GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

EVALUATION OF EFFECTIVENESS OF ETHANOLIC EXTRACT OF MARIGOLD (*CALENDULA OFFICINALIS*) ON BLOOD VALUES IN QUAIL (*COTURNIXCOTURNIX JAPONICA*)

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ABSTRACT
The aim of this study is to examine the effectiveness of ethanolic extract of *Calendula officinalis* on blood values in Quail, 24 birds were used and divided randomly to four equal groups, the first group (A) was a control group that treated with distal water and the other three groups (B), (C) and (D) were treated with plant extract of 50,150 and 300 mg / kg body weight. The results showed increasing in blood values in all treated groups as compares with the control group. Red blood cells (RBC) and white blood cells (WBC) count increased to (5650 ± 129.09) and (9200 ± 151.65) in group (D) respectively while Hb and PCV were (18.13 ± 0.23) and (54 ± 0.73) respectively.

KEYWORDS: *Calendula officinalis*, ethanolic extract, blood, *Coturnixcoturnix japonica etc.*

INTRODUCTION

The plant *Calendula officinalis* belongs to compositae family, it is named in English (Marigold) which is a seasonal plant that spread in the Mediterranean region and Central and Eastern Europe, the plant grows up to about 30 - 60 cm and has a green medium size leaves and stellar shape flowers with colors vary from yellow to orange and Golden[1, 2].

Calendula officinalis has an analgesic effect, anti-ulcer and accelerates wound healing [6]. In British Columbia in Canada the plant used for the treatment of medical conditions in ruminants such as wounds and scratches of the skin, diarrhea, and dyspepsia[7]. It is also used in the same area for the treatment of ear problems in dogs and cats[8]. Several studies have shown that the plant has atumor resistant effect and stimulate lymph cell in vitro [9].The study is aimed to evaluate the effectiveness of alcoholic extract of *Calendula officinalis* on blood values especially that some references indicate the lack of such a study[10].

MATERIALS AND METHODS

Preparation of plant extraction

Aerial parts of the plant where collected from public parks. The plant was diagnosed by Iraqi National Herbs Center, the plant was dried at room temperature and then grinding using electric mill, then extracted in a soxhlet using ethanol 99%, followed by evaporation of the solvent using a rotary evaporator, the extraction was dried using an electric dryer then milled and placed in glass vials and stored until use.

Experimental design

Twenty four female quails (*Coturnix coturnix japonica*) were used in this study, the birds obtained from the Institute of Agricultural Research. Their weights ranged between (220-260 g)and aged 60 days at the start of the experiment, the birds had been left for two weeks for adaption then they were divided randomly into four equal groups: The group (A) was a control group which treated with distilled water only, other groups (B), (C) and (D) were treated with 50, 150 and 300mg/kg of body weight from plant extraction respectively, the treatment continued for two weeks. After the end of the treatment period the birds were slaughtered and 3 ml of blood was collected from each bird in tests tubes containing anticoagulant for blood tests.

Laboratory tests

RBCs count; total WBCs count, differential WBCs count, Hb, PCV, MCV, MCH and MCHC tests were done to evaluate the effect of the plant extraction [11.12].

Statistical analysis

The data was analyzed using the analysis of variance (ANOVA). The significant of differences between means were compared by using with Least Significant Differences (LSD). The analysis of data was conducted by SPSS [13].

RESULTS & DISCUSSION

Results of present study revealed that the differences between RBCs count in the control group (5141.66 ± 100.34) and group D (5650 ± 129.09) was significant ($P < 0.05$) (Table 1), as well as Hb (18.3 ± 0.23) in group D and (11.35±0.40) control group.

PCV was increased in the group D (54 ± 0.73) as compared to control group (41.66 ± 0.66) as well as in groups (B) and (C). Depending on the increasing in the RBCs count, Hb and PCV were increased in the (MCV), (MCH) and (MCHC) to (95.63 ± 0.98), (32.11 ± 0.62) and (33.58 ± 0.49) respectively, in the group (D) as compared with control group which was (81.05 ± 0.59) and (22.08 ± 0.94) and (27.26 ± 1.23), respectively (Table1).

