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Antioxidant and Immunostimulant Effects of Basil (*Ocimum basilicum*) Against Gibberllic Acid and Auxin Supplemention in Broilers Ration

[1]K. Kahilo, [2]H. Elkany, [3]K. Sadek and [1]A. Kheir-Eldeen

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Kafer ELsheikh University, Egypt
[2]Department of Poultry Diseases, Faculty of Veterinary Medicine, Damnhour University, Egypt
[3]Department of Biochemistry, Faculty of Veterinary Medicine, Damnhour University, Egypt

Abstract: The present study was conducted to evaluated the antioxidant and immunostimulant effects of basil aganist gibberllic acid and auxin supplemention in broilers ration. Seventy five clinically healthy, one day old broilers chicken were used in this study and divided into 5 groups, 15 birds each. Group 1 served as a control; group 2 received basal ration and 75 ppm [GA3] in drinking water; group 3 received basal ration and 75 ppm [IAA] in drinking water; group 4 received basal ration supplemented with 5 ml Ocimum basilicum [O. basilicum]/ kg &75 ppm [GA3] in drinking water and group 5 received basal ration supplemented with 5 ml [O. basilicum] / kg &75 ppm IAA in drinking water. At the end of experiment (6 weeks) and night fasting blood samples were collected from wing vein from all birds of each group. Experimental testes as alanine aminotransferase [ALT], aspertate aminotransferase [AST], S.Creatinine, Urea, cholesterol [S.T.Ch], triacylglycerid [TAG], total protein, albumine [A], globulin [G], A/G ratio, IgG, IgM. Tissue as (liver, thymus, muscle and bursa) obtained for measurement glutathione s transferase [GST], reduced glutathione [GSH], glutathione peroxidase [GPx], super oxide dismutase [SOD], catalase [CAT], malondialdehyde [MDA]. The results showed that, GA3 & IAA groups induced significantly decreased Immunity (IgG, IgM) level and antioxidant activity (SOD, CAT, GSH, GPx, GST). but, significantly increased when basil is added, increased s. cholesterol, TAG level, Blood urea and ALT in GA3 group, AST decreased in group containing basil and creatinine level not affected in all groups. MAD increased in group treated with GA3, IAA. but, decreased in group treated with basil. The findings of the present study suggest that immunity dysfunction, oxidative stress, are primary interacting mediators in the pathogenesis of broilers. Histophathological examination refered to muscle of a chicken of GA3 group showing lytic necrosis with infiltration of macrophage while Muscle of a chicken of IAA + basil group showing normal histological structure. From the obtained data, we can concluded that the feeding of basil has a good effects on antioxidant enzyme concentration and immunostimulant against effect of GA3 and IAA included in this study. The effects were pronouced after six weeks of feeding.

Key words: Plant growth regulator · Immunostimulant · Basil · Broilers · Antioxidant

INTRODUCTION

Plant growth regulators [PGRs] have been widely employed in recent decades to improve crops quality and yield. In the future the amounts of these substances placed into the environment may exceed those of insecticides [1]. The effect of (PGRs/phytohormones) on plants is well understood and are extensively used in agriculture, however Knowledge of the effects of phytohormones on animals, lacking. Although different phytohormones have been investigated on insects for their specific effects, reports concerning their use on animals remains limited [2 - 4].

Gibberellic acid [GA3] occurs naturally in the seeds of many species and is produced commercially by growing Gibberella fujikuroi fungus cultures. It is heat resistant, not losing its activity after 4 hours at 100°C [5].

Corresponding Author: K. Kahilo, Department of Biochemistry, Faculty of Veterinary Medicine, Kafer ELsheikh University, Egypt.

Auxins are a class of [PGRs] and morphogens located in shoot and root meristematic tissue, young leaves and in mature root cells. It is positively influence cell enlargement, bud formation and root initiation, also promote the production of other hormones and in conjunction with cytokinins, they control the growth of stems, roots and fruits and are capable of converting stems into flowers. Furthermore, induce sugar and mineral accumulation at the site of application. The most common Auxins found in plants is [IAA] [6].

