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A Arivuchelvan
Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

S Murugesan
Dean (I/C), Veterinary College and Research Institute, Theni, Tamil Nadu, India

Corresponding Author:
A Arivuchelvan
Professor, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Effect of *Ocimum sanctum* on biochemical parameters of broilers treated with high doses of gentamicin

A Arivuchelvan and S Murugesan

Abstract
The purpose of the study is to evaluate the protective effect of *Ocimum sanctum* in broilers exposed to high dose of gentamicin which is common in poultry practice of Tamil Nadu. Biochemical parameters such as ALT, AST, creatinine, uric acid, sodium and potassium were estimated at regular interwell. Two hundred and seventy day old broiler chicks of either sex were randomly divided into nine groups of 10 each with three replicates. Different doses of gentamicin (30mg/kg & 50mg/kg) single intramuscular injection, different inclusion level of *Ocimum sanctum* crude extract (1 and 2%) in feed and their combinations were tested. The results of the study revealed that gentamicin treatment produced significant increase in the serum ALT, AST and significant decrease in the serum sodium and potassium level. Gentamicin also increase the serum uric acid and creatinine level. The groups treated with *Ocimum sanctum* showed significant improvement in all the above parameters, which clearly supports the protective effect of *Ocimum sanctum*.

Keywords: *Ocimum sanctum*, gentamicin, serum biochemical parameters, broiler

Introduction
Intensive poultry production with fast growing strains and high stocking densities are usually susceptible to infectious agents due to varied reasons. Antibiotics especially gentamicin is used frequently by poultry farmers at very high doses from the day of hatching to culling/marketing. The impact analysis of this practice reveals many negative effects namely failure of treatment, escalation of treatment cost, adverse effect of chemotherapeutic agents esp. organ toxicity and mortality in flocks some times. Many medicinal plants showing hepatoprotective and nephroprotective activity have been used instead of drugs because of their low toxicity for the host system, adequate absorption and capability to reach the target organ without much degradation by host enzymes. *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 ethno-medical uses such as hepatoprotective, antihyperlipidaemic, myocardial salvaging and immunostimulant effect in man and animals. Hence, the present was carried out to explore the possible hepatoprotective and nephroprotective effect of *Ocimum sanctum* in broilers treated with high doses of gentamicin.

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 shown below. 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.

S. No	Treatment	Experimental group
1	T1	Normal control
2	T2	Gentamicin- 30mg/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 - 50 mg/kg + *Ocimum sanctum* - 1% level
8	T8	Gentamicin - 30mg/kg + *Ocimum sanctum* - 2% level
9	T9	Gentamicin - 50mg/kg + *Ocimum sanctum* - 2% level

Blood samples were collecte in clean dry test tubes, allowed to clot and centrifuged for ten minutes at 3000 rpm. The serum was separated and the following estimations were done, at the end of the second week, fourth week and sixth week. ALT and AST by DNPH colorimetric method (Reitman and Frankel 1957) [10]. Serum sodium and potassium were estimated during flame Photometer. Serum urea and Creatinine by Diacetyl monoxime (DAM) Method (Marsh *et al* 1965)

Results

Alanine Aminotransferase

Serum ALT values are presented in Table 1.There was no significant difference in the mean ALT values at the age of two weeks. At fourth week the mean serum ALT values for the groups T1 to T9 were 14.29 ± 1.46, 20.29 ± 1.73, 22.33 ± 1.91, 14.57 ± 0.97, 15.19 ± 1.01, 17.86 ± 0.80, 17.36 ± 1.04, 14.86 ± 1.18 and 16.57 ± 1.27 units/ml respectively. A highly significant (P≤0.01) increase in ALT level was observed in gentamicin control groups (T2 and T3) compared to normal control group. There was no significant difference between normal control group, *Ocimum sanctum* control group and treatment groups (T6, T7, T8 and T9) of which T8 showed better reduction in ALT value. There was no significant difference between T2, T6, T7 and T9. The groups T2, T3 and T6 did not differ significantly among themselves. There was no significant difference in the mean serum ALT values at the age of six weeks.

