

## **Protective Effect of Propolis and Folic Acid on Rabbits Treated with H<sub>2</sub>O<sub>2</sub> and its Effect on the Adrenal Gland**

**Hind A.A.Al-Ahmed<sup>1</sup>, Zainb A.H.Al Mousawi<sup>2</sup>, Ahlam A. Al-rikabi<sup>3</sup>**

Department of physiology and Biochemistry, college of veterinary medicine  
Basrah, university of basrah , Iraq  
[h.alahmed57@gmail.com](mailto:h.alahmed57@gmail.com)

### **Abstract**

The study aims to identify the influences of folic acid and propolis administration in rabbits treated with H<sub>2</sub>O<sub>2</sub> and its effect on the adrenal gland. The adrenal gland has a component structure. It plays a role in a defensive role during stressful situations and facilitates reactions concerning fight or flight decisions. The adrenal cortex cells synthesize glucocorticoids (corticoids) and hormones that help to cope with stressful situations. The adrenal medulla cells secrete catecholamine class hormones, such as epinephrine and norepinephrine, that assist instantaneous responses. An experimental study design was done to determine the effect of propolis and folic acid on rabbits' protective effect and physiological from January 2019 to January 2020. Twenty local rabbits were ten male rabbits, and ten female rabbits were used in this experiment placed in metal cages under controlled conditions and environment. Twenty mature, healthy rabbits were split into two groups. Group 1 worked as a control, while group 2, administration folic acid (8.3 µg/kg B.W.) and Propolis-water-extract (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) orally for the twelve weeks. The mean age of rabbits is (7 M ± 2 M) and an original weight before the study (2.9 Kg ± 0.05 Kg). The current study shows that the supplement of rabbit propolis and folic acid has a useful impact on reproductive functions, hormonal parameters. The effect of the folic acid and propolis content of significant amounts of antioxidant such as including minerals, vitamins, enzymes and phenolic ingredients. Therefore the recommended that folic acid and bee propolis doses orally every day to the rabbits to enhance productive and reproductive function traits. But, additional experiments, studies are necessary with large sample with multiple various dosages.

### **Introduction**

The anatomy of the adrenal gland structure of the right Gland and left adrenal gland. The left part sits laterally to the cranial and aorta to the left renal vein. The right part is more cranial and located dorsolateral(1)(2). the adrenal gland it plays a defensive role during stressful situations and facilitates reactions concerning fight or flight

decisions(3). The adrenal cortex cells synthesize glucocorticoids (corticoids) and hormones that help to cope with stressful situations(4). Adrenal medulla cells secrete this hormone catecholamine class hormones, such as epinephrine and norepinephrine, that assist instantaneous responses(5)(6). In the 1960s, the adrenal corticoid response to EMF stimulation was first examined in the late (7)(4). The adrenal disease such as testosterone-generating adrenal tumors have been so-called in numerous kinds of animals however, there is not much information available for rabbits (8)(5). The Propolis is a honeybee produce with many chemical and organic composition (9) (10). Propolis affects an antioxidant effect decays have high substance of polyphenolic syntheses, with terpenoids flavonoids, tannins, and phenolic complexes with free of charge-radical activity. Various properties of the pharmacological and biological of Propolis have been observed, including immune-modulatory, anti-fungal, anti-inflammatory, anti-bacterial antioxidant, antiviral, and anti-carcinogenic properties and stimulation adrenal hormone (5)(11). Folic acid, which is also referred to as Vitamin B9, was identified as a member of co-factors implicated in Metabolism and the cellular processes of single carbon such as biosynthesizes of purine, thymidylate, and methionine (12)(13). Vitamin B9 or folic acid deficiency causes general health problems such as weakness, fatigue, and premature birth. Folic acid is an essential valve of utero spinal cord enhancement and neurogenesis hippocampal. High-level of folic acid and little folate levels can harm to health of the body and link to several diseases(14)(15).

