

COMPARISON THE EFFECIANCY OF LEVAMISOLE AND DRY *Punica granatum* PEEL AGAINST *Ascaridia galli* IN LAYER HENS THAT NATURALLY INFESTED

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ABSTRACT:

This study was conducted to evaluate the efficiency of Levamisole drug and *Punica granatum* dry peel against *Ascaridia galli* that naturally infested Issa brown layer hens type in multi concentrations in order to have best results, at 30 , 31 and 32 week of age, after taken 70 naturally infested hens then divided into 7 groups each one have 10 replicates . In this study Levamisole hydrochloride (Uvemisole)® was orally administered at doses (10, 20, 30 mg / kg), also *Punica granatum* dry peel orally administered to another group at doses (0.5, 1, 1.5 gm / kg), both were given twice per day for five days, the parameters (Body weight , number of parasite egg per gram, PCV, Total Serum Proteins) were evaluated in first day of each weeks .The results showed Levamisole and *Punica granatum* dry peel concentrations (20, 30 mg / kg) and (1.5 gm / kg)respectively gave significantly ($p<0.05$) improving in body weight gain , decrease egg per gram and increasing PCV and total Serum protein in weeks 31 and 32 , following the concentration (10 mg / kg) Levamisole and (1 gm / kg)*Punica granatum* which gave same result but lesser than above concentrations, while the concentration (0.5 gm / kg) *Punica granatum* didn't reveal any significant changes compared with control groups (non-treated) .The highest efficacy percentage appeared in Levamisole (30 mg / kg) group in each weeks, while lowest percentage revealed at *Punica granatum* (0.5 gm / kg) group per weeks. The result improved that the Levamisole in concentrations (20, 30 mg / kg) and *Punica granatum* dry peel (1.5 gm / kg) were significantly ($p<0.05$) improved studied standers for naturally infested layer hens with *Ascaridia galli* in weeks 31 and 32 of age after twice orally administration per day for continuous five days per week .

مقارنة كفاءة الليفاميزول و قشور الرمان الجافة ضد الاسكاريديا جالي المخمجة بصورة طبيعية للدجاج
البياض

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الخلاصة:

تم إجراء هذه الدراسة لمقارنة كفاءة عقار الليفاميزول و قشور ثمار الرمان الجافة ضد ديدان الاسكاريديا جالي المخمجة بصورة طبيعية في الدجاج البياض نوع أيزا البني بعمر (30، 31 و 32 أسبوع) بعد ان تم اخذ 70 طير مصاب وقسمت الطيور إلى سبعة مجاميع يواقع 10 مكررات لكل مجموعة، في الدراسة تم إعطاء الليفاميزول

هيدروكلورايد (يوفيميزول)[®] عن طرق الفم بعدة جرعات (10, 20 و 30 ملغم/كغم) ، كما تم إعطاء مجموعة أخرى من الطيور مسحوق قشور الرمان الجافة بجرعات (1, 0.5 و 1.5 غم/كغم من وزن الجسم) ، كل الطيور المعاملة تم إعطائها الجرعة فمويًا مرتين يوميًا ولمدة خمسة أيام متتالية ، وتم حساب المقاييس التالية في اليوم الأول لكل أسبوع من التجربة (وزن الجسم ، عدد بيض الطفيلي في الغرام الواحد من الزرق ، نسبة الخلايا الدمية المتراصة و البروتين المصلي الكلي) . بينت النتائج إن عقار الليفاميزول و قشور ثمار الرمان بتركيز (20 و 30 ملغم/كغم) و (1.5 غم/كغم) على التوالي أعطت أفضل نتائج و بصورة معنوية ($p < 0.05$) في تحسين الزيادة بوزن الجسم ، خفض عدد بيوض الطفيلي في الغرام الواحد من الزرق ، زيادة نسبة الخلايا الدمية المرصوصة و البروتين المصلي للأسابيع 31 و 32 من العمر ، وبعدها جاءت التراكمات 10 ملغم/كغم للليفاميزول و 1 غم/كغم لقشور الرمان الجافة و التي أعطت نفس النتائج لكن أقل بصورة معنوية عن التراكمات السابقة . بينما التركيز 0.5 غم/كغم لقشور الرمان الجافة لم يظهر أي تغيرات معنوية مقارنة مع مجموعة السيطرة (غير المعاملة) . أظهرت مجموعة الليفاميزول (30 ملغم/كغم) أعلى نسبة كفاءة مقارنة بالمجاميع الأخرى للأسابيع 31 و 32 من عمر الطيور ، في حين أظهرت مجموعة قشور الرمان الجافة (0.5 غم/كغم) أقل نسبة كفاءة مقارنة ببقية المجاميع ولكلا الأسابيع . أثبتت النتائج إن إعطاء الليفاميزول بتركيز (20 و 30 ملغم/كغم) و قشور الرمان الجافة بتركيز (1.5 غم/كغم) قد حسن وبصورة معنوية ($p < 0.05$) المعايير المدروسة للدجاج البياض المخمخ طبيعيًا بديدان الاسكاريديا جالي بعمر 31 و 32 أسبوع بعد الإعطاء مرتين في اليوم ولمدة خمسة أيام بالأسبوع .