In the recent years, the antioxidant and antimicrobial potential of plants have attracted the attention of scientific community. The antioxidants may be useful in retarding oxidative deterioration of food materials especially those with high lipid contents [7]. Also protect the living cells from oxidative damage that occur due to formation of free radicals and reactive oxygen species during metabolic activity. This oxidative damage of cellular constituents lead to cell injury leading to cell death which is associated with pathogenesis of various chronic diseases like carcinomas, coronary heart disease and many other health problems related to advance age [8].

Ocimum basilicum L. commonly called as Sweet Basil b elongs to family Lamiaceace is native plant of Indo-Malayan region. It is called the "king of herbs" which contains plenty of phyto chemicals with significant nutritional as well as antioxidant capabilities and health benefits [9]. Sweet basil is cultivated for production of essential oils, dry leaves as a culinary herb, condiment/spice or as an ornamental plant. It is used as an ingredient in various dishes and food preparations, especially in the Mediterranean cuisine [10]. Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactogogue, stomachic and tonic agents [11].

This study was planned to throw light on the effects of *Ocimum basilicum* as antioxidant & immunostimulant against effect of GA3& IAA.

MATERIALS AND METHODS

A total of 75 broilers chicken were used for performing this study. The birds were allocated in to5 groups 15 birds per each as follow:

- First group (group 1): kept on basal diet. Serve as control group.
- Second group (group 2): kept on basal diet and 75 ppm [GA3] in drinking water.
- Third group (group 3): kept on basal diet and 75 ppm [IAA] in drinking water.
- Fourth group (group 4):Kept on basal diet and 5 ml [O. basilicum]/ kg &75 ppm GA3] indrinking water.
- Fivth group (group 5): Kept on basal diet and 5 ml [O. basilicum] / kg &75 ppm [IAA] in drinking water.

At the end of experiment (6 weeks) and night blood samples were collected from wing vein from all birds of each group. Blood samples collected in centrifuge tubes and incubated at 37°C temperature for minimum 10 minutes for coagulation. Coagulated blood was centrifuged at 3000 r.p.m for 15 minutes and then serum was carefully separated from the clot and stored at -20°C tell assay performance of (other experimental testes) as ALT, AST, Creatinine, Urea,, S.T.Ch, TAG, total protein, albumine, globulin, A/G ratio, IgG, IgM.

Tissue as (liver, thymus, muscle and bursa) obtained for measurement (GST, GSH, GPx, SOD, CAT, MDA).

ALT, AST [12], blood urea [13], creatinine [14], t.Ch [15], TAG [16], total serum protein [17], albumin [18], globulin [19], CAT [20], GPx [21], GST [22], GSH [23]. MDA [24, 25].

RESULTS AND DISCUSSION

The increasing use of this substance agriculture making it as an interesting subject to investigate its possible adverse effects on different organ as liver as one of the main target organs for different xenobiotics, kidney and muscle.

Histophathological examination refered to muscle of a chicken of GA3 group showing lytic necrosis with infiltration of macrophage while Muscle of a chicken of IAA + basil group showing normal histological structure.

The obtained data showed that, the serum ALT level significantly increased in group fed diet contain (GA3) and non significantly differ in all treated groups. This result agree with Hassan and Al-Rawi [26] and Hussein *et al*. [27] who reported that ALT significantly increased by addition of GA3. Mourão *et al*. [28] found that IAA administration did not show any alterations in ALT. Also, this result was in agreement with Osman *et al*. [29] who reported that sweet basil not significantly affect ALT. In contrary, Muthu *et al*. [30] found that administration of GA3 significantly decreased the level of ALT in serum after the third week of treatment.

