 0.57, 6.22, 2.16, and 0.27 mg/g DM, respectively (Tables 2 and 3). *Alpinia officinarum* includes bioactive components as well as antioxidant qualities, according to current research. These findings are in line with those of Abdullah *et al.,* (2015), Basri *et al.,* (2017), and Rachkeeree *et al.,* (2018), who revealed that *Alpinia officinarum* has more bioactive components such flavonoids, phenolic acids, and alkaloids, as well as, flavones such galangin, alpinin, and kaempferol.

1- *Impact of Alpinia galanga (AGR) and Zn on some biochemical blood parameters:*

Table 4 shows the effect of dietary AGR with or without Zn on biochemical blood plasma. In comparison to the other groups, the groups supplemented with AGR1, Zn, AGR1+Zn, and AGR2+Zn had the highest significant values of TP and Alb, whereas the control group had the lowest value for the previous

Table 2. The total concentration of major bioactive components identified in *Alpinia galanga* (AGR) on dry matter bases.

Alpinia galanga rhizome (AGR) mg/g DM				
Total phenols	**Carotenoids**	**Total flavonoids**	**Tannins**	**Saponins**
12.34	0.57	6.22	2.16	0.27

Table 3. Chemical constituents identified by gas chromatography and mass spectrometry

Peaks	**Compounds**	**Retention time (min)a**	**Peak area (%)**	**Molecular formula**
1	Glycyl-D-asparagine	4.138	50.21	C6H11N3O4
2	Benzenepropanal	9.051	37.35	C9H10O
3	**3**-phenyl-2-butanone	11.880	20.49	C10H12O
4	Eucalyptol	5.470	13.89	C10H18O
5	Pyranone	9.174	7.743	C6H8O4
6	α-Terpineol	10.519	9.09	C10H18O
7	Fenchyl acetate	11.508	5.44	C12H20O2
8	**5**-Hydroxymethylfurfural	12.840	11.28	C6H6O3
9	Cinnamic acid	17.957	3.82	C9H8O2
10	Thymol	21.19	25.25	C10H14O
11	Carotol	25.894	17.44	C15H26O
12	Palmitic acid	39.062	4.26	C16H32O2

treatments and the greatest concentrations of AST and ALT. In comparison to the control group, AST and ALT concentrations were low in the same groups. Globulin was recorded unsatisfactory levels in all groups.

Due to the presence of various bioactive components, *Alpinia galanga* is well-known as a medicinal herbal in several traditional methods of treating a wide variety of diseases. Our findings are in agreement with Ganguly *et al.*, (2002), who found that increasing TP and Alb concentrations indicate AGR's function in improving liver efficiency. Given the total bioactive compounds such as phenols, total flavonoids, and antioxidant activity content of Alpinia species, this bioactive component has a significant impact on the liver and kidney in animals, resulting in a reduction in liver function enzymes (ALT and AST) in rats (Negm and Ragheb, 2019). On the other hand, Abdel-Azeem and Basyony (2019) found that

Table 4. Effect of supplemented diet with *Alpinia officinarum* and Zn on some biochemical blood parameters of Californian rabbit bucks

Treatment	Parameters				
	TP, (g/dl)	Alb, (g/dl)	Glo, (g/dl)	AST, (U/L	ALT, (U/L)
C	6.58[c]	3.65[b]	2.93	34.26[a]	34.42[a]
AGR_1	7.29[b]	4.44[b]	2.85	25.31[b]	28.45[b]
AGR_2	7.37[a]	4.71[a]	2.63	26.21[b]	26.35[b]
Zn	7.41[a]	4.83[a]	2.58	25.41[b]	26.44[b]
AGR_1+Zn	7.42[a]	4.89[a]	2.53	25.0[b]	25.7[b]
AGR_2+Zn	7.61[a]	5.29[a]	2.32	23.7[b]	27.0[b]
SEM	**0.34**	**0.18**	**0.21**	**1.16**	**1.79**
P-value	**<.0021**	**<.0011**	**0.643**	**<.0031**	**<.0034**

** a-d: Means with different superscripts within the same column differ significantly at ($P \leq 0.05$).
C: Control. AGR1: *Alpinia galanga* powder in diet as 50mg/kg BW, AGR2: *Alpinia galanga* powder in diet as 100 mg/kg BW, Zn: 200mg Zn/litter of drinking water, AGR1+Zn= *Alpinia galanga* powder in diet as 50mg/kg BW + 200mg Zn/litter of drinking water, AGR2+Zn= *Alpinia galanga* powder in diet as 100 mg/kg BW+200mg Zn/litter of drinking water, TP: Total protein, Alb: Albumin, Glo: Globulin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase.

plasma Glo, Alb, and TP levels were significantly higher in chicks who consumed a diet enriched with Alpinia extract.