INTRODUCTION :

Poultry rearing affecting by different and various conditions like environment, management, type of nutrition etc. The diseases considered one of the most important factors in poultry rearing ,one of that diseases was internal parasites especially nematodes which showed great challenge to poultry industry for many years although using antinematodes and disinfectant but it shows sever threatening to that industry by causing production losses in the range of 10 – 20 % due to impaired feed conversion , reduced growth , egg production and increase mortality (Magwisha et al.,2002). Ascariasis considered one of the most important disease caused by poultry nematodes in which infest different types of birds , the infestation mainly occur in adult or layer poultry, in our country considered one of the most important nematodes that infest chickens leading to economic losses (الخالدي،1996) ,which can be compare to losses occurred by coccidia in weight losses , decrease meat and egg production and mortality of birds (Al-Taif,1972). The highest rate of infestation mainly occurred in May (60%) and in summer more than rainy winter (Hanan et al.,2005), also the infestation commonly observed in layers conventionally in door as well as in free range and organic system also the incidence was high in deep litter and cage system (Permin et al.,1999). The life cycle is simple and direct ,infective eggs containing the L2 larvae, hatch in either the proventriculus or the duodenum of the susceptible bird , the prepatent periods is about five to six weeks (Ruff et al.,1997). In order to control that helminthes had been used chemical drugs as antihelminthic, past years used Tetrachloroethylene compounds and carbontetrachloride , those drugs were dangerous if it use in overdoses or not affected if it use in low and insufficient doses, also it give poor results. Then used several drugs instead of above component like Piprazine adipate, Tetramizole ,Fenbendazole and Levamisole (Maqbool et al.,1998; Yazwinski et al.,2009). Levamisole considered as one of broad spectrum

antinematodes that had been used to treated nematodes that infested the intestinal tract of farm animals and poultry (Aiello,1998). Also used as immunomodulator when making a viral vaccination to poultry (Dalvi,1990). The modern studies focus on using natural substances founding in fruits to treat the ascariidiosis and compare their anthelmintic efficacy with anthelmintic drugs, like using the roots of *Thespesia lampas* to treated infestation of *Ascaridia galli* and *Raillietina spiralis* (Satish et al.,2009). Also using Pineapple powder and Neem leaves then comparative their anthelmintic efficacy with *Piprazine hydrate* (Gautam et al.,2010) Using oven dried ground latex of *Paw paw* (Adu et al.,2009) The studies also compare the efficacy of leaves extract to Neem and Bishkatali with Piprazine and Levamisole against *A. galli* (Begum et al.,2010) also benefit from citrus peels extract (Anas et al.,2012) and using Pumpkin oil to compare them with classical anthelmintic like Parazitol, Levamisole and Albendazole (Llie et al.,2007) *Punica granatum* had been used as antibacterial and antifungal in many researches (اشواق، و اخرون 2009; Al-Zorecky,2009; Chung et al.,1998; Said et al.,2009; Panichayupa et al.,2010; Pranay et al.,2011; Ahmet et al.,2009; Lasur et al.,2012; Laurylene et al.,2003), also used to inhibit the activity of *Entamoeba histolytica* parasite (Ilham et al.,2008), beside it's antioxidant activity and good potency for cancer prevention (Afaq et al.,2003), as well as cold water *Punica granatum* peel extract of treated *Echinococcus granulosus* in albino mice (زينب، 2006), there was important role in treatment the calves infested with nematodes (Pradhan et al.,1992). In experiment conducted on back yard chickens, water suspension of dried pomegranate fruit rind powder shows good results in *vivo* and in *vitro* against *A. galli* (Areej et al.,2007). As known the pomegranate tree can be divided in to several anatomical compartments like seed, peel, juice, flower, leaf, bark and roots each of which has interesting pharmacologic activities (Chaturvedula et al.,2011), the other potential therapeutic properties of pomegranate are wide range including treatment and prevent cardiovascular disease, diabetes, dental condition, erectile dysfunction, protection from ultraviolet radiation, infant brain ischemia and obesity (Nagaraju et al.,1990; Mohammed et al.,2010). As many studies had been occurred for the evaluation of anthelmintic activity of various plant materials by observing several parameters included expulsion of worms from their hosts or reduction in the number of egg per gram of feces (EPG) passed by the infected compared with commercial anthelmintic treated poultry.

