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Effect H₂O₂ And Diffrient Levels of *Heliotropium pacciferum* Plant Powders on Some Production Performance And Blood Biochemecal Properties in Broiler

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Abstract

A study was conducted over the period from 6 June to 10 July 2016 at the special poultry farm in Kifri city-Kurdistan Region- Iraq to invest the effect of using H₂O₂ to induce oxidative stress and different levels of *Heliotropium pacciferum* plant powder in the ration on some production performance and blood serum biochemical properties of broiler. One hundred and eighty, one-day-old chicks (ROSS) were used and randomly with three replications distributed into six treatment, each containing 10 chicks. The treatments were, control, H₂O₂ with drinking water and the third, fourth, fifth, sixth treatment were levels of plant powders (2.5, 5, 7.5 and 10) gm/kg forage sequentially given as ad libitum.

The results showed significant effect for plant powder levels on live body weight reached, (1840.44±26.0)gm, and dressing percentage (62.59%) were birds given 7.5gm/kg, while higher percentage of mortality rate were birds given H₂O₂ reached (8.16%). Also significant effect observed on biochemical blood serum properties when birds given different levels of plant powder, the treatment with H₂O₂ gave high value of cholesterol and less value of HDL.



reached (194.83 ± 8.32 , 29.60 ± 1.86) mg/dl consequently however the treatment 7.5gm/kg gaved less value from triglyceride and VLDL reached (74.04 ± 13.82 , 14.8 ± 0.76) mg/dl consequently and high value from LDL reached (69.88 ± 0.76) mg/dl .

Key words: Alkaloid, pacciferum, chicken, oxidative stress.

Introduction

The Genus *Heliotropium* include 22-320 species, its plant has scorpion inflorescence (Al-mosawi, 1987) with medicinal and economical value in the world (Abbas, 1991 & Othman, 1989), it contain alkaloids and glycosides materials such the species *H. lasocarpium* which grow in desert regions with sandy and clay soils and on farms (Al-Blesh, 2012).

The poultries consider one of the important animal protein source, There for many applicated researches done on this field to find substitute remedy for diseases and food problems (Aleas & Alabed, 2010) and all the researches concentrate about plants and medicinal herbs because it doesn't have any side effect (Abraheem & Puttr, 2008; An-niaumy, 2009; AOA, 1999) instead of synthetic drugs which has side effect transport to human by animal (Abraheem & Gsaan, 2009).

Eating feed which grantee the life for us, produce in same time material causes oxidity has negative effect on the body. The food enter to metabolism attacked by free radical consisting in atoms or molecules has more of free unstable electron on the external orbit it is constantly in research for missing electron which pulle from adjacent molecules and this cause destroying natural cell molecules of body (Bo-Alkhandool, 2011). There are many type of free radical such as Active oxygen (ROS) may be radicals or non radicals in many shaped such as super oxide anion O_2^- , single oxygen O^1_2 , Hydrogen peroxide (H_2O_2), Hydroxyl radical (OH) and Nitrate oxide (NO) (Shama, 2015). the source of this radicales may be internal produced by Mitochondria or NADPH oxidase enzyme and NOS enzyme (Adams *et al.*, 2001) or it comes from external source such as food, smoking, toxicity of ozon layer and exposes to rays (Rakove *et al.*, 1991; Gilbert & Colton, 1999).

The balance between active oxygen type production and anti oxidative give grantee for continous natural physiology body processes (Ozgan *et al.*, 2006) and the over oxygen types production lead damage on molecule level causes damage for tissues and hapning more diseases (Bin-Salama, 2012).

The researches find in the plant from active new molecules and crude materials which used in pharmacology for synthetic derived drugs from plants (Medjdoub, 2006) this plants produce many compounds by secondary metabolism processes (Mohammad, 2013) such as phenols, terpenoids, steroids and alkaloids (Zegheb, 2013) and probably this compounds being a big source of difference medicinal factors (Boukri, 2014) this secondary compounds reach over 2000 (Kanoun, 2011).