The serum AST level non significantly change for group fed diet contain (GA3) while, significantly decreased in groups containing (IAA, GA3& basil, IAA & basil). These results agree with Çelik and Kara [31] who found that IAA was decrease AST. But disagree with results obtained by Ali *et al*. [32] who found that GA3 significantly decreased AST.

Non significant change in creatinine allover the experimental period. These results were agreed with El-Sebai *et al*. [33], Morshed *et al*., [34], Osman *et al*. [29] who found that, GA3, IAA, basil had no effect on blood creatinine. Blood urea non significantly changes in all over experimental period except for group (GA3) there is significantly increase in urea content. This result was in agreement with Osman *et al*. [29] who reported that sweet basil non significantly change urea.

Similarly, Ali *et al*. [32], Hassan and Al-Rawi [26] they found that increase in urea content in groups treated with GA3. The elevated blood urea is correlated with an increased protein catabolism in the mammalian body or more efficient conversion of amonia to urea as a result of increased synthesis of enzymes involved in urea production [35].

The values of T.Ch & TAG non significantly increase in all group except for group containing (GA3). These results agree with the results obtained by Muthu *et al*. [30], Ali *et al*. [32], Hassan and Al-Rawi [26] they showed that GA_3 caused significant increase in T.Ch and TAG may be related to the mobilization of this compound from membrane stores due to increased lipid peroxidation or due to the activation of 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase responsible for increased synthesis of cholesterol [36]. Moreover, Osman *et al*. [29] reported that sweet basil non significantly affect serum T.Ch, TAG. while, Sadek *et al*. [37] showed that lipid lowering effects reflected in decreased serum TAG and T.Ch when basil found in diet of broilers.

The obtained data revealed that there is significantly increased in group (IAA& basil, GA3& basil) for total protein and non significantly changes in albumin. The values of globulin and A\G ratio significantly increased noticed in group (GA3& basil, IAA& basil) for globulin and A\G ratio if compared with non treated one. These results agree with Soliman *et al*. [38] who found that non significant alterations in plasma total protein and globulin in rat received GA3 in drinking water, Muthu *et al*. [30] reported that total serum protein remained stationary for all doses of GA3 treatment indicating that the protein metabolism was not affected by GA3 in male albino rats, Osman *et al*. [29] reported that sweet basil increased globulin production by the liver which reflects a good hepatic function of these birds and correlates very well with high immunity status of these birds. In the contrary, Elkomy *et al*. [39] reported that, GA3 has increased total blood protein summarized causes of raised blood total protein concentration with GA3 as dehydration.

The value of IgG, IGM significantly decreased in group receiving GA3, IAA and significantly increased in groups when basil were added. This explain the effect of basil as immunostimulant. These results agree with, Ali *et al*. [32] who found that significant decrease in immunoglobulin (IgG, IgM) in injected groups with all plant promoters when compared to control group which demonstrated that these promoters interact with immune cell and cause disturbance in immune system.

In the present study the antioxidant enzyme as (GST, GSH, GPx, SOD, CAT) significantly decreased in groups containing (GA3& IAA) and significantly increased in group (GA3, basil& IAA, basil) in organ as (liver, thymus, bursa, muscle). These results come in accordance with Soliman *et al*. [38] who reported that the GSH levels were significantly depleted in the spleen, lungs and stomach, SOD significantly decreased in the spleen, heart and kidney of rats treated with GA3, CAT decreased in lung of treated rats. Muthuraman *et al*. [40] who found that GSH, CAT content was reduced by GA3. Hussein *et al*. [41] found that statistically significant decreases in the mean values of GPx, SOD, CAT enzymes activities in suckling rats as well as in their mothers treated with GA3, Trodui *et al*. [42] reported that GA3 treatment in pregnant Wistar rats from the 14th day of pregnancy until day 14 after delivery revealed in erythrocytes a significant a decrease in antioxidant enzyme activities such as GPx, SOD, CAT. Ali *et al*. [32] found that decrease in erythrocyte GSH, SOD of the treated rats with GA3, IAA in comparison to that of the control group, Hassan and Al-Rawi [26] found that GA3 lead to decrease in GSH activity, CAT. Moreover, Sadek *et al*. [37] showed that Supplemented groups with basil has antioxidant activity revealed in significant increase in GSH, SOD, CAT in all examined tissues in comparison with those of control group, These findings closed to Politeo *et al*. [43] who found that free volatile aglycones of basil possess good antioxidant properties in two different methods as the 2,2/-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH) and ferric reducing/antioxidant power assay (FRAP) in comparable with that of the essential oil and well-known antioxidant butylated hydroxytoluene (BHT).