MATERIALS AND METHODS :

The study was conducted in Al-Suwayrah 2nd for poultry production (Shabaan Abbas for commercial egg production) about 25 km North Al-Suwayrah city, at production farm number 4, in May 2012, by using 70 Issa brown hens, divided into 7 groups each one have 10 replicates at 30 weeks of age, all them had been infested naturally with *Ascaridia galli*, the hens were putting in battery brooders with marked 2 cages for each treatment (5 hen in each cage). The groups were divided in to :-

A- Control group :- (which not treated)

B- Levamisole groups :-Which subdivided depending on drug concentrations to:-

- 1- Levamisole 10 mg/kg body weight
- 2- Levamisole 20 mg/kg body weight
- 3- Levamisole 30 mg/kg body weight

The drug was given orally , twice per day for five days .

C- Dry *Punica granatum* peel groups(P.g.) :- Which subdivided depending on the concentration in to :-

- 1- P.g. 0.5 gm/kg body weight
- 2- P.g. 1 gm/kg body weight
- 3- P.g. 1.5 gm/kg body weight

Water suspension of dried pomegranate fruit-rind powder was administrated orally , twice per day for five days . All hens were provided feed and water as recommended in management guide . In the study had been used Uvemisole® (1 liter) which contain Levamisole hydrochloride , equivalent to Levamisole 100 mg (each 1 ml. of Uvemisole® ,contain 118 mg. of active ingredient) , the drug produced by UVIDECO / Jordan .

The dose rate for individual bird was calculated on body weight as per manufacturer recommendation , oral administrated by using syringe and directly to crop .

For preparation dust of pomegranate fruit-rind , the fruit- rind were dried in the shade at room temperature then dried rind cut into small pieces and pulverized with a blender , then sieved and preserved in air light plastic container . All tests had been conducted at the first day of each weeks .

TESTS

Body weight :- Body weight taken at the first day from each week of age (30,31 and 32) of experiment for each individual bird .(Body weight in infested hens less than the recommended in the management guide)

Egg counting (Egg per gram of feces) (EPG) :-

The fecal samples were examined through Mc Master egg counting technique (Soulsby,1982). Further fecal samples of birds in all groups were collected on the first day from each weeks of experiment , two grams of feces placed in small screw capped plastic bottle contain glass beads , 60 ml of saturated sodium chloride solution was added in the bottle . The contents were shaken gently to break up the feces , then the mixture was allowed to stand for 15– 20 minutes. The supernatant was withdrawn using Pasture pipette to fill one chamber of MC Master egg counting slide , after focusing a corner , the egg were counting by moving the chamber up and down . The counting was repeated for the second chamber , the total number of eggs counted two chambers was multiplied by 50 to get the number of eggs per gram of feces . as the formula below (Soulsby,1982) :-

$$E.P.G = N \times 50$$

N = number of eggs counted in to two chambers .

