Pharmacological evaluation of (garlicin)® In Saso chickens experimentally infected with IBDV.

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Abstract
The present study was designed to study the immunostimulating effect of Garlicin following experimental infection with IBDV at 7 days of age. A pre-experiment to evaluate the safety of Garlicin on chickens health was conducted using one day old broiler chicks (cobb). Garlicin 1 g. /L for 17consecutive days was administered .Body weight, hemogram, liver and kidney functions were determined. Medicated broilers had a non-significant higher body weight gain 7 days from the onset of medication. The experimental study was performed using 110 one day old Saso chicks. 30 were kept as negative control The remaining 80 chicks were infected with a field strain of IBDV(10^3 EID50/0.1ml/chick) at the age of 7 days to induce immunosuppression. After four days of challenge bursal body weight index (BBI) was calculated for five chicks from each group , and was found (0.478) indicating successful induction of immunosuppression. Challenged birds were a liquated into three groups. The 1st group chicks were positive control, the 2nd group received LaSota vaccine following IBDV challenge, the3rd group received garlicin treatment (1 g. / L drinking water for 10 days simultaneously with LaSota vaccine) .The erythrogram in the three IBDV challenged groups showed a significant decrease in the RBCS count , hemoglobin concentration (HB) , Packed cell volume (PCV%) and mean corpuscular hemoglobin concentration (MCHC) compared to control. The leukogram in the three IBDV challenged groups showed mild significant leukocytosis, heterophilia and lymphocytosis in the positive control , and pronounced significance in the 2nd and 3rd IBDV infected groups but lymphocytosis was higher in the 2nd group compared to the 3rd group. Obtained results showed that garlicin did not show the expected improvement in the serologic response, (BBI) or hemogram but it had a hepatoprotective and nephroprotective value.

Key words: immunosuppression, Garlicin , IBDV .

Introduction

Garlic (Allium sativum) has attracted the attention of modern human medicine because of its wide spread health use, since it help to maintain good health warding off illness and providing good vigor, this was attributed to its antioxidant effect, beside its ability to reduce the risk factors of cardiovascular diseases and cancer in humans **Petkov(1986), Borek (2001) .**Garlic can help the stimulation of immune functions and can help in the detoxification of foreign compounds beside its hepatoprotective and antimicrobial effects **Banerjee and Maulik (2002)**.The key active ingredient in garlic is a powerful plant chemical called allicin which rapidly decomposes to several volatile organosulphur compounds with bioactivities. Allicin has antimicrobial effects *in vitro* against many viruses, bacteria, fungi and parasites. Dried, powdered and oil preparations of garlic have no significant antimicrobial activity **Chang and Cheong (2008)**.The object of our research was to study the immune stimulating effect of garlic extract in immunosuppressed chicks experimentally infected with IBDV, as well as its effect on the growth performance beside the antioxidant effect and some biochemical parameters.

Materials and Methods.

Materials.

Specimens.

Specimens were collected from experimental birds as scheduled in (table **-1)**

Experimental birds.

One day old chicks.

Thirty., day old broiler chicks "cobb" obtained from a private source were used in the pre- experiment to examine the safety of garlicin for chicks , beside 110 day old Saso chicks, were used to investigate the immunostimulating effect of the garlicin. The experimental design of the present work is as described in **(table-1)**.Birds were reared in floor pens under strict hygienic condition.

Ration

Starter pelleted broiler ration "3000 k Cal energy, 21% protein and 3.6% fat "EL-Eslamiya company"

Viruses.

Virulent IBDV field isolate previously isolated and identified **Bayoumie and Mohamed (2008)**., this isolate was used in the present study, its titer was $10^{5.5}$ EID_{50} /0.1 ml. Challenge dose was 10^3vp/ chick

NDV LaSota vaccine strain (intervet, Lot 10631 kg 01, EXP.2/2013) was used. Vaccinated birds were receiving 10^6 EID50/0.1 ml individually via eye drops

Haemagglutinating antigen.

Allantoic fluid from chicken embryos inoculated with La Sota NDV was as haemagglutinating antigen for HI-test **Allan et al (1978).**

Garlicin.

Garlicin ® is a water soluble powder (WSP) garlic extract (Allicin C6H10S3)(Active) 25% for Vet .use ,Shandong Luxi Animal Medicine Share CO, LTD,PRC .Batch NO. (20090301) Mfd .date(3/2009).Exp .date(3/2012) .The recommended dose was 1g./L DW for 7 successive days.

Methods.

Pre-experiment (safety trial).

Thirty., day old broiler chicks were reared, 10 chicks were used as negative control while the remaining 20 were given the tested product at a rate of 1 g/L Via drinking water for 17 day . .