Alkaloids are one of the important secondary compounds which consist of more than 200 and its concentrate about 15% from dry weight of plant (Mauro, 2006) distinctive with higher toxicity because of higher biological activity and vigor of physiological effect (Abo-



Zaid,2006) and more uses from another compounds (Husain,1981 , Mukherjee et al.,2001).now yet the number of drived alkaloids reach more than 2000 alkaloids (Alshamaa.1989),therefor the direction was about medicinal plants and herbs such as using seed powder emolusion aqueous of *Avena sativa* L.,*Anithemis nobilis* L. and *Capsicum conicum* (Putrs,2007) ,boiling aqueous extraction of *Cyperus longus* L tubbers(Al-niaumy,2009),*Nigella sativa* L. powder(Al-khufajee,2005) Glycyrrhiza glabra L and Diopyrus kaki L extracts(Husain et al.,2010) and extracts of Chicory local leaves(Al-Ethary,2012).

Material and methods

The study was conducted over the period from 6 June to10 July 2016 at the special poultry farm in Kifri city-Kurddistan Region- Iraq to invested the effect of using different leavels of of *Heliotropium pacciferum* plant powders in the ration on some production performance and blood serum biochemical properties of broiler and using H₂O₂ to induse oxidative strees.One hundred and eighty,one-day-old chicks(ROSS) were used and randomly with three replicaties distributed into six treatment,each containing 10 chicks.The treatments were,control,H₂O₂ with drinking water and the third, fourth,fifth, sixth treatment were leavels of plant powders 2.5,5,7.5 and 10 gm/kg mash sequently gived as (ad libitum) on initiar mash from age (1-10) day,and from age (11-24) day gaved on growth mash and on age (25-35) day gaved definitive mash table(1) according to Ross 308 guide for 2007.

Table 1.percentage of enterning forage material with chemical material.

Mashesmaterial	Primary mash(1-10)day%	Growth mash% (11-24)day	Definitive mash(25-35)day
Corn	40	56.5	59.5
Wheat	11	-	-
Soy bean48%	38	32	29
Vegetable oli(sunflower)	5	5.5	5.5
Protein	5	5	5
Gypssium rock	0.7	0.7	0.7
Salt	0.3	0.3	0.3
Total	100	100	100

Calculated chemical composition

Crut protein%	24.90	22.16	20.97
Energy(kg/kcal)	3055	3152	3179
Licin%	1.32	1.20	1.12
Mithionin%	0.52	0.50	0.48
Mithionin+Cystin%	1.10	1.12	1.13
Calsium%	1.15	1.17	1.16
Phosphor%	0.36	0.33	0.32



-Studed chracteristics:-

Active plant ingrediants:

Diagnosis of plant active ingrediants done by High Performance Liquid Chromotography (HPLC) apparatus .The plant dried at the shade and milled then 10gm from the sample put in 50 ml boiled water (90-100C⁰) for 3 hours then extracted whattman papers no.1 the extraction collected and put in closed glass tube in order measuring the concentration of active ingriadents by HPLC apparatus which supplied by Shimadzu company (Japan) type,LC-10A 2000 supplied with spectrum scale (Spectro photo meter – spd – 10A – UV),Asample size 20µl injected on Fast liquid chramotographic column (LC) with diamention(50×4.6mm I.D) by the injector type (Rheodyn-712)and the data recorded by calculator which drewed the pick area and retention time.Astandard solution of *Heliotropium lasiocarpium* plant used and sperated py HPLC apparatus and identification the pick area and retention time of standard solution and comparing it with the pick area and retention time of studed plant sample at the same condition .

