Antioxidant properties of *Ocimum sanctum* in broilers treated with high doses of gentamicin

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Abstract

The present research work was carried out to study the antioxidant effect of *Ocimum sanctum* in broilers exposed to high doses of gentamicin, which is common in poultry practice of Tamilnadu. Two hundred and seventy day old broiler chicks of either sex were randomly divided into nine treatment groups of 10 each with three replicates. Different doses of gentamicin (30 mg/kg & 50 mg/kg) single intramuscular injection, different inclusion level of *Ocimum sanctum* crude extract (1% & 2 %) in feed and their combinations were tested. The results of the study revealed that gentamicin treatment produced significant increase in the lipid peroxidation level, and significant decrease in the antioxidant enzymes *viz.* superoxide dismutase and glutathione peroxidase. *Ocimum sanctum* inclusion showed significant reversal in all the above parameters, which clearly supports its free radical scavenging property when co-administered with high doses of gentamicin.

Key words: Antioxidant, Broiler chicks, *Ocimum sanctum,* Gentamicin

1. Introduction

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide and hypochloric acid) produced during aerobic metabolism in the body, can cause oxidative damage of macromolecules. Oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, aging, diabetes mellitus, cancer and immunosuppression (Aruoma, 1998). Antioxidants both exogenous and endogenous, whether synthetic or natural can be effective in preventing free radical formation by scavenging them. Gentamicin, highly used antibiotic in poultry industry is known for its adverse effects especially nephrotoxic potential. Gentamicin facilitates the generation of free oxygen radicals responsible for nephrotoxicity and ototoxicity (Enver Yazer *et al*., 2003). Recently there has been growing interest in natural antioxidants of plant origin because they have greater application in poultry industry as feed additive. Spices and herbs are recognized as source of natural antioxidant and thus play an important role in the chemoprevention of disease and aging. The most effective path to eliminate and diminish the action of free radicals, which cause the oxidative stress, is antioxidative defense mechanism. *Ocimum sanctum* Linn. (Lamiacea) commonly known as holy basil in English, Tulsi in Hindi and Tamil is an Indian medicinal plant, which is known to have many ethno-medical uses such as hepatoprotective, antihyperlipidaemic, myocardial salvaging and immunostimulant effect in man and animals. Hence, the present study was carried out to explore the possible antioxidant properties of *Ocimum sanctum* in broilers treated with high doses of gentamicin.

2. Materials and methods

Two seventy commercial day old, unsexed broiler straight run chicks (Vencob strain) belonging to a single hatch, obtained from a commercial hatchery at Namakkal was used for the experimental study. All the chicks were reared under standard and uniform managemental conditions throughout the experimental period of six weeks.

The chicks were weighed, wing banded and reared in battery brooders. On eighth day, the chicks were randomly divided into nine treatment groups of ten each with three replicates. Broiler starter and finisher mash, free of toxins and pesticide residues purchased from a commercial feed manufacturing unit at Namakkal was used as a basal diet for formulating the experimental diet. The broiler starter and finisher mashes were fed *ad libitum* to the birds from 1 - 28 and 29 - 42 days of age respectively. The birds were subjected to respective treatments from eighth day to 42nd day as per table 1.

The crude extracts of the herbal plant *Ocimum sanctum* received as gratis from M/s. Himalayas, Bengaluru, and the commercially available gentamicin was used for the experimental study. A survey was conducted to fix the dose of gentamicin

and was injected on 15th day at the rate of 30 and 50mg/kg body weight as single intramuscular injection. Other control birds received equal quantity of normal saline. Inclusion levels of plant extract were fixed as per the literature. Experimental diets containing *Ocimum sanctum* (crude extract) at 1 & 2 per cent levels were prepared and fed to the respective treatment groups. At the end of forth and sixth week six birds from each treatment group were sacrificed (two from each replicate). Kidney samples were collected for antioxidant enzyme estimation. The homogenates were prepared with 50mM phosphate buffer for estimation of Glutathione peroxidase (Rotruck *et al.,* 1973) and Superoxide dismutase (Marklund & Marklund, 1974). Blood samples were collected at the end of fourth and sixth week. Serum lipid peroxide level was estimated as per Ohkawa *et al.* (1979). The data were statistically analysed by completely randomized block design (Snedecor & Cochran, 1989).

Table 1. *Experimental design*

S. No.	Treatment	Experimental group
1	T1	Normal control
2	T2	Gentamicin - 30 mg/kg
3	T3	Gentamicin - 50mg/kg
4	T4	Ocimum sanctum -1% level
5	T5	Ocimum sanctum -2% level
6	T6	Gentamicin - 30 mg/kg + Ocimum sanctum -1% level
7	T7	Gentamicin - 50mg/kg + Ocimum sanctum -1% level
8	T8	Gentamicin - 30mg/kg + Ocimum sanctum -2% level
9	T9	Gentamicin - 50mg/kg + Ocimum sanctum -2% level

3. Results and discussion

Serum lipid peroxide level, kidney glutathione peroxidase and superoxide dismutase levels are presented in table 2, 3 and 4 respectively .