Experimental Trial

One hundred and ten day old Saso chicks were used , 30 were kept as negative control .The remaining 80 chick were challenged with a field isolate of IBDV at 7 days of age to induce bursal atrophy and create immunosuppression ,five chicks were sacriefied from each group to perform BBI: as an indicative for successful immunesuppression. The immunosuppressed birds were divided into three equal groups.The 1st group was (IBDV challenged only i.e. positive control), the 2nd group (IBDV challenged ,then La sota vaccinated), the 3rd group (IBDV challenged ,then LaSota vaccinated simultaneously with garlicin medication **(table-1). Serology.**

HA and HI tests for NDV, were performed according to **Villegas (1991) .**

Table (1). Shows the experimental design on Saso birds

DIARY	-ve control	+ve control (1st group)	IBDV Infected chicks , then vaccinated with LaSota (2nd group)	IBDV Infected chicks , then vaccinated with LaSota + garlicin (3rd group)
7th DAYS	---	IBDV challenge		
12th DAY	B:B INDEX			
13th DAY	---	-------	LA SOTA	LA SOTA+Garlicin
23rd DAY	Evaluation of the haemagglutination inhibition (HI) titer			

Hematological assay

Experimental birds were sacrificed individually to obtain blood. Samples of blood were collected on EDTA and another samples were processed to obtain sera and plasma samples for determination of clinical chemistry parameters, blood films were prepared for differential leukocytic count. The erythrocytic ,and total leukocytic counts were performed as described by **Harrison and Harrison (1986*)***. Hemoglobin estimation was performed according to **Feldman et al (2000)**. Packed cell volume (PVC),(MCH) , mean corpuscular volume (MCV),Mean corpuscular hemoglobin concentration(MCHC) were calculated according to **Young (2001).**

Clinico -chemical assay: Serum Alanine aminotransferase (ALT) and aspartate aminotrans-

ferase (AST) were performed according to **Henry (1974)** as an indicative for liver functions , while estimation of serum uric acid and serum creatinine were performed according to **Tietz (1976)** as an indicative for kidney functions . Serum Alkaline phosphatase were determined according to **Tietz (1976)**

Oxidant/anti-oxidant markers:L- Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase activity(CAT) were colorimetrically assayed according to **Sinha (1972), Satoh (1987), Packer and Glazer (1990).**

Evaluation of immunosuppression .

Immunosuppresion following infection with IBDV, was evaluated using (HI for NDV) as described by **Villegas(1991)**., and **(BBI)**as described by **Lucio and Hitchner (1979).**

Performance

The body weight (BWT), body weight gain (BWG) .were used as indicators for performance

Statistical analysis.

Data were statistically analyzed as described by **Snedecor and Cochran (1967)** using MSTAT–C computer program

Results

Results of the present work are illustrated in tables (2-8).

Table (2). shows the performance of cobb broiler chicks in the pre- experiment

Criteria	(-ve Control)	Garlicin 1 gm. / lit.
B.WT.at one day old	51.93±6.35	
B.WT.at 4 day old. (Before medication starts .)	66.05±2.12	69.7±1.53 N.S.
B.WT.at 11 day old.(7 day after medication)	156.85±6.6	159.35±6.12 N.S.
B.WT. gain. (7 day after medication.)	84.882	90.1
% of B.WT. gain. (7 day after medication)	56%	73%
B.WT. at 16 day old (12 day after medication.)	263.05±10.31	230.8±3.06**(p≤0.01)
B.WT .gain at 16 day old (12 day after medication)	200.1	160.6
% of B.WT. Gain (12 day after medication.)	133%	111.9%
B.WT. at 21 day old (17 day after medication)	636.75±17.4	534.4±10.63***(p≤0.001)
B.WT .gain from initial WT	555.1	466.3
% of B.WT. gain from initial WT	370.5%	325%

N.S. (non-significant)

Table (3). Hemogram and some biochemical parameters of the garlicin treated group compared to control in the pre -exp.(cobb chicks). (Mean ± S.E),(N=5)

Parameter group	(-ve control)	Garlicin treated group
RBCs 10^6/UL	3.02±0.03	2.6±0.03***
Hb gm/dl	11.92±0.3	10.9±0.3*
PCV%	34.8±.6	29.3±0.03***
MCV Fl	115.2±2.5	74.2±3.4***
MCH Pg%	39.47±1.09	41.9±2.5
MCHC%	34.3±1.15	37.2±3.5
TLC	25.0±0.13	26.9±0.53*
Heterophil	9.63±0.11	10.11±0.14*
Lymphocyte	12.88±0.18	14.23±0.27*
Monocytes	1.42±0.06	1.7±0.05*
Eosinophil	0.8±0.04	0.74±0.07
Basophil	0.17±0.04	0.12±0.03
AST (U/I)	70.9±5.12	92.1±2.03**
ALT (U/I)	16.67±1.23	17.28±1.25
ALP (U/I)	49.2±2.7	58.0±1.4
Creatinine (mg/dl)	1.07±0.05	1.16±0.07
Uric acid (mg/dl)	4.37±0.72	4.41±0.3

*(p≤0.05), ** (p≤0.01), *** (p≤0.001)

Table(4). Mean ± S.E of Body wt., Bursal wt., Bursal body weight ratio and Bursal body weight index of saso chicks 4 days after challenge with IBDV at 7 days of age (before medication).