Table 2. chromatographics separate condition

Column	Revers phase column(50×4.6mm I.D)
Mobile phase	Distill water :Ethanol 70%
	2 :80v/v
Following rate	10 ml/min
Type of detector	Ultra violate ray 254 nm
Temperature	30 C ⁰
Fast of recorder paper	10 ml/min
Size of injected sample	50 mg/ml



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Concentration of compounds in the sample calculated by the equation:

pick area of compound

Conc. Compound in the sample= _____ × standard pattern conc. × dilution factor

pick area of standard sample

Productivity characteristics:

1-Live body weight: At the end of experiment bird weight taken and following equation applied to know bird weight rate on one replicate:

Total of birds weight in replication

Bird weight rate= _____

No. birds in replication

2-Mortality rate: daily mortality recorded and calculated as percentage over experiment period for all replicate and following equation applied to know mortality rate:

Total mortality on a week

Mortality rate percentage= _____

No. of total bird

3-Dressing percentage: six birds, three male and three female were taken from each replicate in order to calculate dressing percentage at the end of fifth week. Live weight were taken then slaughtered, intestines removed and interior eating parts separated, the heart, liver and gizzard and dressing percentage calculated according to the equation below:

Cleaned slaughter weight(gm)

Dressing percentage without eating parts= _____

Live weight(gm)



Biochemical blood parameters:

At 35 day four birds were taken from each replicate and blood were pulled from brachial vein of the bird and collected in test tube without deterrent coagulate. (EDTA) and flowing tests done in laboratories Kifri hospitale and biology department laboratories - education college for pure science-Tkrit University ,the tests included:

-Cholesterol estimation:cholesrol measured by using equipment test (kit) produced by spinereact,S,A,Spain according the method which depends to enzymatic analysis of triglycerides into glycerol,where mixed enzymatic reagent(R2 compsed of (Peroxidase,Cholestroloxidase,Cholesterol esterase and Aminophenozone at concentrate 1250u/l,300u/l,300u/l and 0.4 mmol/l concequently) with same size from reagent buffer (R1) composed of (Pipes Ph 6.9,Phenol and Sodium chadate surfactant at concentrate 90 mol/l,26mmol/l and 3.7mmol/l concequently) and the two solutions shaken to prepar working solution,then taked three test tube and put in each one 1.0 ml from working solution then addition10 μ from blood serum on one of tubes and 1 μ from standard solution (Cholesterol 200mg/dl μ or 5.17 mmol/l) to second tube,then shak slowly and left it in water path on 37C⁰ for five minutes ,the reading for on absortion done on the wave length 500 nm and cholesterol concentration measured using aquation below:

A sample

$$\text{Cholesterol conc. (mg/dl)} = \frac{\text{sample reading}}{\text{standard reading}} \times n(200\text{mg/dl or } 5.17\text{mmol/l})$$

A standard

-Determination of serum triglyceride: triglyceride measured by using equipment test (kit) produced by Italian company (Giesse Diagnosticsns)according to the method depen on enzymatic analysis of triglyceride to the glycerol by taken three test tube and addition on them blood serum ,standard solution TG (glycerol) and distill water concequently then addition 1 ml from solution declare (TG) composed of (Good buffer,Magnesium chloride,Adenosine tri phosphate,4-Aminoati pyrine,Toos,Lioprotein Lipase,Glycerol Chinase,Glycerol-3-phosphate oxidase and Peroxidase) at concentrate 100mmol/l,15mmol/l,4mmol/l,1 mmol/l,0.1mmol/l,2500 μ /l,1000 μ /l,5500 μ /l and1800 μ /l concequently and after slow shaking tubes left at laboratories temperature for 15 minutes and reading absorption on wave length 505 nm then triglycereds calculated according the aquation below:

Asample

$$\text{Triglycereds conc. mg/dl} = \frac{\text{sample reading}}{\text{standard reading}} \times \text{standard conc. (200mg/dl)}$$

Astandard

Blood serum HDL cholesterol:measured by using equipment test (kit) produced by Italian company (Giesse Diagnosticsns)by taken 50 μ /l from each of blood serum,standard solution and distill water concequently on three test tube and addition 1 ml from working



solution(cholesterol Enzymatic solution) and shaking it then but it on the water path at 37 C⁰ for 5 minute and reading the absorption at the wave length 500 nm by using the aqation below:

$$\text{HDL cholesterol (mg/dl)} = \frac{\text{Asample}}{\text{Astandard}} \times 500 \times 1.1$$

500:mean standard solution conc.