Packed Cell Volume (PCV)% :-

Place the open end of capillary tube in blood oozing from wing vein (Brachial vein punctured with sterile needle) allow blood to move in to the tube via capillary action until 2/3 of the tube is filled , then seal one end of the tube with plastic clay , allow tubes to spin in microhaematocrit centrifuge for 3 – 5 min .Immediately after centrifuging read PCV% from scale of microhaematocrit reader (Compbell,1988) .

Total Serum Protein (T.S.P):-

For biochemical test, blood was collected from individual bird from each group from wing vein , two milliliters of blood in sterile test tube without anticoagulant then allowed to clot .Serum was separated out and kept at -20C° until analysis , total protein (g/dl) was estimated by Biruet method as described by (Bush,1975) using Randox diagnostic kit.

The Efficacy :-

The efficacy was measured on the percentage of egg reduction for treated birds relative to not treated control group , as following below formula (Froylan et al.,2011) :

$$\text{Efficacy} = \frac{\text{Number EPG in untreated group} - \text{Number EPG in treated group}}{\text{Number EPG in untreated group}} \times 100$$

Statistical analysis :

All results had been analyzed statistically according to completely randomized design , then determine less significant difference (LSD) in level of (0.05) in order to compare between the means(الراوي،1980) .

RESULTS :

In the present study the result shows there was no significant difference at the week 30 of age as show in (Table1) in all characteristics, while in the weeks 31 and 32 as appear in (Tables 2 and 3), there was a significant differences (P< 0.05) appears between the parameters in each group .

In body weight the best increase significantly(P< 0.05) appear in Levamisole group (20 and 30 mg/ kg) and *Punica granatum* (1.5gm / kg) as compare with another groups , then the increase significantly occur also in Levamisole group (10 mg/kg) and *Punica granatum* group (1gm / kg) compared with control group .

In EPG there was significant decrease (P<0.05) clearly at the groups of Levamisole (20 and 30 mg/kg) and *Punica granatum* (1.5 gm / kg) among other groups , also decrease significantly (P< 0.05) in groups of Levamisole (10 mg / kg) and *Punica granatum* (1gm / kg) compared with control and *Punica granatum* (0.5 gm / kg).

The results revealed there were significant (P < 0.05) increasing in P.C.V percentage in treated groups with Levamisole (20 and 30 mg/ kg) and *Punica granatum* (1.5gm/kg) comparing other groups , there was elevation PCV % in groups , Levamisole (10 mg/kg) and *Punica granatum* (1gm/ kg) significant more than control and *Punica granatum* (0.5gm / kg).

There was significant ($P < 0.05$) elevation in TSP appear in groups Levamisole (20 and 30 mg / kg) and *Punica granatum* (1.5g / kg) more other groups , also there was significant ($P < 0.05$) TSP elevation in groups of Levamisole (10 mg / kg) and *Punica granatum* (1gm / kg) more than control and Punica (0.5gm /kg) . which they didn't show any significant changes in all treatment as revealed in Tables 2 and 3.

In Table 4 can see the percentage of drug efficacy in both weeks 31 and 32 , recognize in Levamisole group (30 mg/kg), then in (20 mg/kg) , then *Punica granatum* group with (1.5 gm./kg) was the higher efficacy while in (0.5 gm/kg) shows the lowest percentage of efficacy.

Table (1) :Comparison between Levamisole and Punica granatum on some characteristics of Issa Brown layer hens at 30weeks of age .

no	Para meter	Control	Levamisole (mg/kg)			<i>Punica granatum</i> (gm/kg)			LS D
			10	20	30	0.5	1	1.5	
1	BWT	1823.125	1790.375	1832.500	1844.125	1799.375	1802.000	1798.750	N.S
2	EPG	900. 750	893. 125	909. 875	893. 750	898. 000	897. 500	888. 500	N.S
3	PCV	24. 625	25. 100	23. 375	23. 000	24. 000	22. 750	22. 375	N.S
4	TSP	2.630	2.554	2.571	2.588	2.593	2.578	2.585	N.S

N.S: No Significant different between means, BWT: Body Weight (grams), EPG: Egg Per Gram of feces , PCV: Packed Cell Volume (%) , TSP: Total Serum Protein (g/100ml), LSD: Less Significant Difference