Group parameter	B.WT	BUR.WT	B.B. RATIO	B.B.I
(-ve control)	70	0.29	0.00426	
IBDV challenged	69.25	0.1425	0.00204	.4788

Table (5). Mean ± S.E of Body wt., Bursal .wt., Bursal body weight ratio and Bursal body weight index of saso chicks in the different experimental groups (after garlicin medication) (N= 5).

Groups / Parameters	(-ve control)	(+ve control)	IBDV Infected chicks , then vaccinated with LaSota	IBDV Infected chicks , then vaccinated with LaSo-ta + garlicin
B.WT	197.00±14.27 [a]	152.4±5.19 [b]	139.2±5.11 [b]	153.2±7.53 [b]
Bu.WT	1.06±0.08 [a]	0.13±0.02 [b]	0.24±0.03 [b]	0.19±0.02 [b]
B.B ratio	0.538±0.001 [a]	0.085±0.001 [b]	0.172±0.002 [b]	0.124±0.002 [b]
B.B.I.	1	0.155	0.31	0.232

Table (6). Geometric mean titer of Hemaglutination inhibition values of saso chicks in the different experimental groups (Mean ± S.E), (n= 5)

Groups / Parameters	-ve control	+ve control	IBDV Infected chicks, then vaccinated with LaSota	IBDV Infected chicks , then vac-cinated with LaSota + garlicin
HI	0	0	5.4±0.68 [a]	4.8±0.66[a]

Table (7). Hemogram of saso chicks in the different experimental groups. (Mean ± S.E) ,(n= 5)

Groups / Parameters	(-ve control)	(+ve control)	IBDV Infected chicks , then vaccinated with LaSota	IBDV Infected chicks , then vac-cinated with LaSota + garlicin
RBCS 10^6/UL	3.00±0.09 [a]	2.46±0.23 [b]	2.47±0.07 [b]	2.49±0.05 [b]
Hbgm/dl	9.39±0.13[a]	7.14±0.43 [c]	7.56±0.17 [b]	7.48±0.26 [b c]
PCV%	33.4±0.75 [a]	30.0±0.66 [b]	31.0±0.71 [b]	30.8±0.37 [b]
MCV FL	111.3±2.87 [b]	121.9±0.8 [a]	125.5±1.49 [a]	123.5±1.14 [a]
MCH Pg%	31.3±0.73 [a]	29.0±0.93 [a]	30.6±0.77 [a]	30.0±0.26 [a]
MCHC%	28.11±0.32 [a]	23.8±0.86 [b]	24.39±0.63 [b]	24.33±0.37 [b]
TLC	18.9±0.25 c	21.5±0.44 b	24.1±0.45 a	22.92±0.55 a
Heterophil	5.54±0.32 b	6.13±0.36 a	6.96±0.27 a	6.75±0.08 a
Lymphocyte	10.93±0.19 c	12.76±0.18 b	14.31±0.4 a	13.2±0.42b
Monocytes	1.44±0.08 a	1.56±0.1 a	1.69±0.12 a	1.74±0.1 a
Eosinophils	0.91±0.08 a	0.94±0.04 a	1.06±0.07 a	1.11±0.14 a
Basophils	0.08±0.04 a	0.09±0.05 a	0.09±0.06 a	0.12±0.06 a

Table (8). Mean ± S.E of some anti-oxidant and some biochemical parameters of the different experimental groups (n= 5).

Groups / Parameters	(-ve control)	(+ve control)	IBDV Infected chicks , then vaccinated with LaSota	IBDV Infected chicks , then vaccinated with LaSota + garlicin
MDA mmol/L	42.37±3.67 [a]	58.25±7.49 [a]	21.67±3.27 [b]	43.09±5.06 [a]
CAT µ/gm Hb	0.64±0.07 [b]	1.05±0.06 [a]	1.03±0.04 [a]	1.01±0.13 [a]
SOD µ/gm Hb	0.44±0.03 [b]	0.57±0.04 [a]	0.58±0.03 [a]	0.56±0.03 [a]
AST (U/I)	69.84±10.75 [c]	229.96±34.08 [b a]	269.18±13.81 [a]	167.54±27.34 [b]
ALT (U/I)	16.0±1.4 [b]	23.6±1.8 [a]	18.8±1.2 [b a]	17.6±2.2 [b]
ALP (U/I)	52.2±4.02 [c]	101.3±5.24 [a]	143.38±9.57 [a]	75.76±5.9 [b]
Creatinine (mg/dl)	1.19±0.13 [a]	1.41±0.22 [a]	1.55±0.07 [a]	1.4±0.25 [a]
Uric acid (mg/dl)	6.17±0.59 [c b]	8.93±0.93 [a]	8.94±0.71 [a]	7.57±0.72 [b a]

Different letters in the same row indicate significance($p \leq 0.05$)

Results and Discussion.