1.1:mean dilution factor

Blood serum LDL cholesterol: measured by using the form below (Freiedwold et al.,1972):

$$\text{LDL (mg/dl)} = \text{Total cholesterol} - \text{HDL cholesterol} - \text{Triglyceride}$$

Blood serum VLDL cholesterol: measured by using the form below (Freiedwold et al.,1972):

$$\text{VLDL (mg/dl)} = \frac{\text{Triglycerides}}{5}$$

5

The data analyside statistically by using complete Randomized Design (CRD) and leastsignificant diffrenece compared between means by using Duncan multiple test(Duncan,1955) at the leavel 5% by using statistical program (SAS) 2001.

Results and Discution

Figure (1) and table (3) shows retention time and pick area of standard solution which injected to the HPLC apparatus and figure (2) with table (4) shows type of alkaloids compounds on the studed plant pattern with retention time ,pick area and its concentration, the compounds were indicine,supinine,indicine-N-oxide,heleurine,heliotrine and lendilofidine with concentrate reached(23.27%,5.66%,2.87%,2.34,48.05%,17.78 %) consequently

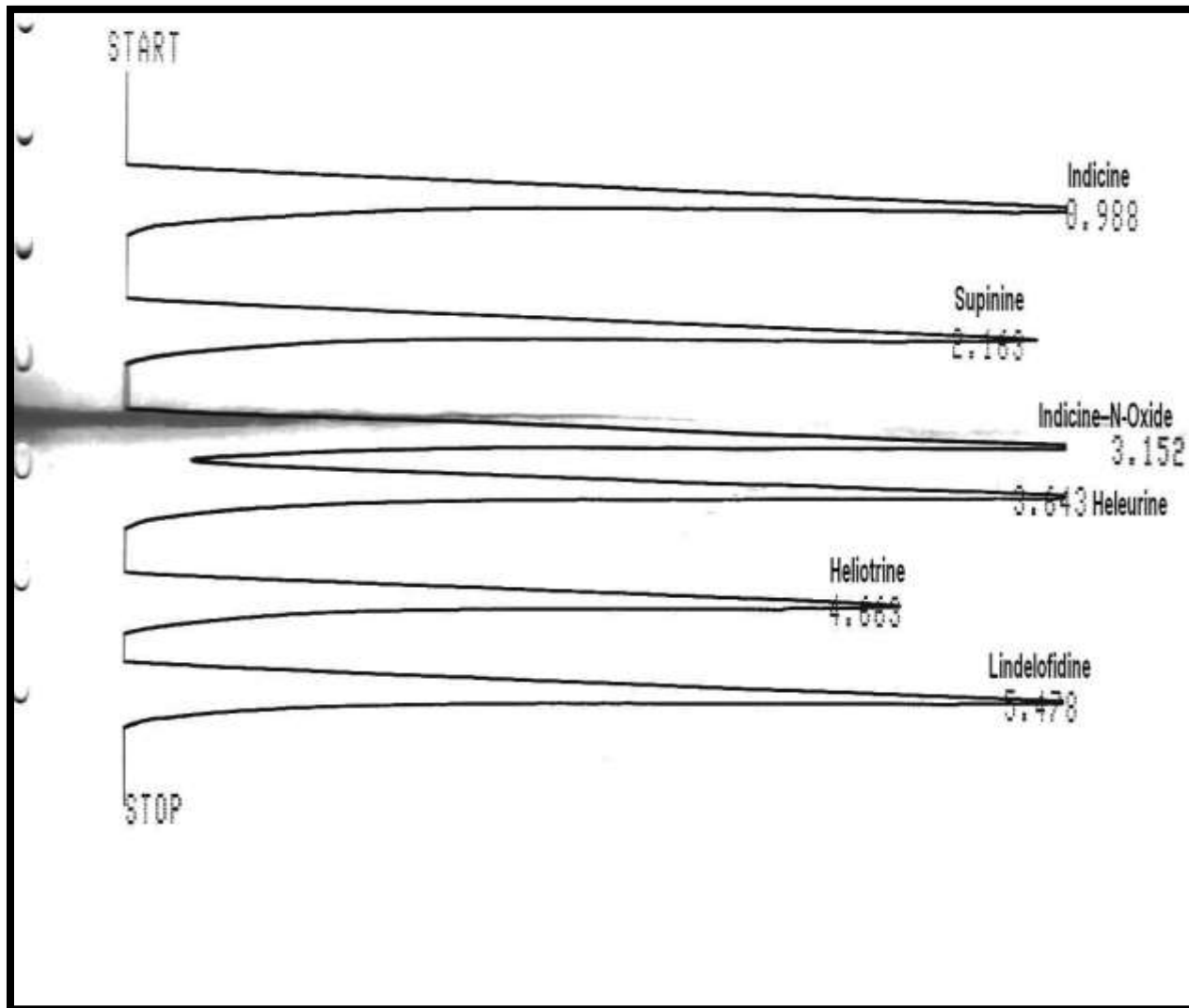


Fig.1. Retention time and pick area of H.bacciferum(standard solution)



Table 3.Values retention time,pick area and concentration of H bacciferum(standard solution

Seq.	Chemical component	Retention type	Pick area	Concentration
1	Indicine	0.988	39148	50mg/ml
2	Supining	2.16	32824	50mg/ml
3	Indicine-N-oxide	3.15	36977	50mg/ml
4	Heleurine	3.64	36748	50mg/ml
5	Heliotrine	4.66	27478	50mg/ml
6	Lindelofidine	5.47	34983	50mg/ml