Table 1: Effect of *Ocimum sanctum* on serum ALT level (Units/ml) (Mean ±S.E) in gentamicin treated broilers

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II week	14.00±1.50	14.57±1.21	16.29±0.89	14.14±1.45	14.57±1.23	14.43±1.15	15.86±1.24	15.57±1.07	16.29±1.43
IV week**	14.29^{c}±1.46	20.29^{ab}±1.73	22.33^{a}±1.91	14.57^{c}±0.97	15.19^{c}±1.01	17.86^{abc}±0.80	17.36^{bc}±1.04	14.86^{c}±1.18	16.57^{bc}±1.27
VI week	14.57±1.23	14.14±1.45	16.29±1.70	15.86±1.06	15.71±1.23	14.14±0.86	15.43±1.04	14.71±0.81	14.86±1.39

n = 6

**Overall means bearing different superscripts between columns differ significantly (P≤ 0.01)

Aspartate aminotransferase

Serum AST values are presented in Table 2. There was no significant difference in the mean AST values at the age of two weeks. At fourth week, mean serum AST values for the groups T1 to T9 were 129.87 ± 5.26, 158.09 ± 2.47, 163.97 ± 5.57, 129.16 ± 3.48, 126.17 ± 4.91, 136.83 ± 4.91, 137.60 ± 3.63, 130.11 ± 5.02 and 136.41 ± 4.17 units/ml respectively. A highly significant (P≤0.01) increase in serum AST value was noticed in gentamicin control groups compared to normal control group. There was no significant difference between control group, *Ocimum sanctum* control group and the treatment groups (T6, T7, T8 and T9). There was no significant difference in the mean AST values at the age of six weeks.

Table 2: Effect of *Ocimum sanctum* on serum AST level (Units/ml) (Mean ±S.E) in gentamicin treated broilers

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II week	128.09 ± 5.25	128.97 ± 6.31	126.33 ± 7.01	128.02 ± 6.39	129.67 ± 4.95	129.23 ± 3.74	129.26 ± 6.44	127.91 ± 6.71	127.14 ± 5.22
IV week**	129.87^{b} ± 5.26	158.09^{a} ± 2.47	163.97^{a} ± 5.57	129.16^{b} ± 3.48	126.17^{b} ± 4.91	136.83^{b} ± 4.91	137.60^{b} ± 3.63	130.11^{b} ± 5.02	136.41^{b} ± 4.17
VI week	126.17 ± 4.91	127.27 ± 6.04	129.00 ± 6.80	129.29 ± 8.24	128.71 ± 5.13	129.43 ± 6.88	127.46 ± 5.87	129.71 ± 6.66	127.43 ± 5.09

n = 6

**Overall means bearing different superscripts between columns differ significantly (P≤ 0.01)

Kidney function profile

Uric acid

Uric acid levels were estimated in the serum of all the groups at the end of the second, fourth and sixth week and presented in Table 3. There was no significant difference in the mean values of serum uric acid at the age of second week. The mean values of uric acid for the treatment T_1 to T_9 at the end of the fourth week were 12.46± 1.19, 20.21± 1.35, 22.91 ± 1.60, 12.49± 1.19, 12.78 ± 1.13, 17.91 ± 1.31, 18.58 ± 0.92, 13.79 ± 1.50 and 14.09 ± 1.04 mg/dl respectively. A highly significant (P≤0.01) increase in serum uric acid level was noticed in gentamicin control groups compared to normal control group. There was no significant difference between normal control group, *Ocimum sanctum* control groups and treatment groups T8 and T9.The treatment group T6 did not differ significantly from the gentamicin control group (T2). The group T7 did not differ significantly from the gentamicin control groups T2 and T3.Among the treatment groups T6, T7 and T9 did not differ significantly among themselves. The groups T7 and T8 differed significantly among themselves. There was no significant difference in the mean values of serum uric acid at the age of sixth week.

Creatinine

Serum creatinine values are presented in Table 4. Mean serum creatinine levels did not differ significantly between the groups at the end of second week. At the end of fourth week the mean serum creatinine values for the treatment groups T1 to T9 were 0.73 ± 0.04, 1.03 ± 0.07, 1.24 ± 0.09, 0.76 ± 0.09, 0.73 ± 0.04, 0.82 ± 0.09, 0.73 ± 0.11, 0.73 ± 0.04 and 0.74 ± 0.13 mg/dl respectively. A highly significant (P≤0.01) increase in serum creatinine value was observed in T3 compared to normal control group. T2 also recorded apparent increase in serum creatinine level but it did not differ

significantly from normal control as well as T3. There was no significant difference between normal control group, *Ocimum sanctum* control groups and treatment groups (T6, T7, T8 and T9). Mean serum creatinine values did not differ significantly between the groups at the end of sixth week.