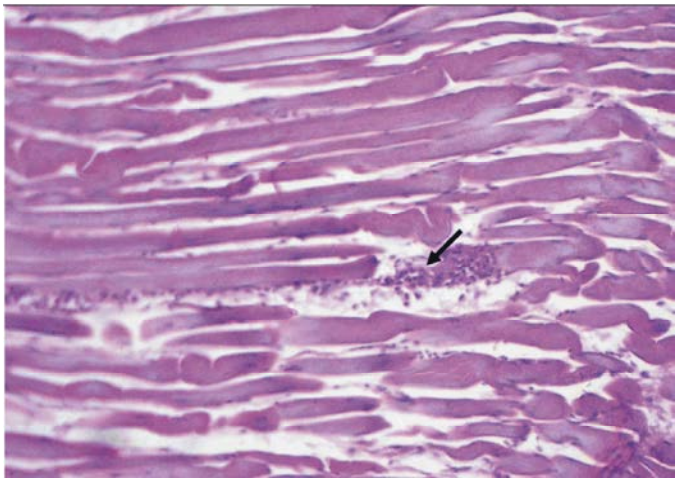

Fig. 1: Muscle of a chicken of GA3 group showing lytic necrosis with infiltration of macrophage (arrow). H&E. (x160)

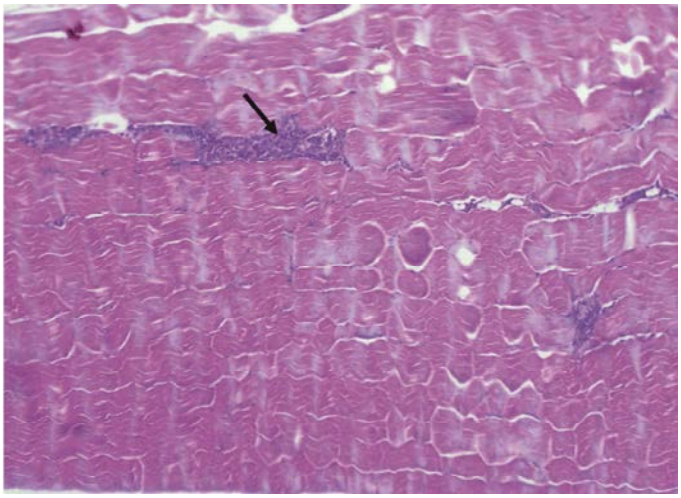

Fig. 2: Muscle of a chicken of IAA group showing lytic necrosis with infiltration of phagocytic cells (arrow). H&E. (x160)

Table 1: Effect ofGA3&IAA and basil treatment ALT, AST, Creatinine, Urea, Cholesterol, TAGin broilers