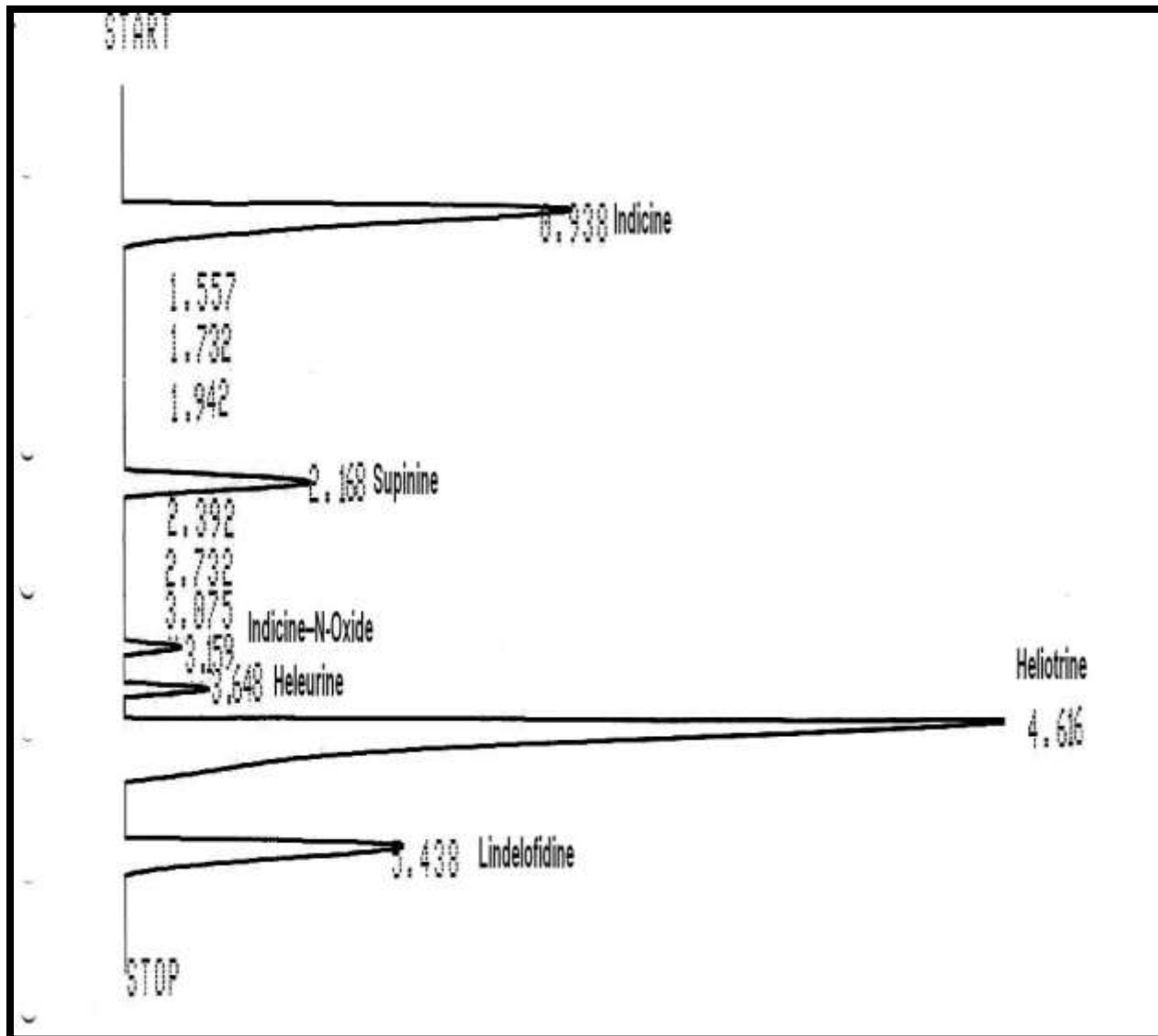


Fig.2. Retention time and pick area of *H. bacciferum* (plant sample)



Table(4) Values retention time , pick area and concentration of

H.bacciferum

Alkaloids	Pick area of Plant compounds	Pick area of standard compounds	Standard conc. (mg/ml)	Compounds conc.(mg/ml)	Compounds percentage(%)
Indicine	12950	39148	50	66.15	23.27
Supinine	2642	32824	50	16.09	5.66
Indicine-N-oxide	1510	36977	50	8.16	2.87
Heleurine	1227	36748	50	6.67	2.34
Heliotrine	18761	27478	50	136.55	48.05
lindelofidine	8842	34983	50	50.55	17.78

Table (5) shows effect of treatments on the some broiler productivity properties. The highest live body weight reached (1840.44±26)gm when broiler gaved (7.5) gm /kg from plant powder with mash, while least value were (1667.15±21.71)gm when broiler gaved H₂O₂ with drinking water, the same table shows least value of mortality rate when broiler gaved different level from plant powders with mash while highest value reached (8.16±0.72%) when broiler gaved H₂O₂ with drinking water. From same table we saw high value of dressing percentage reach (62.59±0.07%) when broiler gaved (7.5)gm/kg from plant powder with mash and low value reach (52.86±0.33%) in the H₂O₂ treatment. 1840.44±26.0a1974.33

Table 5. Effect of difference treatmentS on the productivity properties.