The main active component of garlic is allicin, which is an organosulfur compound **Ankri and Mirelman. (1999), Miron *et al* (2000).** When a garlic bulb is damaged separate segments containing the non-protein amino acid alliin and the enzyme allinase are opened, resulting in the production of allicin in a rapid self-limiting reaction **Haciseferogullari *et al* (2005).**The effect of allicin on avian cells has not been described. However, because of its higher level of organization, avian cells are probably also less sensitive to allicin than microbe **Velkers *et al* (2011)**.

Birds in the pre- experiment given garlicin at the rate of 1 g./L. had a non-significant increase in body weight gain at the 7th days from the onset of medication as compared to the negative control . latter on this increased weight gain had significantly declined compared to control after extending the medication period up to 12 and 17 days **(table-2).**

The examined hemogram of chicks in the pre-experiment showed a significant decrease in RBCS count, HB concentration, PCV%, MCV. **(table-3)** this agree with the previous findings of **Ademola *et al* (2004)** whom reported slight decrease in total erythrocytic count and haemoglobin in garlic powder treated birds, they mentioned that this effect could be due to the presence of hemolytic bioactive constituents in garlic or their respective metabolites.

The examined leucogram in the pre- experiment showed a significant increase in the TLC, heterophils ,lymphocytes and monocytes count **(table-3)** .Similarly **Ademola *et al* (2004), Prasad *et al* (2009**) reported increase in total white blood cells, lymphocytes and heterophils in garlic powder treated birds as compared to the control. These data, therefore, support the earlier reports of **Sumiyoshi (1997)** whom recorded that garlic extract stimulates immune functions. This observation may also explain the role of garlic in activating the natural killer cells, the function of T lymphocytes and the increased level of interleukin-2 **Tang (1997)**.

As for the clinicochemical parameters, significant increase in AST level was observed in the garlicin treated group in the pre-experiment **(table-3)**. This agree with the previous report of **Mahmoud *et al* (2008)** whom demonstrated that dietary garlic supplementation had significantly ($P < 0.05$) increased the level of AST while ALT concentration was not affected**.**

The effect of bird strain on the response to a specific garlic preparation should not be ignored **McCaleb (1993)**, this fact may be used to interpret the mild difference observed between the two bird strains used in the present study (cobb broilers and saso breed) . It worth to mention that the observed picture of liver and kidney functions in the cobb broilers is the result of extending medication for 17 consecutive days . It should be noted that cobb broiler chicks were used to obtain better look on the performance parameters since the native breed mix leads to great variance and non homogencity in their growth performance.

IBD is a highly contagious viral disease of young chickens which is characterized by destruction of the lymphoid cells in the bursa of Fabricius., other lymphoid organs are also affected but to a lesser degree., the disease has a worldwide distribution and causes severe economic loses to the commercial poultry industry **Hamoud *et al* (2007) .** Immunosuppression is a common sequllea for IBD infection **Lukert and Saif (1997).** Clinical IBD is most commonly recognized in susceptible 3-6 weeks old chicks in both field or experimental infection .,one day old chicks seldom show any clinical signs or mortality. **Faragher *et al* (1974), Wyeth and Cullen (1976), Meulemans *et al* (1977), Hirai *et al* (1979), Fadly and Nazerian (1983)** beside **Bayoumie *et al* (2009)** found that IBDV infection in the first week caused severe defect in the humeral immune response .

The second experiment was performed to study the immunostimulating potency of the garlicin . 110 Saso chicks were bred, 80 birds of them were challenged with a field isolate of IBDV at the 7th days of age to induce bursal atrophy and create immunosuppression , the remaining 30 chick were kept as negative control**. Lucio and Hitchner(1979)** used the

(BBI) as an indicator for immunosuppresion specially if it was lesser than (0.7).In the present study five chicks from each group were used to study the (BBI) 4 days post challenge at 7 days of age and its value was 0.4788 which is indicative for successful induction of bursal atrophy and consequent immunosuppression **(table-4)** . The inoculated native Saso chicks at 7 days of age did not express any clinical signs or mortality which agree with the previous finding of **Bayoumie *et al* (2009)**. The (immunosuppressed) birds were a liquated into three groups 1st group (IBDV challenged only i.e. positive control), 2nd group (IBDV challenged, then La sota vaccinated),3rd group (IBDV) challenged ,then LaSota vaccinated simultaneously with garlicin medication **(table, 1).**

The hemogram of the of the three IBDV challenged group showed a significant decrease in the RBCS count, HB concentration, PCV%, MCHC % and a significant increase in MCV in these groups too compared to control **(table, 7)** this agree with the previous finding of **Bayoumie *et al* (2009) .**

There is wide variation in the normal leucograms among birds of the same species therefore the values of diagnostic importance must differ greatly from normal values **Campbell (1994).**The leukogram of the experimental groups showed a significant increase in TLC ,heterophila and lymphocytosis in the three IBDV challenged groups this increase was mild for the positive control only and was pronounced in the 2nd and 3rd groups vaccinated with LaSota for the TLC and heterophils since they received two antigenic stimulus .Lymphocytes were higher in the 2nd group compared to the 3rd group **(table-7)**. This could explain the lower serologic response in the 3rd group **(table-6)**.Thus it obvious that garlicin did not cause the expected improvement in the serologic response in the treated group.