The folic acid have antioxidant ability involved in its protecting effect. The effect of FA in enzymatic activities, serum biochemical, and lipid peroxidation, the rabbits have not been investigation yet (14)(10). The adrenal gland or the following species within the order Lagomorpha Rocky Mountain pika (*Ochotona princeps*), mountain hare (*Lepus townsendii*), snowshoe rabbit (*Lepus americanus*), desert jackrabbit (*Lepus arizonae*), cottontail rabbit (*Sylvilagus floridanus*), and pygmy rabbit (*Sylvilagus idahoensis*). (16)(17) In reviewing the literature on the adrenal gland, the writer has been unable to find a record or any histological investigations on members of the order Lagomorpha other than the domesticated rabbit(18)(19). In most of the glands examined, the medulla was surrounded by the cortical tissue, except in the hills region, where the sizeable medullary vein empties into the adrenal vein. The medulla is not precisely centrally located but is in the hole against

the surface of the gland. Propolis and folic acid supplements enhance Metabolism, promote energy, ease so-called adrenal fatigue, or provide "adrenal support." The adrenal gland makes hormones like Cortisol, regulating the Metabolism and managing stress, and aldosterone, which controls blood pressure (20) (21).

## Methods

The present study was done and planned at the Animal house. The experimental work was carried out on the rabbits at a private rabbit farm from January 2019 to January 2020. The study aims to identify the influences of folic acid and propolis administration on rabbits treated with H<sub>2</sub>O<sub>2</sub> and its effect on the adrenal gland. The local committee approved the experiments' design, and the protocol of the National Institutes of Health (NIH) conformed to the guidelines (22). The rabbits were separately stored in steel cages. H<sub>2</sub>O<sub>2</sub> was obtained as a 30% solution from Mallinckrodt, Inc. (St. Louis, Mo.). Methylmercuric hydroxide (97%) was purchased Ventron Alfa Products (Beverly, Mass.).

In specific experiments, a nearly constant concentration of H<sub>2</sub>O<sub>2</sub> (0.05 to 0.07 mm) was maintained in the culture media.. Twenty local rabbits were ten male rabbits, and ten female rabbits were used in this experiment placed in metal cages under controlled conditions, and environmental (twelve hours light/dark rotation) at with a humidity of (60±5) % and at (25±1°C) and maintained in separate cages with diameter (55 x 50 x 40) centimetre and left for two weeks for acclimatization before use. At the end of the culture time, the experimental tube was unstoppered briefly to sample for measurement of H<sub>2</sub>O<sub>2</sub> concentration and insert a vial of methyl benzethonium hydroxide to absorb CO<sub>2</sub>. Blanks were run both with and without H<sub>2</sub>O<sub>2</sub> in the medium. Experiments were conducted, which showed that nearly 100% of labelled CO<sub>2</sub> in the medium was subsequently recovered in the methyl benzethonium hydroxide. The spectrophotometric method described by Pirie measured the concentration of H<sub>2</sub>O<sub>2</sub> in the medium.

After the addition of 0.1 ml of sample (diluted if necessary), after 15 days of acclimatization, they were separated into two groups. The first one was administrated Normal saline and the second group, folic acid (8.3 µg/kg B.W.), and Propolis-water-extract (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) orally for the 12 weeks. The body weight of the rabbit was measured before starting treatment and feed intake. At the end of the twelve weeks, the animals

were anaesthetized by administering ketamine (5 mg/kg). The adrenal glands were removed from all rabbits. The volumes of the adrenal gland medulla and cortex were measured by using a serological counting technique. The provided Propolis by the Honeybee, the Propolis forty percentage was dry in five °C and storage in four °C in black wrapped bottles till administration. The temperature of the water was kept at 28°C. The rabbit was regularly checked for health for any mortality.