Treat. Properties	Control	H2O2	plant powder levels(mg/kg)mash			
			2.5	5	7.5	10
Live body weight (gm)	1811.33±21.71b	1667.15±15.088c	1821.33±11.355b	1840.44±26.0b	1974.33±46.4a	1836.33±11.2b
Mortality rate(%)	4.33±16.0b	8.16±0.7a	22.33±0.00c	2.33±0.03c	2.00±0.03c	2.33±0.08c
Dressing percentage(%)	57.43±0.33b	52.86±0.12c	57.74±0.11b	58.34±0.03b	62.59±0.07a	58.22±0.11b

*the value refer to the rate±stander error

*similar letters in one class means no significant difference at level(0.05>p)

From table (6) we saw as significant effect of plant powders and H₂O₂ on biochemical properties, the least percentage of cholesterol were in the H₂O₂ treatment reached (194.83±8.32) mg/dl while highest percentage were (170.71±15.99)mg/dl when broiler gaved (2.5gm/kg) plant powder, the same treatment showed highest level from triglyceride reached (120.02±11.42) mg/dl while least percentage were (74.04±13.82) mg/dl when added (7.5)gm



from plant powder with(1 kg) mash .the same table appeared least percentage of HDL were (29.60 $\pm$ 1.86) mg/dl while highest value of LDL reached (53.21 $\pm$ 9.54) mg/dl were used (7.5mg)plant powder with mash.The same treatment gaved lest percentage fromVLDL were(14.8 $\pm$ 0.76)mg/dl while high value were (24.0 $\pm$ 1.66)mg/dl at the H2O2 treatment .