Regarding role of Zn, our results are harmonize with results obtained by Ahmed *et al.,* (2020) who showed that the rabbit consumed nano-zinc oxide significantly improved serum ALT, AST, urea, and creatinine. Also, Boiko *et al*., (2020) recorded that the weaning rabbit drinking different level of zinc citrate affect the content of the TP and activity of liver enzymes in the blood of the experimental groups. It has to be renowned that the greatest effect was exerted on level of TP and activity of ALT, alkaline phosphatase enzymes in animals that consumed the smallest amount of zinc citrate.

2- ***Impact of Alpinia galanga (AGR) and Zn on immunity status:***

The results presented in Table 5 showed a significant improvement of IgG in the groups supplemented with AGR2, Zn, AGR1+Zn and AGR2+Zn.While, groups Zn, AGR1+Zn and AGR2+Zn recorded highly significant differences of IgM. On the other hand, groups AGR1+Zn and AGR2+Zn showed improved of SRBS antigen compared to the control group. Harmony with our results, Flavonoids discovered in *Alpinia galanga* exhibit strong immunological stimulating effects in mice, as well as an effect on antibody production to T-dependent antigen SRBCs, which requires the cooperation of T-lymphocytes and macrophages (Alok *et al.,* 2012). *Alpinia galanga* contains many bioactive

Table 5. Effect of supplemented diet with AGR and Zn on immune response in the blood plasma of Californian rabbit bucks

Treatment	Parameters		
	IgG	**IgM**	**SRBS /reaction index**
C	158.13[c]	51.43[c]	0.00
AGR_1	188.22[b]	65.89[b]	38.2[c]
AGR_2	211.43[a]	63.39[b]	44.7[b]
Zn	215.17[a]	69.19[a]	14.2[d]
AGR_1+Zn	205.57[a]	70.68[a]	65.6[a]
AGR_2+Zn	212.13[a]	73.51[a]	71.10[a]
SEM	**3.255**	**0.132**	**2.45**
P-value	**<.0029**	**<.0034**	**<.0045**

** a-d: Means with different superscripts within the same column differ significantly at (P≤0.05). C: Control. AGR1: *Alpinia galanga* powder in diet as 50mg/kg BW, AGR2: *Alpinia galanga* powder in diet as 100 mg/kg BW, Zn: 200mg Zn/litter of drinking water, AGR1+Zn= *Alpinia galanga* powder in diet as 50mg/kg BW + 200mg Zn/litter of drinking water, AGR2+Zn= *Alpinia galanga* powder in diet as 100 mg/kg BW+200mg Zn/litter of drinking water. IgG= immunoglobulin G,IgM=: Immunoglobulin M. sheep red blood cell (SRBC): Delayed type hypersensitivity response to SRBC was induced in mice.

component such as tannins, alkaloids, flavonoids, vitamins and saponins are the important ingredients, this component have immunomodulatory activity due to down-regulated chemokine expression (CXCL1, CCL4, CCL5, CXCL5, CXCL10) and cytokines (IL-6, IL-10, IL-1β, IL-12p70, TNF, IL-1α) (W Zeng *et al*., 2018), immunostimulants activity by stimulating mononuclear cells to secrete cytokine IL-1β, IFN-γ, and TNF-α (Liao *et al*., 2015).

Regarding to the role of Zn, Haase *et al.,* (2007); Hassan *et al.,* (2017) recorded that zinc has a vital part in the transcription of polynucleotides and the expression of genes in cells, as well as in the activation of humoral and cellular immune factors. Also, Ezzat *et al*., (2019) reported that diet supplemented with zinc methionine improved serum IgG and IgM were augmented significantly in supplemented groups compared with the control. Also, zinc has an effect on thymulin secretion by the thymus gland, which increases T-cell production. As a result of the zinc deficit, the thymus misfired, affecting normal immunological function significantly (Mocchegiani *et al*., 1998). However, another study by Li *et al.,* (2016) recorded that consumed nano zinc (nZnO) or ZnO supplementation of weanling piglet diets increased γ–globulin and IgG.