Table (2) : Comparison between Levamisole and Punica granatum on some characteristics of Issa Brown layer hens at 31weeks of age

no	Para meter	Control	Levamisole (mg/kg)			<i>Punica granatum</i> (gm/kg)			LSD
			10	20	30	0.5	1	1.5	
1	BWT	1828.750 c	1880.250 b	1954.0 00 a	1973.0 00 a	1832.75 0 c	1890.500 b	1978.870 a	59.3086
2	EPG	976.675 c	491.875 b	134.37 5 a	109.12 5 a	860.500 c	414.500 b	264.125 a	120.884
3	PCV	23.337 c	26.725 b	31.200 a	30.500 a	23.400 c	26.555 b	29.883 a	3.1136
4	TSP	2.634 c	3.164 b	3.435 a	3.599 a	2.685 c	3.178 b	3.466 a	0.2117

BWT: Body Weight (grams), EPG: Egg Per Gram of feces , PCV: Packed Cell Volume (%) , TSP: Total Serum Protein (g/100ml), LSD: Less Significant Difference

Table (3) : Comparison between Levamisole and Punica granatum on some characteristics of Issa Brown layer hens at 32weeks of age

no	Parameter	Contr ol	Levamisole (mg/kg)			<i>Punica granatum</i> (gm/kg)			LSD
			10	20	30	0.5	1	1.5	
1	BWT	1815. 000 c	1958.0 00 b	2012. 750 a	2020. 875 a	1827. 000 c	1947.7 50 b	2015. 000 a	53.983
2	EPG	960.5 00 c	413.62 5 b	67.50 0 a	47.12 50 a	837.2 50 c	326.25 0 b	88.37 5 a	92.467
3	PCV	23.87 5 c	29.875 b	33.50 a	34.62 5 a	24.50 c	28.750 b	34.87 a	3.330
4	TSP	2.659 c	3.295 b	3.560 a	3.689 a	2.771 c	3.333 b	3.619 a	0.1392

BWT: Body Weight (grams), EPG: Egg Per Gram of feces , PCV: Packed Cell Volume (%) , TSP: Total Serum Protein (g/100ml), LSD: Less Significant Difference

Table (4): Comparison between percentage of efficacy to Levamisole and *Punica granatum* in 31 and 32 weeks of age

weeks	Levamisole (mg/kg)			<i>Punica granatum</i> (gm/kg)		
	10	20	30	0.5	1	1.5
31	49.64%	86.24%	88.83%	11.89%	57.57%	72.96%
32	56.94%	92.97%	95.09%	12.83%	66.03%	90.37%

Discussion:-

The results showed changes occurred in parameters due to using multi concentrations and that changes are directly proportional to increase the dose of administration which mean it dose dependent. The results obtained from the experiment revealed there was no significant differences ($P < 0.05$) in week 30 of age between all groups, that due to experimental design in which the parameters measured in the first day of each week, so can consider that measures as a control reading to indicate the changes in treatment groups to followed weeks.

Body weight :-

Levamisole produced enhanced body weight gain in doses (20 and 30mg/kg) in all weeks, and these result similar to the results of many studies(Begum et al.,2010;Porchezian et al.,2006;Phiri et al.,2007), as well as best improve in body weight gain occurred in *Punica granatum* group with (1.5 gm/kg) compare with other groups, that elevation in body weight due to enhance body parameters occurring after significant

($P < 0.05$) decrease in EPG which refers to great decrease in *A. galli* numbers that infested birds which cause reduction in food intake , injury to intestinal wall and hemorrhage resulting in poor weight gain of host as (Samad et al.,1986) shows . Chickens infested with *A. galli* have lost body weight significantly due to lack of absorption of nutrient, electrolytes and vitamins as result of intestinal obstruction , and that agree with (Haseeb et al.,2002) that found significant decreased in body weight, concentration of vitamin A and carotenoids of serum liver after infestation with *A. galli* , and that obvious in control group which has less body weight than other groups.