Table 6. values of some biochemical properties(mg/dl) affected by different treatments on blood serum of broiler

Treat. Properties	Control	H2O2	plant powder levels(mg/kg)mash			
			2.5	5	7.5	10
Cholestrol	175.4 $\pm$ 8.26b	194.83 $\pm$ 8.32a	170.71 $\pm$ 15.99 b	174.0 $\pm$ 3.83b	175.22 $\pm$ 3.83b	179.04 $\pm$ 6.60b
Triglycerides	107.60 $\pm$ 9.94b	120.02 $\pm$ 11.42 a	99.20 $\pm$ 6.22b	85.25 $\pm$ 3.39b	74.04 $\pm$ 13.82c	94.25 $\pm$ 25.51b
HDL	35.06 $\pm$ 3.78a	29.60 $\pm$ 1.86b	34.73 $\pm$ 3.26a	34.94 $\pm$ 3.16a	31.30 $\pm$ 2.07b	35.61 $\pm$ 6.8a
LDL	31.74 $\pm$ 2.83e	45.21 $\pm$ 4.80c	36.78 $\pm$ 9.54d	53.21 $\pm$ 9.54b	69.88 $\pm$ 0.76a	49.18 $\pm$ 1.32c
VLDL	21.52 $\pm$ 1.65b	24.0 $\pm$ 1.66a	19.84 $\pm$ 3.19c	17.17 $\pm$ 0.09cd	14.8 $\pm$ 0.76e	18.85 $\pm$ 1.32c

***the value refer to the rate $\pm$ stander error**

***similar letters in one class means no significant difference at level(0.05>p)**

Th high value of livebody ,dressing percentage and less value of mortality and improving other biochemical properties may attribute to the role of plant extract in scavenging free radical from cells whereas more study prooffe dthat some of eatable plant or some of its isolated ingrediants has antioxidant properties(Tsao et al,2004) and antioxidant material which available in fruits and vegetables may be responsible about scavenging effect of free radicals(Halliwell,1994) the natural antioxidant in foods do as redactance factors (Parasakthy et al,1996)and the high physiological effect of alkaloids(Abo-Zaid,2005),also role of alkaloids in reducing due to its potential antioxidant (Blazorics et al,2003) and role of plant extracts in reducing synthsed lipids in liver tusses and leassing enzymes level which assist fatty acids synthetic (Al-Ehari,2012) .The rich plant extracts with antioxidants do to inhibition harmful oxidation for lever tussues by scavenging of generated free radical DNA and then leasssing cholestrol generation by inhibition Hydroxymethyl gultry-CoA reductase enzyme and increasing throw yellow acids with stool where convert big amount from cholesterol into yellow acids ,destroy cholesterol ,inhibition lipid absorption and inhibition lever cholestrol generation



(Belal,2011) ,also the results above may attribute to the content of plant powder vitamins such as E ,C and carotenoids which has role in forming antioxidant system (Surai et,al,2009) and participation enzyme of glutathione peroxidase with vitamin E and C in prokenfree radical reaction chains (Al-Drajee and Rad,2012) and role of alkaloids in scavenging DPPH radical (Benabdesselem,et al,2007) and free radicals (Gilani et al,2005).The results agree with other studies such as Al-Hamadany and Abdula-Rahem (2009)whom refer to significant leas on peroxy nitrait radical by using garlic powder in chicken forage,also agree with Abraheem(2009) whom see negative effect for abstracts of roots,steams and leaves of alkaloids materials,and with results showed effect of alkaloids in scavenging free radicals activity(Tiony et al,2013).

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