3- ***Impact of AGR and Zn on lipid profile*****:**

Table 6 shows that all groups using AGR with or without Zn had significantly lower concentrations of TG, cholesterol and LDL, while HDL-c and

Table 6. Effect of supplemented diet with AGR and Zn on lipid profile in blood of Californian rabbit bucks

Treatment	Parameters			
	TG, mg/dl	**TC, mg/dl**	**HDL-c, mg/dl**	**LDL-c, mg/dl**
C	96.81^{a}	88.27^{a}	32.52^{b}	36.39^{a}
AGR_1	78.10^{bc}	77.34^{b}	44.13^{a}	17.59^{b}
AGR_2	74.18^{b}	74.24^{b}	43.21^{a}	16.19^{b}
Zn	72.74^{b}	71.44^{b}	41.92^{a}	14.19^{b}
AGR_1+Zn	72.54^{b}	70.98^{b}	42.44^{a}	14.03^{b}
AGR_2+Zn	71.32^{b}	71.23^{b}	43.48	13.49^{b}
SEM	**2.29**	**1.49**	**2.89**	**1.74**
P-value	**<.0042**	**<.0022**	**<.0045**	**<.0035**

** a-d: Means with different superscripts within the same column differ significantly at ($P \leq 0.05$).
C: Control. AGR1: *Alpinia galanga* powder in diet as 50mg/kg BW, AGR2: *Alpinia galanga* powder in diet as 100 mg/kg BW, Zn: 200mg Zn/litter of drinking water, AGR1+Zn= *Alpinia galanga* powder in diet as 50mg/kg BW + 200mg Zn/litter of drinking water, AGR2+Zn= *Alpinia galanga* powder in diet as 100 mg/kg BW+200mg Zn/litter of drinking water, TG: Total glycerids, TC: Total cholesterol, HDL-c: High density lipoprotein, LDL-c: Low density lipoprotein.

TAC were significantly increased compared to the control group. These findings are consistent with those of Kaushik *et al.,* (2013), who discovered that the AGR extract reduced total cholesterol, TG and LDL while, increasing HDL. Furthermore, Abdel-Azeem and Basyony (2019) found that supplementing a diet with *Alpinia galangal* extract (AGRE) significantly reduced plasma total cholesterol, TG, LDL, and total lipids. Considerable, Negm and Ragheb (2019) found that supplementing AGRE led at drop in blood lipid profiles TG and TC, as well as a large increase in HDL-c. Our findings were agree with Kumar and Alagawadi's (2013) which revealed that galangin enhanced liver function and serum lipid profile. According to Janten *et al.,* (2005) who found that the rhizomes of *Alpinia officinarum* lowered serum TG and TC while increasing serum HDL levels in mice. Regarding to the role of zinc, the findings of this study agree with those of Boiko *et al.,* (2020), who discovered that supplementing drinking water of rabbits with nano-zinc citrate reduced cholesterol, TG, and lipid hydroperoxides. Interestingly, Xu *et al.,* (2015) who emphasized that in lipid disturbance rabbits, zinc protected the liver, decreased TG, and elevated HDL-C. In vivo, zinc inhibited the expression of matrix metalloprotease 2 (MMP2) and matrix metalloprotease 9 (MMP9).

4- ***Impact of AGR and Zn on antioxidant status:***

The effect of AGR and Zn on antioxidant capacity was summarized (Table 7) as significant increases in CAT, GSH, SOD, and TAC while diminishing MDA

Table 7. Effect of supplemented diet with AGR and Zn on antioxidant status in blood of Californian rabbit bucks

Treatment	Parameters				
	CAT, µmol/ml	**GSH, µmol/ml**	**SOD, (µ /dl)**	**TAC, µmol/ml**	**MDA, nmol/ml**
C	13.13^{c}	13.29^{c}	16.11^{b}	1.26^{c}	5.97^{a}
AGR_1	15.95^{b}	15.12^{b}	17.60^{b}	1.73^{ab}	4.68^{b}
AGR_2	16.94 ^{b}	17.76^{b}	17.62^{b}	1.95^{ab}	4.32^{b}
Zn	15.88^{b}	16.58^{b}	16.89^{b}	1.88^{a}	4.01^{b}
AGR_1+Zn	19.86 ^{a}	21.85^{a}	19.82a	1.95^{a}	3.37^{c}
AGR_2+Zn	21.41 ^{a}	22.12^{a}	20.12a	1.99^{a}	3.54^{c}
SEM	**0.86**	**0.92**	**0.89**	**0.54**	**0.74**
P-value	**<.0083**	**<.0047**	**<.0048**	**<.0023**	**<.0032**

** a-d: Means with different superscripts within the same column differ significantly at (P≤0.05).
C: Control. AGR1: Alpinia galanga powder in diet as 50mg/kg BW, AGR2: *Alpinia galanga* powder in diet as 100 mg/kg BW, Zn: 200mg Zn/litter of drinking water, AGR1+Zn= *Alpinia galanga* powder in diet as 50mg/kg BW + 200mg Zn/litter of drinking water, AGR2+Zn= *Alpinia galanga* powder in diet as 100 mg/kg BW+200mg Zn/litter of drinking water.CAT: Catalase, GSH :Reduced glutathione, SOD: Superoxide dismutase, TAC: Total antioxidant capacity .MDA: Malonylaldehyed.