Table 3: Effect of *Ocimum sanctum* on serum uric acid level (mg/dl) (Mean ± S.E) in gentamicin treated broilers

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II week	11.76 ±1.22	11.74 ±1.09	12.54 ±1.08	13.66 ±1.09	14.31 ±1.12	13.21 ±2.03	12.29 ±1.39	12.08 ±1.17	12.97 ±1.23
IV week**	12.46^{e} ±1.19	20.21^{ab} ±1.35	22.91^{a} ±1.60	12.49^{e} ±1.19	12.78^{e} ±1.13	17.91^{bcd} ±1.31	18.58^{abc} ±0.92	13.79^{de} ±1.50	14.09^{cde} ±1.04
VI week	14.33 ±1.30	13.48 ±1.71	14.53 ±1.06	15.87 ±1.73	15.27 ±1.87	12.32 ±1.69	15.86 ±1.78	12.07 ±1.56	14.13 ±1.52

n= 6
** Overall means bearing different superscripts between columns differ significantly (P≤ 0.01)

Table 4: Effect of *Ocimum sanctum* on serum creatinine level (mg/dl) (Mean ± S.E) in gentamicin treated broilers

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II week	0.61 ±0.04	0.63 ±0.07	0.67 ±0.08	0.66 ±0.05	0.54 ±0.07	0.65 ±0.05	0.47 ±0.08	0.68 ±0.08	0.52 ±0.11
IV week**	0.73^{bc} ±0.04	1.03^{ab} ±0.07	1.24^{a} ±0.09	0.76^{bc} ±0.09	0.73^{bc} ±0.04	0.82^{bc} ±0.09	0.73^{bc} ±0.11	0.73^{bc} ±0.04	0.74^{bc} ±0.13
VI week	0.66 ±0.05	0.76 ±0.14	0.89 ±0.07	0.55 ±0.13	0.71 ±0.08	0.76 ±0.09	0.76 ±0.08	0.73 ±0.09	0.76 ±0.07

n= 6
** Overall means bearing different superscripts between columns differ significantly (P≤ 0.01)

Serum electrolytes

Sodium

Mean serum sodium level are presented in the Table 5.Mean serum sodium level did not differ significantly between the groups at the end of second week. At the end of fourth week mean serum sodium levels for the treatment groups T1 to T9 were 145.05 ± 3.63, 123.80 ± 3.84, 118.10 ± 3.75, 147.99 ± 4.18, 148.77 ± 4.99, 132.73 ± 4.81, 133.46 ± 4.80, 144.66 ± 3.71 and 135.25 ± 5.36 mmol/l respectively. A highly significant (P≤0.01) decrease in serum sodium level was recorded in gentamicin control group compared to control group. There was no significant difference between control group, *Ocimum sanctum* control groups and treatment groups (T6, T7, T8 and T9). Treatment groups T6, T7 and T9 did not differ significantly from gentamicin control group (T2). But T6 and T7 did not differ significantly from the gentamicin control groups (T2 and T3).Mean serum sodium level did not differ significantly between the groups at the end of sixth week.

Potassium

Mean serum potassium level are presented in the Table 6 Mean serum potassium level did not differ significantly between the groups at the end of second week. The mean serum potassium levels for the treatment groups T1 to T9 at the end of fourth week are 4.55 ± 0.09, 3.49 ± 0.11, 3.34 ± 0.11, 4.51 ± 0.08, 4.49 ± 0.08, 4.05 ± 0.03, 3.56 ± 0.11, 4.32 ± 0.09 and 3.85 ± 0.12 mmol/l respectively. A highly significant (P≤0.01) decrease in serum potassium level was noticed in gentamicin control groups (T2, T3) compared to normal control group (T1). Among the treatment groups T6 and T9 did not differ significantly among themselves, similarly T7 and T9 did not differ significantly. Also the treatment groups T6 and T8 did not differ significantly among themselves. Mean serum potassium level did not differ significantly between the groups at the end of sixth week.

Table 5: Effect of *Ocimum sanctum* on serum sodium level (mmol/l/dl) (Mean ± S.E) in gentamicin treated broilers

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II week	142.15 ± 4.30	145.36 ± 4.90	147.32 ± 4.92	143.00 ± 4.93	146.76 ± 5.11	143.11 ± 4.34	143.70 ± 4.37	146.95 ± 4.32	147.35 ± 5.23
IV week**	145.05^{a} ± 3.63	123.80^{bc} ± 3.84	118.10^{c} ± 3.75	147.99^{a} ± 4.18	148.77^{a} ± 4.99	132.73^{abc} ± 4.81	133.46^{abc} ± 4.80	144.66^{a} ± 3.71	135.25^{ab} ± 5.36
VI week	143.11 ± 4.34	143.44 ± 3.22	147.20 ± 3.42	141.87 ± 4.90	143.77 ± 5.79	148.34 ± 3.60	146.04 ± 4.14	142.39 ± 4.35	145.46 ± 7.17

n= 6
** Overall means bearing different superscripts between columns differ significantly (P≤ 0.01)

Table 6: Effect of *Ocimum sanctum* on serum potassium level (mmol/l/dl) (Mean ± S.E) in gentamicin treated broilers