### **Blood collection**

The site for blood collection in a rabbit depends on the amount and number of samples needed and whether the rabbit will be anaesthetized for blood collection. The sample of blood collected of the rabbit from the marginal ear vein blood samples in the anaesthetized rabbit can be used for single or repeat. In the experiment, blood samples (10 ml) were collected from each (ten males and ten females) rabbit and serum isolation by centrifugation at 3000 gram for fifteen minutes. The centrifuged blood at 1400 ×g for fifteen minutes and used to measure serum testosterone levels. The rabbit lab tests found they all contained thyroid hormone, and most had at least one steroid hormone. After six treatments do at day fifteen. The samples, blood collected in heparinized and non-heparinized container tubes for serum and plasma.

### **Biochemical analysis**

All biochemical blood is (serum Albumin, Oxytocin, T4 (ug/dl), Cortisol, serum C'sterone, LH, T3, T4, serum estradiol, FSH, Prolactin, Progest, Testost, α1-globulin) was determined using commercial kits.

### **Statistical analysis**

All reported data were analysed for a t-test was used to analyse data using computer bundled software (SPSS) (Statistical Research Packages) (V19). The findings were described as Mean ± S.D. significant,

### **Results**

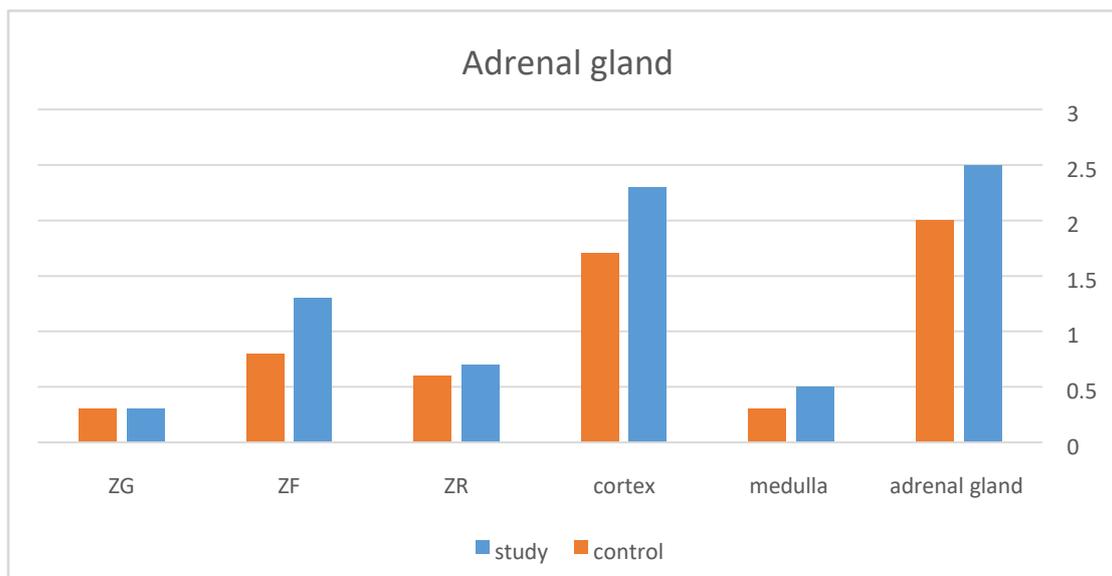
The presented study included Twenty mature, healthy rabbits were split into two groups. Group 1 worked as a control, while group 2, administration folic acid (8.3 µg/kg B.W.) and Propolis-water-extract (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) orally for the twelve weeks. The mean