in the AGR1+Zn and AGR2+Zn supplemented groups. While, the groups treated with AGR1, AGR2, and Zn showed no significant differences, the similar trend was observed for CAT, GSH, SOD, TAC, and MDA at all parameters measured, however, are improved was noticed in the experimental groups than in the control one. Previous study are agree in our results by Mahae and Chaiseri (2009) who recorded that the highest significant activity levels of SOD and GPx concentration detected in group supplemented with *galanga* may be due to the which considers a rich source of antioxidants that lead to the expulsion of toxins and inhibiting free radicals. Also, recent studies and agree with our result by Abdel-Azeem and Basyony (2019) who found that supplementing a diet with *Alpinia galanga exetract* (AGRE) significantly raised antioxidant capacity, glutathione S-transferase, superoxide dismutase, catalase, and glutathione peroxidase Likewise. Negm and Ragheb (2019) found that supplementing AGRE increased SOD levels while decreasing MDA levels. Also, Al-Mosawy and Khalid (2021) who documented that the highest significant activity level of SOD and GPx and reduced reversed the H_2O_2 impacts recorded in group consumed *Alpinia galanga* extract in quails.

Regarding, the role of zinc, the findings of this study agree with those Boiko *et al*., (2020) who revealed that increasing glutathione reductase and catalase activity. Also, Mazani *et al.,* (2012) found that Zn supplementation boosted overall antioxidant capacity, glutathione peroxidase, and SOD activity while decreasing MDA levels in the blood serum. On the other hand, Alissa *et al.,* (2009) illustrated that dietary zinc supplementation can modulate SOD activity in rabbits. In addition, Zhao *et al*., (2014) found that the concentration of SOD was significantly increased in serum of broilers after feeding nZnO. in the AGR1+Zn and AGR2+Zn supplemented groups. While, the groups treated with AGR1, AGR2, and Zn showed no significant differences, the similar trend was observed for CAT, GSH, SOD, TAC, and MDA at all parameters measured, however, are improved was noticed in the experimental groups than in the control one.

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Conclusively, it is concluded that rabbit's bucks blood parameters, immunity and oxidative status may be improved with alpinia galanga and zinc supplementation to rations of Californian rabbit bucks.

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تأثير إضافة الخولنجان والزنك على بعض مقاييس الدم وحالة الأكسده والمناعة فى ذكور أرانب الكاليفورنيا

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أستخدم فى هذه الدراسة عدد ستة وثلاثون ذكر من سلالة الكاليفورنيا عمر ٤-٥ أشهر بمتوسط وزن ٢٩٨٠ ± ٣٠.٣ جرام و تم توزيعها عشوائيا الى **ستة معاملات** وبكل معاملة

عدد ٦ ذكور وذلك بهدف دراسة تأثير استخدام الخولنجان المجففة (AGR) وكبريتات الزنك على مقاييس الدم وحالة الأكسدة والصفات المناعية لذكور أرانب الكاليفورنيا.

تم استخدام المجموعة الأولى كمجموعة مقارنة غير معاملة (C). المجموعتان الثانية والخامسة تم امدادهما بمعدل واحد كجم من AGR لكل طن علف (AGR1) بينما المجموعتان الثالثة والسادسة تم امدادهما بمعدل إثنان كجم من AGR لكل طن علف (AGR2). كما تم إضافة الزنك عن طريق مياه الشرب بمعدل ٢٠٠ملجرام زنك (Zn) لكل لتر ماء شرب وذلك خلال فترة الدراسة (شهران).للمجموعات الرابعة والخامسة والسادسة

أوضحت النتائج ان إضافة كلا من مسحوق الخولنجان والزنك معا او احداهما يؤدى الى زيادة معنوية فى كل من TP وAlb و AST و ALT مقارنة بالكنترول. كما لوحظ أن تركيز الجلوبيولين لم يتأثر معنويا ما بين المعاملات. أظهرت المعاملات الثادلثة (AGR2) والرابعة (Zn) والخامسة (ARG1+Zn) و السادسة (ARG2+Zn) تحسنا معنويا فى IgG. وسجلت المجموعات الرابعة (Zn) والخامسة (ARG1+Zn) والسادسة (ARG2+Zn) أعلى قيمة معنوية فى تركيزIgM . وقد لوحظ أن المجموعات التى غذيت على ARG بدون زنك سجلت اقل تركيز معنوي فى كل من TG والكوليستيرول و LDL مقارنة بالمجموعة المقارنة. كما وجد أن جميع المعاملات التجريبية أظهرت زيادة معنوية فى تركيز كل من CAT و GSH و SOD و TAC مقارنة بالمجموعة المقارنة.

التوصية: ادى استخدام مسحوق نبات الخولنجان الى تحسين الصفات الكيموحيوية للدم وحالة الاكسدة والمناعة لذكور ارانب الكاليفورنيا.