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9
II week	4.39 ± 0.10	4.31 ± 0.10	4.50 ± 0.11	4.39 ± 0.13	4.38 ± 0.11	4.35 ± 0.11	4.30 ± 0.11	4.43 ± 0.12	4.32 ± 0.12
IV week**	4.55^{a} ± 0.09	3.49^{de} ± 0.11	3.34^{e} ± 0.11	4.51^{a} ± 0.08	4.49^{a} ± 0.08	4.05^{bc} ± 0.03	3.56^{de} ± 0.11	4.32^{ab} ± 0.09	3.85^{cd} ± 0.12
VI week	4.39 ± 0.13	4.11 ± 0.16	4.21 ± 0.15	4.41 ± 0.10	4.43 ± 0.12	4.45 ± 0.12	4.31 ± 0.12	4.33 ± 0.11	4.39 ± 0.11

n= 6
** Overall means bearing different superscripts between columns differ significantly (P≤ 0.01)

Discussion

ALT and AST

There was no significant difference in the mean ALT values at the age of two and six weeks. A highly significant increase in ALT level was observed in gentamicin control groups compared to normal control group at the end of fourth week. There was no significant difference between normal control group, *Ocimum sanctum* control group and treatment groups of which T8 (Gentamicin 30mg/kg + *Ocimum sanctum* 1%) had shown better reduction in ALT value. Similar increase in ALT was noticed by Saleemi *et al.*, (2009) [11] in broilers treated with gentamicin at the rate of 20 mg/kg and higher. Arvind Soni *et al.*, (2009) also reported increase in ALT and AST in mice treated with amikacin, an aminoglycoside antibiotic.

The increase in ALT and AST may be due to hepatic damage or muscular damage induced by the dose of gentamicin used in this study (Itoh and Okada, 1993) [6].

Decrease in ALT and AST level by *Ocimum sanctum* was in concurrence with the report of Kheir *et al*. (2009) [7]. Further, eugenol and methyl eugenol present in *Ocimum sanctum* could have been responsible for hepatoprotection (Govind Pandey and Madhuri, 2010) [5] and the restoration of ALT and AST level. Pingale *et al*. (2010) [9] also reported that *Ocimum sanctum* had very high potential in healing liver parenchyma as well as regeneration of liver cells.

Uric acid and Creatinine

There was no significant difference in the mean values of uric acid at the age of second week and sixth week. At the end of fourth week a highly significant increase in serum uric acid level was noticed in gentamicin control groups compared to normal control group. The groups treated with higher dose of *Ocimum sanctum* was normal and did not differ from normal control group. Whereas the groups treated with lower dose of *Ocimum sanctum* recorded dose dependent significant increase in uric acid level. The level of uric acid was normal in all the groups at the end of sixth week.

Mean serum creatinine levels did not differ significantly between the groups at the end of second week and sixth week. At the end of fourth week higher dose of gentamicin recorded significant increase in serum creatinine level whereas the lower dose did not differ from the normal control. *Ocimum sanctum* treatment restored the uric acid level in all the treatment groups to normal.

The disturbed concentrating ability of the kidney and the persistence of the drug in serum following succession of therapy might be the reason for the nephrotoxicity leading to increased level of uric acid and creatinine. El Daly *et al.* (1996) [4] supported the hypothesis of alteration of renal protein thiol status playing an important role in the mechanism of gentamicin induced nephrotoxicity. He further stated that hydroxyl radical scavengers and iron chelators were significantly protected against gentamicin induced nephrotoxicity. Main toxicity of gentamicin had been attributed to binding of the strongly cationic drug to the megalin receptor located deep at the base of brush border villi and causing proximal tubular damage (Nagai and Takano, 2004) [8]. The results of the present study are in accordance with Vasilios *et al*, (2003) and Saleemi *et al.* (2009) [13, 11].

Ocimum sanctum contains a number of flavonoids and triterpenes which might hinder the binding of gentamicin to the brush border surface, thereby reducing the kidney damage (Singh *et al.*, 2007) [12]. The nephroprotective effect of *Ocimum sanctum* could be attributed to these active principles.

Serum electrolytes

There was no significant difference in mean serum sodium and potassium level at the end of second and sixth week. Gentamicin induced decrease in serum sodium and potassium was attributed to its nephrotoxic potential and inability of kidney to concentrate electrolytes. Similar findings were reported by Watson *et al.* (1999) [14], Avinash *et al.* (2000) [2], Vasilios *et al.* (2003) [13] and Chou *et al.* (2005) [3]. The serum electrolyte levels were restored by administration of *Ocimum sanctum* due to prevention of proximal tubular damage induced by gentamicin and preserving the concentrating ability of the kidney

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