age of rabbits is ( $7M \pm 2M$ ) and weight of the robot ( $2.9 \pm 0.05$ ) Kg. The serological results showed that the adrenal gland's mean volume is significantly in the study groups linked to the Control group. The body weight gain happened after 12 weeks in the study group more than in the control group. All animals were examined before they were included in this study. Many hormones can be measured in laboratory animals' serum, the more relevant hormones in laboratory animals, including adrenal hormone. The present study shows the propolis supplementation at 150 mg increased plasma total protein and albumin in the study group more than the control group, while The propolis plus folic acid decreased plasma  $T_3$  in the study group the other groups. The effect was the significantly greater  $T_3/T_4$  ratio compared to F.A. Pulse propolis at 150 mg. The Propolis plus F.A. At 150 mg increased the serum albumin and  $\alpha_1$ -globulin than the control group. Testosterone levels were (4.3) (pg/ml) 1.7 mmol/L. The adrenal glands were examined grossly with the aid of examining by microscope. The current results showed that the propolis and folic acid administration in healthy, non-treated rabbits induced a significant elevation in all adrenal gland hormones and activity compared to the control group (table 1).

Table 1: adrenal gland ultrasound measurement means and (length/ width) in study and control sample (rabbits)

Right adrenal gland	The length by the U.S	Width by the U.S
Control sample	0.71±0.1	0.34±0.11
Study samples	0.73±0.15	0.39±0.11
Left adrenal gland	The length by the U.S	Width by the U.S
Control sample	0.73±0.12	0.38±0.12
Study samples	0.76±0.11	0.41±0.13

Adrenal gland measurements in rabbits.



The adrenal gland of the rabbit's sample with a Concentration of GSH in rabbit lenses cultured for 3 hr in the medium in which the concentration of H<sub>2</sub>O<sub>2</sub> was maintained constant (0.05 to 0.07 mm. And component volumes (\*; p < 0.05).

Z.G. Volume was slightly higher in the study group, but the groups' alterations were not significant. Mean Z.F. Volume was significantly higher than in the Cont group, but there was no significant alteration between the count study groups. In the Z.R., the mean volume of cells exhibited a slight decrease in the control, but this difference was not statistically significant. The study group, treated with propolis and folic acid, exhibited significant medulla and cortex. The total volumes of Z.F., Z.R., and medulla decreased more in the control group than in the study group (fig1).

The list of endocrine diseases reported in companion animals is extensive. Endocrine diseases are prevalent in ferrets, with insulinomas (benign, insulin-producing tumours causing hypoglycaemia) and hyperadrenocorticism (caused by increased androgen and oestrogen production by adrenal gland tumours) being most prevalent.

Table 2 Baseline hormonal results before administration of the F.A. and Propolis.

Hormonal Results	Study group	Control group
Cortisol (µg/dl)	3±0.1	3.1±0.2
C'sterone (µg/dl)	1.3±0.1	1.3±0.1
T3 (ng/dl)	143±1	142±1

T4 (ug/dl)	2.3±0.1	2.3±0.1
LH (ng/ml)	21±1	22±1
FSH (ng/ml)	3.1±0.1	3±0.1
Estradiol (pg/ml)	2.6±0.1	2.7±0.1
Progest (Ng/MI)	0.9±0.1	0.8±0.1
Prolactin (ng/ml)	7.5±0.3	7±0.3
Oxytocin	14.5±0.5	15±0.4
Testost (pg/ml)	3.1±0.1	3.2±0.1
Total protein	5.4±1	5.5±0.9
	1.17 ± 0.06	1.18 ± 0.07
α <sub>1</sub> -globulin (g/dL)	2.5±0.5	2.6±0.4
Albuman g/dL	1.8±0.1	1.7±0.1
Glycogen	1.12 ± 0.02	1.13 ± 0.02

Table 3: Comparison between study and control groups after 12 weeks of administrated the F.A. and propolis.