Table 2. *Effect of Ocimum sanctum on serum lipid peroxidation level (nmol/ml) (Mean ±S.E) in gentamicin treated broilers*

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
IV week**	31.31c±1.55	39.25ab± 1.76	41.88a± 1.69	30.38c±1.31	31.25c±1.66	33.88c± 1.29	33.44c±1.29	30.13c± 1.17	33.38c± 1.46
VI week	30.13± 1.62	31.75± 1.60	31.50± 1.30	30.25± 1.64	30.25± 1.67	31.63± 1.76	32.25± 1.44	32.50± 1.78	30.63±1.50
n=6 ** Overall means bearing different superscripts between columns differ significantly (P≤0.01)									

Table 3. *Effect of Ocimum sanctum on kidney glutathione peroxidase level (GSH consumed/min/mg of protein) (Mean ±S.E) in gentamicin treated broilers*

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
IV week**	18.28a± 0.80	13.58bc± 0.92	12.73c± 0.99	18.45a± .53	18.44a± 0.72	16.24ab± 0.72	16.21ab± 0.74	18.10a± 0.60	16.31ab± 0.92
VI week	18.45± 0.53	18.29 ± 0.45	18.13± 0.67	17.76± 1.00	18.08± 0.79	18.20± 0.68	17.48± 0.74	18.45± 0.53	17.64± 0.96
n=6 **Overall means bearing different superscripts between columns differ significantly (P≤0.01)									

At the end of the fourth week dose dependent highly significant (P ≤0.01) increase in lipid peroxidation level was noticed in gentamicin control group. Groups treated with *O. sanctum* alone showed no change and groups treated with combination of gentamicin and *O. sanctum* produced significant decrease in lipid peroxidation when compared to gentamicin control groups. Inclusion of *O. sanctum* produced significant antioxidant effect, which was indicated by the reduction of lipid peroxidation level. At the end of the sixth week lipid peroxidation values were normal in all the groups tested.

Table 4. *Effect of Ocimum sanctum on kidney superoxide dismutase level (Unit/min/mg of protein) (Mean ±S.E) in gentamicin treated broilers*

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
IV week**	1.36a± 0.05	0.90b± 0.04	0.66b± 0.05	1.46a± 0.09	1.41a± 0.06	1.39a± 0.09	1.37a± 0.07	1.37a± 0.07	1.39a± 0.04
VI week	1.47± 0.08	1.37± 0.08	1.45± 0.10	1.43± 0.07	1.42± 0.04	1.39± 0.05	1.38± 0.10	1.44± 0.11	1.39± 0.06
n=6 **Overall means bearing different superscripts between columns differ significantly (P≤0.01)									

A significant decrease in glutathione peroxidase and superoxide dismutase level was observed in gentamicin control groups compared to normal control. The other groups had shown dose dependent improvement and did not differ from normal control. At the end of 6th week, the antioxidant enzyme levels were restored to normal in gentamicin control groups and did not differ from other groups.

Gentamicin administration might have resulted in the generation of oxidative stress / reactive oxygen species which in turn was responsible for the increase in lipid peroxidation (Ramasamy *et al.,* 2009) and decrease in the antioxidant enzymes superoxide dismutase and glutathione peroxidase (Pedraza - Chaverri *et al.,* 2000 ; Abdel- Raheem *et al.,* 2008).

Ocimum sanctum treatment significantly prevented the rise in lipid peroxidation levels suggesting that it attenuates the excessive formation of reactive oxygen species. Further, it increases the level of superoxide dismutase and glutathione peroxidase, which in turn will scavenge the reactive oxygen species generated by gentamicin. This is in agreement with the observation of Vara Prasad Reddy *et al.* (2009). The constituents of *Ocimum sanctum* interrupt the free radical chain of oxidation by donating hydrogen from phenol's hydroxyl group thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids (Tabassum *et al.,* 2009). *Ocimum sanctum* constituents such as flavonoids (orientin and vicenin), phenolic compounds (eugenol, crisilineol, apigenin), and anthocyanins are known to augment reduced glutathione and antioxidant enzyme levels and scavenge the lipid peroxides (Gupta *et al.,* 2002).

4. Conclusion

The results of the study revealed that gentamicin treatment produced significant increase in the lipid peroxidation level, and significant decrease in the antioxidant enzymes *viz.* superoxide dismutase and glutathione peroxidase. *Ocimum sanctum* inclusion showed significant reversal in all the above parameters, which clearly supports its free radical scavenging property when co-administered with high doses of gentamicin.

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6. References

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