	ALT, AST, Creat			Urea, T.chol, TAG		
Groups	6 weeks	6 weeks	6 weeks	6 weeks	6 weeks	6 weeks
Group 1 (control)	10.68±2.44b	235.00±16.64a	0.29±0.08	10.40±1.21b	137.40±4.45b	67.00±6.94b
Group 2 (GA3)	38.00±0.71a	187.40±19.91ab	0.26±0.01	14.40±1.96a	188.60±10.54a	150.80±24.73a
Group 3 (IAA)	11.98±0.81b	162.60±14.02b	0.18±0.04	10.40±0.68b	179.00±18.83ab	91.00±9.79b
Group 4 (GA3& basil)	9.40±0.60b	175.60±21.82b	0.27±0.03	8.20±0.86b	155.80±19.81ab	85.40±33.81b
Group 5 (IAA& basil)	10.00±1.04b	142.80±13.71b	0.24±0.01	10.00±0.89b	145.40±10.14ab	80.00±11.42b

Means within the same column carrying different letters are significantly different (P< 0.05).Values represented by means ± standard error.

Table 2: Effect ofGA3&IAA and basil treatment T.protein,Albumin,Globulin,A/G ratio,IgG IgM in broilers.

	T. protein albumin globulin			A/G ratio IgG IgM		
Groups	6 weeks	6 weeks	6 weeks	6 weeks	6 weeks	6 weeks
Group 1 (control)	2.74±0.08c	0.82±0.16ab	1.92±0.17b	0.46±0.12ab	561.67±4.41a	66.00±3.06b
Group 2 (GA3)	2.92±0.20bc	1.18±0.12a	1.74±0.21b	0.74±0.13a	333.33±6.01b	26.33±3.18c
Group 3 (IAA)	2.72±0.12c	0.90±0.21ab	1.82±0.28b	0.62±0.20ab	253.33±26.03c	23.33±4.91c
Group 4 (GA3& basil)	3.30±0.20ab	0.54±0.19b	2.76±0.30a	0.23±0.09b	610.67±32.26a	81.67±3.28a
Group 5 (IAA& basil)	3.78±0.24a	0.90±0.07ab	2.88±0.24a	0.32±0.04b	620.00±25.17a	84.33±2.33a

Means within the same column carrying different letters are significantly different (P< 0.05).Values represented by means ± standard error

Table 3: Effect ofGA3&IAA and basil treatment GST,GSH,GPx in broilers

	6 week (GST)				6 week (GSH)				6 weeks(GPx)			
Groups	Liver	Thymus	Bursa	Muscle	Liver	Thymus	Bursa	Muscle	Liver	Thymus	Bursa	Muscle
Group 1 (control)	8.83±1.62a	1.30±0.10b	0.77±0.03b	0.77±0.09b	15.37±1.69b	16.90±1..65b	40.47±0..87c	40.73±5.19b	49.00±5.20b	45.00±2.89b	49.67±4.70b	40.67±1.67c
Group 2 (GA3)	2.73±1.27b	0.97±0.22b	0.17±0.07c	0.43±0.09b	7.23±1.40c	11.93±0..78c	17.57±3.95d	27.30±2..22c	30.67±5.61c	18.33±3.28c	32.33±4.06c	23.77±2.83d
Group 3 (IAA)	1.67±1.17b	0.83±0.07b	0.13±0.03c	0.50±0.15b	6.47±0.84c	11.53±1..30c	20.40±0.31d	23.60±1..81c	28.30±3.27c	25.10±4.42c	24.33±4.33c	19.33±0.67d
Group 4 (GA3& basil)	11.33±0.98a	2.43±0.23a	1.63±0.19a	1.43±0.23a	33.10±1.57a	29.33±2..22a	48.57±1.80b	52.53±1..64a	69.30±4.39a	78.00±11.27a	64.07±6.64a	60.33±3.38a
Group 5 (IAA& basil)	11.83±1.71a	2.43±0.32a	1.97±0.37a	1.63±0.30a	28.47±4.47a	32.63±1..33a	58.77±0.03a	56.30±2..03a	72.23±8.12a	54.00±7.21b	68.97±3.07a	51.97±3.55b

