

رقم البحث (4)

CLINICOPATHOLOGICAL STUDIES ON THE EFFECT OF GROWTH PROMOTERS POMEGRANATE AND ECHINACEA EXTRACTS IN BROILER CHICKENS.

BY

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ABSTRACT

Fifty broiler chicks were used to evaluate the effects of *Punica granatum* (*P. granatum*) peel and *Echinacea purpurea* (*E. purpurea*) extracts on growth performance and some hematological, biochemical and immunological parameters. At one week of chicken ages, chicks were divided into five equal groups. Control group (C) received water only while others received the extract in drinking water for four weeks. Second and third groups (P1 and P2) were administered 0.1% and 0.2% of *P. granatum* extract, respectively. Fourth and fifth groups (E1 and E2) were administered 0.1% and 0.2% of *E. purpurea* extract, respectively. Blood samples were collected at the 5th week post experiment. There was a significant improvement in body weight and weight gain of chicks received 0.1% of each medicaments (P1 and E1). Total leukocytic and lymphocytic counts were significantly elevated in P2 and E2 groups. Total protein value showed a significant elevation in E1 group. Meanwhile, glucose value was significantly decreased in P2 group. The nitric oxide value was significantly decreased while unchanged bactericidal test result in all groups. Although the lysozyme activity was significantly increased in all groups except in P1 group. Oral using of *P. granatum* and *E. purpurea* could improve the growth performance and some immunological parameter in broiler chickens.

INTRODUCTION

Medicinal plant with minimal side effects is an excellent choice for improving growth performance of chickens. One of the most common plants in America and Europe is the purple coneflower Echinacea (Asteraceae family) (Thomson, 2004 and Charrois et al, 2006). The main active principles of *E. purpurea* are alkamides, glycoproteins, caffeic acid derivatives and polysaccharides. Pomegranate (*P. granatum* L. family puniceae) is a delicious fruit broadly cultivated in Egypt. The fruits peels contain substantial amount of tannins and polyphenols. Polyphenols pointed to the potent antioxidant, antimutagenic, and antiviral effects (Thomson, 2004; Parmar and Kar, 2007). Echinacea and pomegranate had immunomodulatory, anti-inflammatory, a potent antioxidant and antibacterial action. Moreover, *E. purpurea* had interferon like effect (Bone, 1997, Thomson, 2004 and Cravotto et al, 2010).

The present study aimed to evaluate the growth performance and immunomodulatory effect of *P. granatum* and *E. purpurea* extract in broiler chickens.

MATERIALS AND METHODS

Plant extracts:

Fresh pomegranate fruits from local market were peeled off manually, air dried, grounded and extracted in methanol (100g/600 mL). The yield was stored at -4°C for future use (Parmar and Kar, 2007). The dry extract (*E. purpurea*) of the capsule (Immulant) was obtained from Arab Co. for Pharmaceuticals & Medicinal Plants ME.

Chickens and ration:

Fifty, one day old Cobb chicks were obtained from a local commercial hatchery, housed in battery system. Chicks were fed on non-made starter rations (protein \geq 21.2%) and vaccinated against Newcastle disease at 7, 18 and 28 days of age. Feed and water were offered ad libitum.

Experimental design:

The chicks were acclimatized for one week and randomly divided into five equal groups (each of 10 chicks). A control group received no medications or additives and the others

received the extract for four weeks and reared for another week. Groups P1 and P2 were administered *P. granatum*, 0.1% and 0.2% respectively in drinking water (Patel et al, 2008). Groups E1 and E2 were administered *E. Purpurea*, 0.1% and 0.2% respectively in drinking water (Allen, 2003). They were vaccinated against Newcastle disease (at 7th, 18th and 28th days old). Five blood samples of each group were taken from the wing vein at 5th week. Serum was stored at -20 °C until assayed.

Growth performance analysis:

Chickens were weighed individually 2 times weekly. Calculation of growth parameters were done according to Nicodemus et al, (1999).