Hormonal	Study group	Control group	p-value
Cortisol (µg/dl)	3±0.3	3.3±0.4	0.9
C'sterone (µg/dl)	1.4±0.1	1.52±0.2	0.8
T3 (ng/dl)	132±5	142±4	00.1
T4 (ug/dl)	2.1±0.1	2.3±0.2	0.9
LH (ng/ml)	35±6	22±7	0.01
FSH (ng/ml)	4.3±1	3±1	0.2
Estradiol (pg/ml)	2.9±0.9	2±0.7	0.1
Progest (ng/ml)	0.9±0.2	0.7±0.3	0.3
Prolactin (ng/ml)	9±2	7±3	0.3
Oxytocin	17.8±2.5	15±2	0.1
Testost (pg/ml)	4.9±1.2	3.2±1.5	0.001
Total protin	10.5±2	5.5±2	0.3

Free amino acids	69.37 ± 0.43	1.17 ± 0.06	0.01
α <sub>1</sub> -globulin (g/dL)	3.5±1	2.6±1	0.1
Albuman g/dL	2.7±1	1.7±0.8	0.01
Glycogen	41.00 ± 1.73	1.14 ± 0.02	0.001

## Discussion

Adrenal gland ultrasound measurement means and (length/ width) in study and control sample (rabbits) summarized in table 1; the rabbit was planned for an exploratory ultrasound to rule out an tumor in adrenal or recognize an additional possible extra source of testosterone. The present study has shown that exposure of lenses to H<sub>2</sub>O<sub>2</sub> results in an increased NADPH production via the HMS pathway. The suggests a role for the enzymes glutathione peroxidase and glutathione reductase in the detoxification of H<sub>2</sub>O<sub>2</sub> in the lens through the following sequence of reactions, where GSSG is oxidized glutathione, as originally suggested by Pirie. Such a mechanism is consistent with many previous findings that lens epithelium contains unusually high concentrations of GSH and phosphorylated pyridine nucleotides and significant activity of G6PD and glutathione peroxidase glutathione reductase (23). There are hormones of the adrenal that can be measured in the serum of laboratory animals, the relevant hormones in laboratory animals, including Cortisol, a glucocorticoid produced by the adrenal cortex's zona fasciculata. It is involved in the stress reaction. Cortisol acts to increase blood pressure and blood glucose and has an immunosuppressive action. (24). The levels of Cortisol are varied depend on environmental influences and diurnal rhythm. Corticosterone is generated by the adrenal cortex and the glucocorticoid in some species, such as rabbits. Cortisol has functions similar to corticosterone (25). The effect on blood parameters was summarized in Table 2. TP, involving of globulin and albumin, is an essential parameter in any animal. Numerous factors such as reproductive status, age of the animal and pregnancy) can affect Total protein levels. The information presented that therapy with high doses of propolis and FA caused significant increases in plasma total proteins at ( $P \leq 0.05$ ) α<sub>1</sub>-globulin, Albuman values compared to the control group (26). Adrenal free amino acids were significantly elevated at ( $P < 0.001$ ). This result is similar to this study Farag S., et al (26). The significant variations were presented in albumin of rabbits treated with propolis and folic acid more than the control group. The Testosterone levels were (4.3) (pg/ml). This study is similar to the study done by.(26). This study shows that pro plus folic acid shows a significant reduction in

the Cortisol ( $\mu\text{g}/\text{dl}$ ) C'sterone ( $\mu\text{g}/\text{dl}$ ). The Cortisol is a glucocorticoid produced by the adrenal cortex's zona fasciculata. This result is in agreement with this study done by Habeeb AA, et al.(27). The mixture of propolis and folic acid had an effect on reduction of the thyroid hormone (T3, T4) in study group than the control group.

## CONCLUSION

The current study shows that supplement of rabbits with propolis and folic acid have a useful impact on reproductive and productive functions, blood hormonal parameters. The impacts of propolis and folic acid substance have antioxidant nutrients effect, with minerals, vitamins, enzymes phenolic and constituents.

## Recommendation

The presented study recommended average doses of propolis (150 mg/kg B.W) and FA (8.3  $\mu\text{g}/\text{kg}$  B.W) by administered orally everyday to the rabbit may improve reproductive and productive and improve the adrenal hormonal and endocrine function. Still, extra experiments studies are required with more study sample.

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