Table 4: Effect ofGA3&IAA and basil treatment SOD,CAT,MDA in broilers

	6 weeks (SOD)				6 weeks (CAT)				6 weeks (MDA)			
Groups	Liver	Thymus	Bursa	Muscle	Liver	Thymus	Bursa	Muscle	Liver	Thymus	Bursa	Muscle
Group 1 (control)	116..67±1.67b	87.33±1..20b	189.00±0.58c	119.33±2.33b	39.00±1.15b	43.33±0.88b	81.00±2.31a	73.00±2.51b	7.60±0.20c	8.87±0.90b	16.73±0.57a	9.13±0.17b
Group 2 (GA3)	90.67±0..33c	73.33±2..03c	175.00±2.89d	91.00±2..08c	28.67±1.45c	39.00±0.58c	70.00±0.58b	65.00±2.64c	11.20±1.66b	14.43±2.16a	18.60±0.74a	14.93±1.58a
Group 3 (IAA)	91.00±0.58c	73.33±0..88c	169.67±0..33d	88.33±0..88c	28.67±1.67c	38.00±0.58c	71.33±1.86b	66.67±1.20c	14.30±0.32a	16.97±0.83a	18.23±1.25a	14.13±0.84a
Group 4 (GA3&basil)	127.00±2.52a	97.00±3..61a	202.00±2.52b	126.00±2.08a	43.33±0.67a	50.00±1.15a	84.33±1.86a	79.67±0.88a	7.23±0.33c	7.53±0.58b	12.37±0.03b	7.97±0.44b
Group 5 (IAA&basil)	129.00±0.58a	98.67±0..88a	209.33±1.76a	127.67±1.45a	40.00±0.58ab	49.67±0.33a	86.33±3.18a	82.00±1.73a	6.77±0.64c	8.03±0.33b	10.80±0.95b	7.97±0.79b

Conversely, El-Sebai *et al*. [33] reported that GPx activity has significantly increased due to GA3 treatment, Mourao *et al*. [28] reported that IAA administration at all three doses tested did not produce any alteration compared with the control for GPx, SOD, CAT in mice.

The data obtained reveled that, the value of MDA significantly increased in group containing GA3, IAA and significantly decreased in group containing basil. These results come in accordance with Orrenius *et al*. [44] who found that PGRs compounds including GA3 can accelerate lipid peroxidation up to 65-fold, in different tissues and this was attributed to the formation of OH radicals that may react with the lipids, possibly by hydrogen abstraction leading to oxidative damage within the cell. Hussein *et al.* [41] found that disruption of the hepatic antioxidant enzymes activities with accumulation of MDA indicating GA3 induced oxidative stress and lipid peroxidation in the treated animal livers, Trodui *et al.* [42] reported that GA3 treatment in Pregnant Wistar rats from the 14th day of pregnancy until day 14 after delivery revealed in erythrocytes a significant increase in MAD, Hassan and Al-Rawi [26] found that GA3 lead to elevation in hepatic and renal MDA is associated with generation of ROS which interacts with tissues leading to numerous pathophysiological alterations. Sadek *et al*. [37] showed that supplemented groups with basil has antioxidant activity revealed in significant decrease in MDA level.

Conversely, El-Sebai *et al*. [33] reported that reduction of MDA content as GA3 injection for four weeks reduced blood MDA content significant compared to the control, Muthu *et al*. [30] reported that reduction in MDA content was also noted in all the tissues studied for the different doses of GA3 treatment.

CONCULSION AND RECOMMENDATIONS

From the obtained data, we can concluded that the feeding of basil has a good effects on antioxidant enzyme concentration and immunostimulant against effect of GA3 and IAA included in this study. The effects were pronouced after six weeks of feeding.

- The use of GA3 should be under strict control.
- Periodic monitoring of GA3 concentration in the soil and plants.
- More studies are needed to explore other hazardous effects of GA3 on other body systems and organs.
- Other studies with prolonged periods of administration of GA3 are recommended to learn more about its toxic effects.

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