Hematological, biochemical and immunological analysis:

Erythrograme, blood indices calculation, total leukocyte count (TLC) and differential leukocyte count were done according to Feldman et al, (2000). Serum samples were used for estimation of AST (Randox cat. No (AS 101), ALT (Randox cat. No (AL 100), cholesterol and creatinine (Human), urea and glucose (Diamond), total protein (Spinreact), albumin (Stanbio) and globulin and A/G ratio according to Kaneko et al, (1997). Nitric oxide (NO) was determined by enzymatic colorimetric method by using ready-made kits (Biodiagnostic, Egypt) (Vodovotz, 1996). Lysozyme was determined via turbidometric assay (Sigma, St. Louis, MO) (Zucker et al, 1970). Agar diffusion bioassay (bactericidal test) was estimated according to Lorian (2005).

Statistical analysis:

All data were expressed as $M \pm SE$. All statistical analysis were carried out using SPSS 11.0 for Windows (ANOVA), followed by a post-hoc Duncan test used by SPSS/ computer program version 18 (SPSS). Dissimilar superscript letters in the same column show a significance differences ($P < 0.05$).

RESULTS AND DISCUSSION

Table (1) demonstrated the weekly growth performance. At the end of the 5th week, P1 group shows a significant increase of total and daily weight gains and decreasing of FCR. The improved growth performance may be attributed to the active constituent in the peel. This opinion is supported by **Elswijk et al, (2004)** who reported that pomegranate peel has three estrogenic compounds (Luteolin, quercetin and kaempferol). In addition, the peel also contain tannins which had a marked antimicrobial activity(**Al-Zoreky, 2009**). The growth promoting effect of estrogen did not explained but it was effectual (**Weise et al, 2001**).

In the present results, the E1 group showed prominent improvement of all growth performance profiles except the FCR, without negative effects on liver transaminases (AST, ALT). Echinacea stimulated the secretion of digestive enzymes (**Przybilla and Weib, 1998**) and its effect on the microbial pathogens was due to the presence of active ingredients and phenolic compounds (**Duman et al, 2009**) led to better intestinal health and more tissues protein deposition (**Nasir, 2009**). The present results are in harmony with **Nasir, (2009)** who confirmed that *E. purpurea* juice to have no negative effect on broilers chicken performance but offer better daily weight gain and did not affect liver function. In addition, **Aituan et al, (2009)** recorded a reduction in broilers FCR that administered *E. purpurea* extract orally. Our results disagree with the unchanged performance of turkey-hens administrated Echinovit C and in laying hens received *E. purpurea* juice (**Krauze et al, 2007 and Bohmer et al, 2009**).

In Table (2), there are normal erythrogram in all groups indicates normal erythropoitic tissue of bone marrow and adequate requirements (**Coles, 1986**). Results are in concord with **Aboueilla et al, (2007)**. Leukocytosis and lymphocytosis were significantly appeared in group E2 (table, 3). Echinacoside and caffeic acid included in *E. purpurea*, eliminated the free radicals and prevent blood cell destruction (**Mishima et al, 2004**). Lymphocytosis indicated the immunostimulant role of Echinacea (**Bauer, 1996**) and confirmed with the elevation of lysozyme activity in groups E1 and E2 (table, 5). Its immunostimulatory action was attributed to the lipophilic alkylamides and the polar caffeic acid derivative, cichoric acid (**Bone, 1997**). The results are partially in agreement with **Aly et al, 2008** who reported elevation of lysozyme in fish. While our results are in agreement with **Krauze et al, (2007)** and **Bohmer et al, (2009)** due to the different Echinacea preparation.

