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

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Abstract: The present study was conducted to evaluated the antioxidant and immunostimulant effects of basil agonist gibberlic acid and auxin supplementation 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], aspertonate 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. Histopathological 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 pronounced 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].
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 elong to family Lamiaceae 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 to 5 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.
- Fifth 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 and assayed for the following:

- 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).

**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.

Histopathohological 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 GA3 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 GST, 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, Trodul 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 of GA3&IAA and basil treatment ALT, AST, Creatinine, Urea, Cholesterol, TAG in broilers

<table>
<thead>
<tr>
<th>Groups</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
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<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>10.68±2.44b</td>
<td>235.00±16.64a</td>
<td>0.29±0.08</td>
<td>10.40±1.21b</td>
<td>137.40±4.45b</td>
<td>67.00±6.94b</td>
</tr>
<tr>
<td>Group 2 (GA3)</td>
<td>38.00±0.71a</td>
<td>187.40±19.91ab</td>
<td>0.26±0.01</td>
<td>14.40±1.96a</td>
<td>188.60±10.54a</td>
<td>150.80±24.73a</td>
</tr>
<tr>
<td>Group 3 (IAA)</td>
<td>11.98±0.81b</td>
<td>162.60±14.02b</td>
<td>0.18±0.04</td>
<td>10.40±0.68b</td>
<td>179.00±18.83ab</td>
<td>91.00±9.79b</td>
</tr>
<tr>
<td>Group 4 (GA3&amp; basil)</td>
<td>9.40±0.60b</td>
<td>175.60±21.82b</td>
<td>0.27±0.03</td>
<td>8.20±0.86b</td>
<td>155.80±19.81ab</td>
<td>85.40±33.81b</td>
</tr>
<tr>
<td>Group 5 (IAA&amp; basil)</td>
<td>10.00±1.04b</td>
<td>142.80±13.71b</td>
<td>0.24±0.01</td>
<td>10.00±0.89b</td>
<td>145.40±10.14ab</td>
<td>80.00±11.42b</td>
</tr>
</tbody>
</table>

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

Table 2: Effect of GA3&IAA and basil treatment T.protein, Albumin, Globulin, A/G ratio, IgG IgM in broilers

<table>
<thead>
<tr>
<th>Groups</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>2.74±0.08c</td>
<td>0.82±0.16ab</td>
<td>1.92±0.17b</td>
<td>0.46±0.12ab</td>
<td>561.67±4.41a</td>
<td>66.00±3.06b</td>
</tr>
<tr>
<td>Group 2 (GA3)</td>
<td>2.92±0.20bc</td>
<td>1.18±0.12a</td>
<td>1.74±0.21b</td>
<td>0.74±0.13a</td>
<td>333.33±6.01b</td>
<td>26.33±3.18c</td>
</tr>
<tr>
<td>Group 3 (IAA)</td>
<td>2.72±0.12c</td>
<td>0.90±0.21ab</td>
<td>1.82±0.28b</td>
<td>0.62±0.20ab</td>
<td>253.33±26.03c</td>
<td>23.33±4.91c</td>
</tr>
<tr>
<td>Group 4 (GA3&amp; basil)</td>
<td>3.30±0.20ab</td>
<td>0.54±0.19b</td>
<td>2.76±0.30a</td>
<td>0.23±0.09b</td>
<td>610.67±32.26a</td>
<td>81.67±3.28a</td>
</tr>
<tr>
<td>Group 5 (IAA&amp; basil)</td>
<td>3.78±0.24a</td>
<td>0.90±0.07ab</td>
<td>2.88±0.24a</td>
<td>0.32±0.04b</td>
<td>620.00±25.17a</td>
<td>84.33±2.33a</td>
</tr>
</tbody>
</table>

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

CONCLUSION 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 pronounced 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.

REFERENCES