Reactive oxygen species (ROS) are defined as chemical entities that possess one or more unpaired electron in their outer orbit .Unpaired electrons seeks other electrons to become paired .therefore ,they attack other molecules .A chemical compound becomes a free radical by either gaining an additional electron or by losing one **Murry *et al* (1993).** If reactive species are not scavenged by antioxidants then they can react with other components of the cell. The consequence of such detrimental reactions includes lipid per-oxidation, protein modification and damage to DNA **Ischiropoulos *et al* (1995).**

Munksgaared (1997) classified the antioxidants according to their mode of action into (Scavenging antioxidants , Preventive antioxidants , Enzymatic antioxidants) These systems acts by catalyzing the oxidation of ROS to other molecules e.g. superoxide dismutase, catalase and Glutathione peroxidase (GSH-Px) antioxidants are oxidized by ROS in a biological system, decreasing the rate at which the ROS react with crucial cellular components like lipid membranes, DNA, or proteins. Some antioxidants or its precursors are consumed as part of the diet and are considered as primary defenses against ROS **(Tajima *et al.,* 1983)**. The significant increase in superoxide dismutase (SOD), catalase (CAT) level. In all treatments of the underlying experiment compared to control group could be due to increase in (ROS) **(table, 8)**.The biological system responds to this by increasing the activity of these antioxidant, enzymes in order to be able to combat the increasing free radicals and put it under control **Tisan and Bowen (1995)**

The excessive formation of (ROS) is not the initial step in the pathogenesis of diseases but it causes rapid progression of the disease**.** The most dangerous effect of oxidative stress arises as a consequence of a pathologic event, the antioxidant defense system promotes the regulation and expression of antioxidant enzymes **Frankand Biesalski (1997),Mates *et al*(1999**). SOD reduces superoxide to hydrogen peroxide; catalase and (GSH-Px) reduce hydrogen peroxide from the SOD reaction to water. In addition, glutathione peroxidase can reduce lipid peroxides directly. There is still insufficient knowledge about the kinetics or molecular regulation of these enzymes in mammalian tissues (**Ji, 1995b) .**

The increase in malondialdehyde (MDA) level in the positive control had led to the release of SOD and CAT . The MDA level was non-significant in 3rd group, it also showed a significant decrease 2nd group **(table-8)**.This could be attributed to the synergistic increase in both MDA and CAT activity which overcome the ROS generated due to the further exposure to the viral vaccine stimulus. In a recent study **Avci *et al*. (2008)**demonstrated that garlic significantly lowers plasma and erythrocyte MDA levels while increasing antioxidant enzyme activity in elderly subjects, suggesting its peroxidation reducing effect**.** Whole garlic and aged garlic extract exhibit direct antioxidant effects and enhance the serum levels of two antioxidant enzymes, catalase and glutathione peroxidase **Toro *et al*. (1994) ,Yamasaki and Lau (1997).**The sulfur compounds found in fresh garlic appear to be nearly 1000 times more potent as antioxidants than crude, aged garlic extract **McCaleb (1993).** Although garlic powder represents the composition of garlic cloves better than any other type of processed garlic, some changes do occur during processing. Therefore, the a possible reason for the negative results is that the active constituents may have not been sufficient.

Aspartate Aminotransferase (AST) high activity has been described in association with liver damage **Lumeij (1994)**., (AST) activity greater than 230 IU/L is considered abnormal and it is indicative of liver damage **Lumeij (1994), Riddell (2009), Harris (2001) and Hoefer (2010).**In the present study the three experimental groups showed a significant increase in AST compared to control but the lowest value was observed in the garlicin treated group **(table-8)**,this may be due a hepatoprotective effect of the garlicin.

ALP is involved in energy transfer for Ion exchange across the cell membrane, ALP activity may be elevated due to irritation of cells in different tissues. Pathological elevation of ALP is common in liver diseases, induced stimulation of osteoplastic activity and enteritis **Bayoumie *et al* (2009).**In the present study ALP activity was significantly higher in the IBDV 1st and 2nd group but the 3rd group had low ALP value compared them **(table-8)** this may be attributed to the hepatoprotective effect of the garlicin.

Blood creatinine is derived mainly from the catabolism of creatine found in muscle tissue., phosphocreatine is used to store energy in the muscle and its catabolism to creatinine occurs at a steady rate ., excretion of creatinine occurs solely via kidney **Lumeij (1994).,** creatine is excreted in urine before it has been converted to creatinine so it is not providing accurate assessment for avian renal function ., and there is a slim margin between the physiological and pathological level of creatinine., usually the normal range of creatinine is 1.1-1.4mg /dl **Lumeij (1994)**.Pathologically severe kidney damage can raises creatinine specially if the filtration rate decreased .