Table (4) demonstrated the serum biochemical analysis post 5th week of treatments. Group E1 showed, increase in total proteins. Serum proteins are being synthesized in liver and

usually used as indicators of metabolic and synthetic activity of liver. The present results are in harmony with **Nasir (2009)** results. Serum glucose was decreased in P1 and P2 groups. The total polyphenols and insulin stimulatory activity of the methanolic peel extract may lead to hypoglycemia in rats (**Parmar and Kar, 2007 and 2008**). There is a variation in the normal circulating glucose concentration in recent reports for chickens (156 and 330 mg/dl) (**Scanes, 2008**). On the contrary, our results are somewhat disagreement with **Patel et al, (2008)**.

Nitric oxide production was determined as biomarkers for immunomodulation (**Goel et al, 2002**). The NO level was decreased in groups E1 and E2 groups (Table, 5). Echinacea inhibited NO production in vitro by decreasing the production by macrophages via modulation of NO yield (**Zhai et al, 2009**). Conversely, it did not modify nitrites level in chickens with coccidiosis (**Allen, 2003**). This may be attributed to different period of application.

The NO level was decreased in P1 and P2 groups. Pomegranate juice possesses potent inhibitor of superoxide anion-mediated disappearance of NO (**Ignarro et al, 2006**). **Shukla et al, (2008)** revealed this to the inhibition of IL-1 β -induced NO in rabbits chondrocytes stimulated (24 hours) with human IL-1 β . The extract exerted potential NO inhibition in vitro and in vivo (**Lee et al, 2010**). The suggested immunomodulatory action of extract confirmed by elevation of the TLC, lymphocytes and lysozyme activity in P2 group (Tables, 3 and 5). Lysozyme is a vital substance of the natural immune response with both anti-bacterial and antiviral activity (**Desvignes et al., 2002**). Irradiated rats suffered from leukocytes apoptosis, that was prevented by *P. granatum* (**Toklu et al, 2009**).

In conclusion, the *P. granatum* and *E. purpureae* have a promising growth performance and immunomodulatory effects in broiler chicken. More studies are needed for selection of appropriate dose, duration of treatment, and possible side-effects.

Table (1): Growth performance profiles (Mean \pm S.E) of chickens treated with *P. granatum* and *E. purpurae* extracts.