TABLE 1: Effect of plant extract on the some blood profile

Test	Control group	Group B	Group C	Group D
RBC count($x10^3/mm^3$)	5141.66±100.34c	5300.0±129.74b	5291.66±83.08 b	5650.00±129.09 a
Hb(g/dl)	11.35±0.40d	12.41±0.36c	15.08±0.31 b	18.13±0.23 a
PCV (%)	41.66±0.66c	44.33±1.20c	49.33±0.98 b	54.00±0.73 a
MCV (Fl)	81.05±0.59b	83.96±3.70b	93.26±1.69 b	95.63±0.98 a
MCH (Pg)	22.08±0.94c	23.40±0.49c	28.51±0.88 b	32.11±0.62 a
MCHC (g/dl)	27.26±1.23c	28.26±1.28bc	30.58±0.62 b	33.58±0.49 a

Small letters refer to significant differences between groups ($P \leq 0.05$)

The statistical analysis showed significant differences ($P \leq 0.05$) between the treated groups and control group for all blood profile criteria.

Although there is no study shows the effects of *Calendula officinalis* on blood values in quail [10], but the effect of the plant in this study could be due to antioxidants materials that is available in the plant such as flavonoids and cartenoids[14]. These materials are working to reduce the harmful effects on the cells and deal with physiological and pathological conditions due to cell respiration which lead to free radicals production [15]. The antioxidant effectiveness of *Calendula officinalis* was confirmed by [16]. The results of this study was disagree with that found by[17] who reported that blood values in mice were not affected by extraction of the plant and this may be due to physiological and functional differences between rats and birds.

The results show increasing in total and deferential WBCs count in group (D) with increasing of plant extract concentrations. The total WBCs count was 9200 in group (D) while it was 5120.83 in control group. Similar trend was showed in number of Monocyte (10 ± 0.57), Lymphocyte (38.66 ± 1.78), Neutrophil (54.33 ± 0.80), Eosinophil (2±0.36) and Basophil (1±0.36). The corresponding means of control group were (7.66 ± 1.17), (35.50 ± 0.84), (46.33 ± 1.05), (3.50 ± 0.42) and (0.66 ± 0.33) respectively. Results also showed significant differences between treated group and control group ($P \leq 0.05$) (Table 2).

TABLE 2: The effect of plant extract on the immune cells

Test	Control group	GroupB	Group C	Group D
WBC count	5120.83±91.83 d	5900.00±129.09 c	7900.00±73.02 b	9200.00±151.65 a
Monocyte (%)	7.66±1.17 b	8.00±1.06 a	8.83±0.47 a	10.0±0.57 a
Lymphocyte(%)	35.50±0.84 b	35.66±0.84 a	38.66±0.49 a	38.66±1.78 a
Neutrophil (%)	46.33±1.05 b	45.66±1.17 b	51.00±1.52 a	54.33±0.8 a
Eosinophil (%)	3.50±0.42 c	3.66±0.33 c	3.80±0.49ab	3.91±0.36 a
Basophil (%)	0.66±0.33 b	0.66±0.42 b	0.83±0.40 a	1.00±0.36 a

Small letters refer to significant differences between groups ($P \leq 0.05$)

The effectiveness of the plant to increase the number of total and differential WBCs count could be attributed to cartenoids which is available in the plant, this material enhance immunity [18], and increase the ability to resist cancer[19]. The results of this study are agreed with other study that showed the increasing in immunity of chicken treated with *Calendula officinalis* extract (20). Also the plant polysaccharide is working as a catalyst for immunity, particularly granular immune cells that led to increase phagocytosis in a ratio of (54-100%) (21) and this may be the reason for increasing the total and deferential WBCs count in this study.

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