Uric acid is the major product of nitrogen catabolism ., its synthesis occurs mainly in liver and in renal tubules **Lumeij (1994).,** 90% of blood uric acid is eliminated by tubular secretion ., if the blood uric acid concentration exceeds its solubility in the serum, gout may occur .,it should be noted that normal chicken uric acid level exceeds 6 times that of human due to the high sodium level in avian plasma and high avian body temperature .,both factors increase the uric acid solubility in serum ., thus normal uric acid level is 2-11 mgm/dl. Age and diet may influence blood uric acid level., Hyperuricemia is a good indicator of renal disease, hyperuricemia can be expected if glomerular filtration decreased below 70-80%.,decreased filtration may occur due dehydration from viral infection., The nephrogenic affinity of IBDV appears to be behind the renal alterations **Eterradossi and Saif (2008),**in the present study the three groups challenged with IBDV had a significantly higher uric acid values but the group receiving garlicin had the lowest value compared to the non-treated group**(table-8)**.

References.

Ademola, S.G;Farinu, G.O;Ajayi-Obe, A.O. and Babatunde, G.M. (2004). Growth, haematological and biochemical studies on garlic

and ginger fed broiler chicken. Moor J. Agric. Res. 5(2).122-128

Allan, W. H.; Lancaster, J. E. and Toth, B. (1978). ND vaccines: their production and use. FAO Animal Production and Health Series No. 10 Rome.

Ankri, S. and D. Mirelman.(1999). Antimicrobial properties of allicin from garlic. Microbes Infect. 1:125–129.

Avci ,A.; Atli, T. and Ergüder, I.B. (2008). Effects of garlic consumption on plasma and erythrocyte antioxidant parameters in elderly subjects. Gerontology 54: 173–176.

Banerjee,S.K. and Maulik,S.K.(2002). Effect of garlic on cardiovascular disorders: a review. Nutr.J.1:4

Bayoumie, H.A.A. and Mohamed,IAA. (2008). Laboratory evaluation of selected disinfectants on gumboro disease virus . Assuit Vet.Med.J.55:231-242.

Bayoumie, H.A.A; kaied, I.H. Galhoom; Omayma, M. samy; Sahar, N .Mohamady and Dina, M. Mahdy (2009). Clinical, hematological and biochemical alterations during the

coarse of induced IBDV in native Saso chicken breed.Zag.Vet.J. 37:186-199**.**

Borek, C.(2001). Antioxidant health effect of aged garlic extract. J.Nutr.,131:10105-10155.

Campbell, T.W. (1994). Hematology :In Avian medicine principles and application, edited by Richie B, Harrisson, G . andHarrissan,.L. wingers publishing company , pp:176-180.

Chang, K.J. and Cheong, S.H. (2008). Volatile organosulfur and nutrient compounds from garlic by cultivating areas and processing methods. Fed. Am. Soc. Exp. Bio. J. 22: 1108.

Eterradossi, N. and Saif, Y.M.(2008). Infectious Bursal Disease :in diseases of poultry.12th ed.,Saif,YM.Pp185.Blackwell Publishing

Fadly, A.M. and Nazerian, K. (1983). Pathogenesis of infectious bursal disease in chickens infected with virus at various ages. Avian Dis.27 :714-723.

Faragher, J.T. ; Allan, W.H . and Wyeth, RJ (1974). Immunosuppressive effect of infectious bursal agent on vaccination against Newcastle disease Vet Rec. 95:385-388.

Feldman ,B.F., Zinkl J.G. and Jain .N.C. (2000). Schalm's veterinary Hematology . 5th Ed , London, New York , TOKYO.

Frank, J. and Biesalski, H. K. (1997). Involvement of reactive oxygen species in the progression of renal disease and the significance of antioxidants in the therapy. Nieremud Hochdruckk Rannkhei Ten, 26 (8). 342 -346.

Haciseferogullari, H.; Ozcan, M.; Demir, F. and Calisir, S. (2005). Some nutritional and technological properties of garlic (*Allium sativum* L.). J. Food Eng. 68:463–469.

Hamoud, M.M., P. Villegas and S.M. Williams(2007). Detection of infectious bursal disease virus from formalin-fixed paraffin-embedded tissue by immunohistochemistry and real-time reverse transcription-polymerase chain reaction. Journal of Veterinary Diagnostic Investigation, 19: 35-42.

Harris , D.J (2001). Avian Clinical Pathology . http :www.vin .com / VINDBP Pub/ search PB / proceedings PR5000/p

Harrison G.J and Harrison L.R (1986). Clinical avian medicine and surgery W.B Saunders company, Philadelphia.

Henry , R.Z (1974). Clinical chemistry , principle and techniques, 2nd Edition , Harper and raw. p.52.

Hirai, K.; Kunihiro, K. and Shimakura, S. (1979).characterization of immunosuppression in chickens by infectious bursal disease. Avian Dis .23:950-965.

Hoefer, H.L.C. (2009). Basic Avian Clinical pathology http: // www heidihoefer .com / pages / birds / Avian – blood – test. Htm

Ischiropoulos, H.; Al.-Mehdi, A. and Fisher, A. (1995). Reactive species in ischemic rat injury: contribution of peroxynitrite. The American Physiological Society, pp L158-L164.