Time/week	Groups	Initial weight/gm	Final weight/gm	Total weight gain/gm	Daily weight gain/gm	FCR gm/gm
Between weeks 0-2	Control	181 \pm 3.32	701 \pm 43.89	520.0 \pm 42.72	37.14 \pm 3.05	2.31 \pm 0.20
	P1 (0.1%)	180 \pm 1.58	625 \pm 52.44	445.0 \pm 53.41	31.79 \pm 3.82	2.77 \pm 0.31
	P2 (0.2%)	180 \pm 2.74	640 \pm 38.41	460.0 \pm 38.99	32.86 \pm 2.79	2.62 \pm 0.24
	E1 (0.1%)	181 \pm 2.45	685 \pm 36.74	504.0 \pm 35.51	36.00 \pm 2.54	2.36 \pm 0.18
	E2 (0.2%)	180 \pm 2.74	699 \pm 36.41	519.0 \pm 38.52	37.07 \pm 2.75	2.30 \pm 0.17
Between weeks 2-4	Control	701 \pm 43.89	1149 \pm 51.66	447.52 \pm 39.65	31.96 \pm 2.83	2.69 \pm 0.24
	P1 (0.1%)	625 \pm 52.44	1116 \pm 64.11	490.71 \pm 35.19	35.05 \pm 2.51	2.42 \pm 0.166
	P2 (0.2%)	640 \pm 38.41	1074 \pm 59.97	434.00 \pm 35.78	31.00 \pm 2.56	2.75 \pm 0.18
	E1 (0.1%)	685 \pm 36.74	1181 \pm 48.53	495.57 \pm 30.14	35.40 \pm 2.15	2.39 \pm 0.15
	E2 (0.2%)	699 \pm 36.41	1179 \pm 54.00	480.20 \pm 24.83	34.30 \pm 1.77	2.46 \pm 0.14
Between weeks 4-5	Control	1149 \pm 51.66	1367 \pm 46.30 ^b	218.48 \pm 21.70 ^b	31.21 \pm 3.10 ^b	4.00 \pm 0.38 ^a
	P1 (0.1%)	1116 \pm 64.11	1395 \pm 52.44 ^{ab}	279.29 \pm 34.79 ^{ab}	39.90 \pm 4.97 ^{ab}	3.20 \pm 0.40 ^{ab}
	P2 (0.2%)	1074 \pm 59.97	1351 \pm 46.65 ^b	277.00 \pm 44.65 ^{ab}	39.57 \pm 6.38 ^{ab}	3.30 \pm 0.43 ^{ab}
	E1 (0.1%)	1181 \pm 48.53	1535 \pm 50.99 ^a	354.43 \pm 62.76 ^a	50.63 \pm 8.96 ^a	2.67 \pm 0.45 ^b
	E2 (0.2%)	1179 \pm 54.00	1445 \pm 67.08 ^{ab}	265.80 \pm 22.79 ^{ab}	37.97 \pm 3.26 ^{ab}	3.29 \pm 0.37 ^{ab}
Between weeks 0-5	Control	181 \pm 3.32	1367 \pm 46.30 ^b	1186.0 \pm 43.31 ^b	33.88 \pm 1.23 ^b	6.06 \pm 0.25 ^a
	P1 (0.1%)	180 \pm 1.58	1395 \pm 52.44 ^{ab}	1355.0 \pm 50.52 ^a	39.71 \pm 1.44 ^a	5.28 \pm 0.22 ^b
	P2 (0.2%)	180 \pm 2.74	1351 \pm 46.65 ^b	1171.0 \pm 44.87 ^b	33.46 \pm 1.28 ^b	6.11 \pm 0.23 ^a
	E1 (0.1%)	181 \pm 2.45	1535 \pm 50.99 ^a	1354.0 \pm 50.26 ^a	38.69 \pm 1.43 ^a	5.29 \pm 0.21 ^b
	E2 (0.2%)	180 \pm 2.74	1445 \pm 67.08 ^{ab}	1265.0 \pm 68.61 ^{ab}	36.14 \pm 1.96 ^{ab}	5.69 \pm 0.31 ^{ab}

Column with different superscripts differ significantly ($p < 0.05$) within the same week.

Table (2): Erythrogram (Mean \pm S.E) of chickens treated with *P. granatum* and *E. purpurae* extracts at 5th week.

Groups	RBC($10^6/\mu\text{l}$)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
C (control)	2.83 \pm 0.27	8.14 \pm 0.85	27.40 \pm 1.78	100.168 \pm 12.28	29.39 \pm 3.00 ^{ab}	29.75 \pm 2.20
P1 (0.1%)	2.75 \pm 0.28	7.30 \pm 1.03	28.00 \pm 2.98	105.322 \pm 15.59	26.36 \pm 2.70 ^b	28.06 \pm 6.10
P2 (0.2%)	2.96 \pm 0.08	7.94 \pm 0.56	32.60 \pm 2.86	110.20 \pm 8.90	26.85 \pm 1.66 ^{ab}	25.53 \pm 3.66
E1 (0.1%)	2.91 \pm 0.20	9.59 \pm 0.77	28.20 \pm 1.02	102.76 \pm 12.00	33.87 \pm 1.44 ^a	34.48 \pm 3.71
E2 (0.2%)	2.72 \pm 0.11	8.86 \pm 0.89	27.80 \pm 1.28	103.11 \pm 7.11	32.75 \pm 3.29 ^{ab}	32.20 \pm 3.45

Column with different superscripts differ significantly ($p < 0.05$) within the same week.

Table (3): Leukogram (Mean \pm S.E) of chickens treated with *P. granatum* and *E. purpurae* extracts at 5th week.