Egg per gram of feces :-

Results showed significant ($P < 0.05$) decreased in EPG that greatly in Levamisole groups (20 and 30mg/kg), this agree with results of (Yazwinski et al.,2009; Aiello,1998; Dalvi,1990; Begum et al.,2012) which showed significant decrease in EPG , due to decreased in numbers of infested worms in alimentary tract , the decrease occur due to anthelmintic activity of Levamisole in which agonist nicotinic receptors and elicit spastic muscle paralysis due to prolonged activation of the excitatory nicotinic acetylcholine receptors on nematodes body wall muscles (Akhtar et al.,1985) .Significant decrease in *Punica granatum* can be observed in (1.5 gm/kg) among other granatum groups and control , this agree with (Areej et al.,2007) who proved in *in vitro* anthelmintic activity against *A. galli* adult worms which was concentration dependant began with paralysis then death of worms . Also the results agreed with (Ozcal et al.,1993) . That results may be due to alkaloids group like pelleterine or isopelleterine (Reed,1995). In study was found certain concentration of aques and alcoholic *Punica granatum* extracts , the parasite completely inhibited by causing clear reduction in RNA and DNA of the parasite by inhibition some important enzymes like polymerase , or may inhibit nucleoprotein from their precursors like riboneucleotide and deoxyribose (Ilham et al.,2008) , also *Punica granatum* contain tannins and fenol compounds which have mechanism of action leading to harmful effect on nematodes by binding with some proteins inside the body and prevent their lyses , so affect on nitrogen and amino acid processing that important to nematode life (Reed,1995).

PCV :

Results showed there were increase PCV% in treated groups comparing to control one which shows significant ($P < 0.05$) decrease in PCV% , that agree with (Deka et al.,2008) and (Hoque et al.,2006), they reported in their researches significant decrease PCV% in infested group with *A. galli* , otherwise the treated groups with Levamisole showed significant increase in PCV% , that may be due to consideration of nematodes infestation one of stressors that associated with free radicals formation which are known to play a role in tissue damages and have adverse effects on erythrocytes , also known that lipid peroxidation increases in plasma and tissues lead to damage to cell membranes (Avellini et al.,1995) , while (Adang et al.,2010) reported there was blood-tinged diarrhea combined with *A. galli* infestation, also there was a necrosis of intestinal villi , glands and muscularis mucosa , all that lead to decrease numbers of red blood cells first directly by

haemorrhage(Haseeb et al.,2002) or indirectly by decrease absorption the essential elements that forming RBCS .The results showed best significant ($P < 0.05$) increase in PCV% occur after treated with Levamisole(20 and30 mg/kg) or with *Punica granatum*(1.5gm /kg), that due to decrease in infested worms, appearing from significant decrease in EPG .

TSP :

Total serum protein showed significant ($P < 0.05$) decrease in control group, this finding was in agreement with the findings of (Soulsby,1982; Deka et al.,2008). Hypoproteinemia might occur due to increased motility of intestine as in diarrhea , in that case the protein get lost from the bowel (Tanwar et al.,2001) , also a considerable loss of tissue protein may occur through leakage in to gut with loss of digestive secretion and mucous due to intestinal parasitism in anemic birds, which also cause inefficient protein absorption and utilization in the system to extent of leading to marked decrease in serum protein (Coles,1986).

The best significant ($P < 0.05$) increase occurred in Levamisole groups (20 and 30 mg / kg) and *Punica granatum*(1.5 g / kg) , that occurred due to direct decreasing in infested worms in birds They were affecting on absorption of amino acids and essential nutrients for protein syntheses (Tanwar et al.,2001) and due to effect of Levamisole and *Punica granatum* by increase the immune response inside infested birds , as a researches showed that *A. galli* can stimulates a strong antibody response (Marcors et al.,2009; Anna et al.,2011), also(Coles,1986) reported that the changes in the level of blood proteins may indicated to some pathological immunoglobulins that results from immunostimulation .

That agree with significant increases in antibodies titer, because its effect as immunostimulator (Dalvi,1990) ,while *Punica granatum* can elevated TSP in treated groups due to its antidiabetic activity which prevent body weight loss and elevated TSP with inhibition to lipid peroxidation(Osama et al.;2010).

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