Ji, L.L. (1995b). Oxidative stress during exercise: implication of antioxidant nutrients. Free Radic. Biol. Med. 18(6). 1079-1086.

Lukert, P.D. and Y.M. Saif, (1997). Infectiousbursal disease. In Dis. of poultry, Eds.,

B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, 10th ed. Iowa State Univ. Press, Ames, pp: 271-738.

Lucio, B. and Hitchner, SB.(1979). IBDV emulsified vaccine effect upon neutralizing antibody level in the dam and subsequent protection of the progeny. Avian Dis .23:466-478.

Lumeij, J.E. (1994). Hepatology and nephrology in avian medicine principles and application, editors Richine B , Harrisson G and Harrisson L. Wingers publishing company , pp:522-555.

Mahmoud, R. El., Hassanien, El. I., El-Kholy, M. El.H. and Soliman, M. H. (2008). Influence Of Dietary Garlic Supplement On Performance, Serum Metabolites, Some Lymphoid Organs And Carcass Traits In Japanese Quails 9th Vet. Med. Zag. Conf. (20-22 Aug 2008) Port-Said**.**

Mates J. M.; Perez-Gomez C. and Nune-z-de Castrol (1999). Antioxidant enzymes and their implications in pathophysiologic processes. Front. Biosci., 15: D339–D345.

Meulemans , G ; Halen ,p.; Vinderogel ,H. and Gouffaux, P.(1977). The immunodepressive effect of IBD agent on vaccinations against NDV. Res.Vet.Sci.22:222-224.

McCaleb R. (1993). Antioxidant, antitumor and cardiovascular actions of garlic. Herbal Gram; 29:18.

Miron, T., A. Rabinkov, D. Mirelman, M. Wilchek, and L. Weiner(2000). The mode of action of allicin: Its ready permeability through phospholipid membranes may contribute to its biological activity. Biochim. Biophys. Acta 1463:20–30.

Munksgaared, (1997). Reactive oxygen species and antioxidants in inflammatory diseases. J. Clin. Periodontol, 24: 287-296.

Murry,R.K.;Granny,D.K.;Mayers,P.A.andRodwell, V.W. (1993). Harpers Biochemistry. 23 Edition .Middle east Edition, Appleton and Lange Librariri du Liban, Beriut, California.

Packer, L. and Glazer, A. N. (1990). *Method in enzymology .vol. 186 part B , Academic press Inc. new York , PP. 251.*

Petkov,v.(1986). Bulgarian traditional medicine: a source of ideas for phytopharmacological investigation. J.Etheniapharmacol. 15:121-132.

Prasad, R.; Rose, M.K.; Virmani, M.; Garg S.L.andPuri J.P.(2009). Effect of Garlic (*Allium sativum*) supplementation on hematological parameters in chicken (Gallus Domesticus). Indian J. Anim. Res., 43 (3). 157-162.

Riddell, G (2009). Comparative anatomy , histology and physiology of the chickens http:// cal.vet.upenn.edu/projects/ poultry / syllabus / pages 37-44.htm.

Satoh, K. (1987). Lipids peroxide (Malondialdehyde) coloremetric methods. Clin. Chem. Acta, 90: 37

Sinha, A. K. (1972). colorimetric assay of catalase . Analytical biochemistry,47,38**9**

Snedecor,GW and Cochran,WG(1967). Statistical methods,6th ed.,Iowa .Univ press, Ames., Iowa, USA.

Sumiyoshi, H. (1997). New pharmacological activities of garlic and its constituents (Review). Folia Pharmacol. Japonica110: Suppl. I, 93-97.

Tang, Z. (1997). Bull. Human Med. Univ. 22: 31246-31248.

Tajima, K.; Sakamoto, M.; Okada, K.; Mukai, K.; Ishizu, K.; Sakurai, H. and Mori, H. (1983). Reaction of biological antioxidants with superoxide generated by cytochrome P-450 model system. Biochem.Biophys. Res. Commun, 115: pp 1002-1008.

Tietz, N. W. (1976). Fundamentals of clinical chemistry, W B. Saunders Philadelphia.

Tisan, L.; Cai, A. and Bowen, R. (1995). Effects of caloric restriction on age related oxidative modification of macro molecules and lymphocyte proliferation in rat. Free Rad. Biol. Med., 19: 859-865.

Torok, B.;Belagyi, J.; Rietz, B. and Jacob R. (1994). Effectiveness of garlic on the radical activity in radical generating systems. Arzneimittel for schung; 44:608-611.

Velkers , F. C.; Dieho , K.; Pecher , F. W. M.; Vernooij , J. C. M.; van Eck , J. H. H. and Landman, Villegas, P.C. (1991). Lab.

manual .avian virus diseases. pp 16-19.Coll. Vet. Med. Athens, Georgia 30602.