Groups	TLC $10^3/\mu\text{l}$	Lymphocytes $10^3/\mu\text{l}$	Heterophils $10^3/\mu\text{l}$	Monocytes $10^3/\mu\text{l}$	Eosinophils $10^3/\mu\text{l}$	Basophils $10^3/\mu\text{l}$
C (control)	23.40 \pm 4.90 ^a	12.67 \pm 2.16 ^a	9.41 \pm 2.73	0.60 \pm 0.16	0.55 \pm 0.29	0.21 \pm 0.16
P1 (0.1%)	27.00 \pm 2.81 ^{ab}	14.32 \pm 2.25 ^{ab}	11.92 \pm 1.24	0.48 \pm 0.19	0.19 \pm 0.19	0.10 \pm 0.10
P2 (0.2%)	36.60 \pm 2.40 ^b	20.24 \pm 1.83 ^b	14.35 \pm 2.63	1.02 \pm 0.39	0.57 \pm 0.14	0.42 \pm 0.18
E1 (0.1%)	28.80 \pm 1.66 ^{ab}	16.97 \pm 0.73 ^{ab}	9.95 \pm 1.06	0.99 \pm 0.26	0.56 \pm 0.15	0.30 \pm 0.10
E2 (0.2%)	35.80 \pm 5.19 ^b	19.95 \pm 3.86 ^b	14.35 \pm 1.76	0.78 \pm 0.27	0.41 \pm 0.19	0.31 \pm 0.13

Column with different superscripts differ significantly ($p < 0.05$) within the same week.

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Table (4): Serum biochemical (Mean \pm S.E) of chickens treated with *P. granatum* and *E. purpurae* extracts at 5th week.

Groups	AST (μ /L)	ALT (μ /L)	Cholest. (mg/dl)	TP (g/dL)	albumin (g/dL)	Globulin (g/dL)	A/G ratio	Glucose (mg/dl)	urea (mg/dl)	creatinine (mg/dl)
C (control)	40.98 \pm 4.88	4.6 \pm 0.63	116.4 \pm 12.08	3.92 \pm 0.27 ^b	2.47 \pm 0.24	1.45 \pm 0.32	2.97 \pm 1.63	220.25 \pm 6.33 ^a	43.4 \pm 4.45	0.32 \pm 0.04 ^{ab}
P1 (0.1%)	40.98 \pm 7.77	2.88 \pm 0.44	100.6 \pm 6.71	4.45 \pm 0.50 ^{ab}	2.78 \pm 0.41	1.67 \pm 0.52	5.51 \pm 4.03	197.25 \pm 6.26 ^{bc}	38.2 \pm 9.57	0.30 \pm 0.02 ^b
P2 (0.2%)	29.71 \pm 6.60	3.87 \pm 0.79	99.6 \pm 8.13	3.97 \pm 0.41 ^b	2.51 \pm 0.14	1.45 \pm 0.48	2.43 \pm 0.55	178.50 \pm 9.08 ^c	46.2 \pm 8.19	0.32 \pm 0.02 ^{ab}
E1 (0.1%)	39.52 \pm 10.74	4.77 \pm 0.46	120 \pm 13.24	5.00 \pm 0.30 ^a	2.79 \pm 0.26	2.2 \pm 0.31	1.39 \pm 0.23	212.5 \pm 4.06 ^{ab}	36.91 \pm 6.71	0.34 \pm 0.01 ^{ab}
E2 (0.2%)	37.04 \pm 5.58	4.55 \pm 1.16	116.2 \pm 4.74	45.46 \pm 0.15 ^{ab}	3.12 \pm 0.21	1.42 \pm 0.26	3.06 \pm 1.26	204.13 \pm 6.45 ^{ab}	43.4 \pm 5.53	0.39 \pm 0.01 ^a

Column with different superscripts differ significantly ($p < 0.05$) within the same week.

Table (5): Serum biochemical immunological parameters (Mean \pm S.E) of chickens treated with *P. granatum* and *E. purpurae* extracts at 5th week.