Wyeth, P.J ; Cullen , G.A (1976). Maternally derived antibody effect on susceptibility of chicks to infectious bursal disease. Avian Path .5:252-260.

Yamasaki, T. and Lau, B.H. (1997). Garlic compounds protect vascular endothelial cells from oxidant injury. Nippon YakurigakuZas-shi; 110 Suppl 1:138P-141P.

Young, D.S. (2001). Effects of disease on clinical lab. tests , 4th ed AACC, London.

التقييم الفارماكولوجي لمستخلص الثوم (الجارليسين)® في الدجاج الساسو المعدي اصطناعيا بفيروس الجامبورو

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الملخص العربي

في دراسة سابقه رُصد فيها التثبيط المناعي بعد ألعدوي الاصطناعية بالجامبورو في كتاكيت الساسو في أقصي صُوره عند العدوى في عمر أسبوع . لذلك رأينا دراسة قدرة الجارليسين علي تحسين الاستجابة المناعية في حالات مماثلة. و تأثيراته علي معدلات النمو و قيمته كأحد مضادات الأكسدة وتأثيره علي صوره الدم وبعض وظائف الكبد و الكلي للكتاكيت المُعالجة.

1-أجُريت دراسة استكشافية لدراسة حدود الأمان للجارليسين علي معدلات النمو و صوره الدم ووظائف الكبد و الكلي في كتاكيت تسمين (كب). أُعُطيت الجارليسين بمعدل 1 جم /لتر ماء شرب لمدة 17 يوم بدأً من اليوم الرابع من العمر .و قد أدي ذلك إلي ارتفاع متوسط وزن الطيور بعد 7 أيام من بداية العلاج ارتفاعاً غير معنوياً مقارنة بضابط التجربة. هذه الزيادة قد انخفضت معنوياً بعد تمديد فترة علاج إلى12 و 17 يوما من بدء العلاج . وبدراسة التغيرات الدموية الحادثة في الطيور المعالجة لوحظ انخفاض ملحوظ في عدد كرات الدم الحمراء و الهيموجلوبين و PCV و MCV و بدراسة leukogram لاحظنا زيادة كبيرة في عدد كرات الدم البيضاء وخلايا الليمف والخلايا الوحيدة والخلايا المغايرة. بالنسبة للتغيرات الكيميائية الحادثة في الطيور المعالجة فقد لوحظ زيادة كبيرة في مستوى (AST) في المجموعة المعالجة بمستحضر الجارليسين يمكن ان نعزيها إلي طول فترة العلاج .2

2- لدراسة تأثير الجارليسين علي المناعة . تم تربيه 110 كتكوت ساسو كالتالي :(30 ضابط سلبي) و (80 أُحُدثت بها عدوي الجامبورو وقد تم التأكد من ذلك من خلال إجراء معامل وزن البرسا علي خمس طيور من كل مجموعة و بعد التأكد من نجاح حدوث التثبيط المناعي تم تقسيم الطيور المثبطة مناعياً إلي ثلاث مجموعات. الأولي ضابط ايجابي و الثانية مصابة بالجامبورو ثم أعطيت لقاح اللاسوتا و الثالثة مصابة بالجامبورو ثم أعطيت لقاح اللاسوتا ثم الجارليسين لمدة عشرة ايام) .في نهاية التجربة تم تقييم الاستجابة السيرولوجية لفيروس اللاسوتا باستخدام اختبار منع التلازن الدموي للنيوكاسل و كذلك تم أعادة تقييم معامل وزن البرسا .لُوحظ انخفاض ملحوظ في عدد كرات الدم الحمراء و الهيموجلوبين وPCVو MCHCفي المجموعات الثلاث التي تم تقطير فيروس الجامبورو بهم مقارنة بالمجموعة الضابطة . كما أظهرت نتائج فحوصleukogram في المجموعات التجريبية زيادة كبيرة في عدد كرات الدم البيضاء و الخلايا المغايرة و خلايا الليمف في المجموعات الثلاثة التجريبية وقد كانت الزيادة في مجموعة الضابط الايجابي اقل منها في المجموعتين اللتين تم تحصينهم بفيروس اللاسوتا، و قد لوحظ أيضا ان الخلايا اللمفية كانت اقل في المجموعة التي تلقت العلاج بالجارلسين مقارنة بباقي المجموعات التجريبية مما قد يبرر انخفاض التتر المناعي للنيوكاسل في هذه المجموعة .و عليه من مراجعة معامل وزن البرسا في المجموعة التي تلقت العلاج بالجارلسين و التتر المناعي للنيوكاسل بها مُقارنة بباقي المجموعات التجريبية نجد انه ليس هناك أي تأثير منشط للمناعة للجارليسين .و ليس له قيمة كمضاد للأكسدة ولكنه حّسن وظائف الكبد و الكلي فقط في المجموعة الثالثة مقارنة بباقي المجموعات الثلاث سس. و يمكن أن نعزي هذه النتائج إلي كونهُ مخلق صناعياً و هو ما تختلف نتائجه عن الثوم الطبيعي و الثوم المجفف .