Groups	Nitric oxide (mmol/L)	Bactericidal test (mm)	Lysozyme activity μ g/mL
C (control)	18.81 \pm 1.62 ^a	12.40 \pm 0.51	1.11 \pm 0.02 ^b
P1 (0.1%)	11.96 \pm 1.14 ^b	11.40 \pm 0.60	1.17 \pm 0.01 ^{ab}
P2 (0.2%)	11.88 \pm 0.40 ^b	11.20 \pm 0.66	1.23 \pm 0.02 ^a
E1 (0.1%)	11.22 \pm 0.07 ^b	11.90 \pm 0.25	1.21 \pm 0.03 ^a
E2 (0.2%)	13.04 \pm 0.96 ^b	12.20 \pm 0.26	1.21 \pm 0.04 ^a

Column with different superscripts differ significantly ($p < 0.05$) within the same week.

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الملخص العربي

دراسات باثولوجيا اكلينيكية على تأثير مستخلص الإكينيسيا و الرمان المحفز للنمو في دجاج التسمين

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تعد النباتات الطبيعية ومخلفاتها الناتجة من فضلات الصناعات الغذائية مصدرا غنياً لتعزيز نمو وزيادة مناعة الحيوانات لإنتاج البروتين الحيواني بأعلى جودة وبأقل التكاليف بعيداً عن الآثار السلبية التي تنتج عن استخدام العقاقير الكيميائية.

وقد أجريت هذه الدراسة لتقييم آثار مستخلصا كلا من قشر الرمان والإكينيسيا بريوريا على دجاج التسمين في النمو (زيادة الوزن اليومية وإجمالي زيادة الوزن ومعدل التحويل الغذائي) وكذلك تقييم صورة الدم وبعض العوامل البيوكيميائية والمناعية. تم تربية عدد خمسون كتكوتا (عمريوم) من نوع كاب لمدة أسبوع ثم قسمت إلى خمس مجموعات متساوية. مجموعة التحكم والتي لم يضاف لها شيئاً في مياه الشرب. المجموعتان الثانية والثالثة تم إضافة مستخلص قشر الرمان بجرعات ٠,١ % و ٠,٢ % من مياه الشرب على التوالي. المجموعتين الرابعة والخامسة تم إضافة مستخلص الإكينيسيا بجرعات ٠,١ % و ٠,٢ % من مياه الشرب على التوالي. أضيفت المستخلصات لمياه الشرب لمدة أربعة أسابيع ثم توقف العلاج لمدة أسبوع ثم أخذت عينات الدم من وريد الجناح. أما بالنسبة للأوزان فقد تم تسجيلها لكل طائر على حده مرتين في الأسبوع وقد أظهرت النتائج الآتي:

حدث تحسناً مؤثراً في الوزن الكلي واليومي ومعدل التحويل الغذائي في الفترة بين الأسبوعين الصفرو الخامس (٥٠) وذلك في مجموعتي قشر الرمان والإكينيسيا المنخفضة الجرعة والتي شهدتا تحسناً معنوياً في الوزن النهائي والزيادتين الكلية واليومية. لم يحدث أي تغير في صورة الدم عدا زيادة كرات الدم البيضاء والخلايا الليمفاوية وذلك في الجرعات العالية لكلا المستخلصين. فيما ازدادت قيمة البروتين الكلي وذلك في مجموعة الإكينيسيا المنخفضة الجرعة. بينما انخفض مستوى سكر المصل في مجموعتي قشر الرمان. وقد بينت نتائج المناعة انخفاضاً معنوياً في قيمة أكسيد النيتريك على عكس قيمة الليسوزيم التي ازدادت في المجموعات عدا مجموعة قشر الرمان المنخفضة الجرعة.

وقد خلصت الدراسة إلى إمكانية استخدام مستخلصا كلا من قشر الرمان والإكينيسيا كمحفزات للنمو ومنشطات للمناعة في دجاج التسمين.