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Haemato-biochemical changes in grower broilers on sub-acute allopurinol toxicity

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Abstract
The experiment was conducted to explore subacute toxic effect of feeding allopurinol in grower broiler birds as an anti-gout medication. Gout is a systemic disease that results from the deposition of monosodium urate crystals (MSU) in tissues. Uric acid is synthesized in liver from purines where the last two reactions is mediated by xanthine oxidase. Allopurinol is an xanthine oxidase inhibitor which is frequently used as an anti-gout drug in human medicine and well used in treating poultry gout during the last few years. In our experiment grower broilers were fed allopurinol @ 250mg/kg b. wt. orally for seven days and thereafter blood was collected to determine haemato-biochemical changes. Biochemical studies revealed that the value of urea increased while creatinine and globulin values were decreased in allopurinol treated group than the control group of birds but the variation was non-significant statistically. Haematological studies showed that there was increase in the value of total leucocyte count and at the same time decrease in total erythrocyte count in birds of treatment group in comparison to control group, though the variation was non-significant. A significant increase in the number of heterophil count was observed but lymphocyte count was significantly decreased. There was no statistical difference in monocyte and eosinophil count. It appears that allopurinol has some irritating nature and mild effect on kidneys though not enough to cause any overt deleterious effect and may be recommended for use in broiler at therapeutic dose.

Keywords: Broilers, Gout, Allopurinol

Introduction
With increase in the human population and simultaneous increase in the demand for meat and eggs, the modern day poultry birds has genetically engineered to increase their production efficiency for meat and egg. In the process to achieve this target, the birds are subjected to stress and most of their vital organs are being directly or indirectly affected with resultant increased frequency of metabolic disorders. This paves the path for greater incidence of one of the important metabolic disorder of poultry i.e. hyperuricemia. In the recent past an increase in the incidence of hyperuricemia in poultry birds has been reported. Hyperuricemia is the main underlying factor for the development of gout. Gout is the clinical term which describes the physiological outcome associated with excessive uric acid accumulation in the body fluids. Gout in India is one of the major causes of heavy mortality and morbidity in poultry leading to economic losses to the poultry industry. It is being frequently reported from different geographical areas of India (Jana *et al*. 2009 and Singh *et al*. 2013) [1, 2]. For management of gout the ultimate way is to lower the serum urate level and dissolve the crystal deposits. This can be achieved by reducing the formation of uric acid by xanthine oxidase inhibitors like allopurinol. Allopurinol (4-hydroxypyrazolo (3,4-d}pyrimidine) is a purine analog; it is a structural isomer of hypoxanthine (a naturally occurring purine in the body) and is an inhibitor of the enzyme xanthine oxidase (Elion, 1978). Allopurinol is being rampantly used in the treatment of gout in poultry medicine. It has been reported safe to use, however, injudicious use for long duration and higher dosage may lead to some adverse effects. The current work was therefore planned to explore the toxic effect of allopurinol in grower broiler birds, if any by feeding persistent higher than recommended dose of allopurinol.

Materials and methods

Day old, apparently healthy 12 Ven-cobbbroiler chicks were obtained from local dealers of Ranchi. The chicks were kept in the experimental animal house of the College under strict hygienic conditions. The experimental room was thoroughly cleaned with water followed by potassium permanganate solution. Two days before the arrival of the birds, the rooms along with accessories were fumigated with 60 grams potassium permanganate and 120 ml formalin per 100 cubic feet area. All the birds were provided with standard poultry ration wholesome water *ad-libitum*. The birds were vaccinated for combined IB and RD vaccine on 3rd day and IBD on 13th day by naso-ocular route followed by R_2B vaccine on 25th day in chilled drinking water mixed with milk powder @ 5gm per 100 birds. At the age of 25 days these grower broiler birds were randomly assigned into two groups and treatment was given as shown in table 1. The birds were weighed daily and their dose was calculated and administered through oral route.

Table 1: Showing experimental plan of the work:

Groups	No. of birds and age	Treatment	Route of administration	Duration of treatment
C	06 (25 day old)	No Treatment	*per os*	7 days
T	06 (25 day old)	Allopurinol @ 250 mg/kg b.wt.	*per os*	7 days

At the end of the experiment, blood was collected from all the six birds of each group for study of certain haematological and biochemical parameters. For haematological study 2.0 ml blood was collected from right jugular vein in a clean dry vial containing 2.0 mg ethylene diaminetetra acetic acid (EDTA) as an anti-coagulant. The haematological attributes studied includes total leucocyte count, differential leucocyte count and total erythrocyte count.

For biochemical studies blood samples was collected in a sterile, clean and dry test tube without using any anticoagulant. For separation of serum, the test tubes were placed in slanted position for half an hour at room temp. After this, the separated serum was centrifuged at 3000 rpm for 5 min. The clear supernatant was collected in eppendorftubes and placed in refrigerated condition (4°C) for further use. The biochemical parameters like urea, creatinine and globulin were studied on all the collected serum samples using tulip's diagnostic kits.

Results and Discussion

Details of biochemical studies have been presented in Table (2). It was found that the level of urea was increased in allopurinol treated group birds in comparison to control birds but the increase was non-significant statistically, though the variation was within the normal limits of broilers. The values of creatinine and globulin was decreased in treated group in comparison to control group but differed non- significantly and lie within the normal range. Urea is the final degradation product of protein and amino acid metabolism. In protein catabolism, the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is synthesized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the body through kidneys. Serum urea level reflects the balance between urea production in liver and elimination by kidneys. High urea level reflects decline in GFR but it may also be due to pre renal causes (like water deprivation, high protein diet etc.) or post renal causes like obstruction in urinary tract etc. The amount of creatinine excreted in the urine is directly related to muscle mass and approximately proportional to the lean body mass. Creatinine estimation is required to assess renal functioning. Kidneys freely filter and secrete creatinine suggesting that no creatinine is reabsorbed by the blood unlike glucose, amino acids and electrolytes that include sodium and potassium. Due to this pattern, creatinine levels in the urine and blood are directly reflective of kidney functioning. A low creatinine level alone does not necessarily mean that there is an underlying health problem. Low creatinine levels are usually not considered bad or troublesome since it indicates that the kidneys are optimally functional. However, since most systems and tissues of the body work in coordination, it is recommended to observe the creatinine concentration if levels remain low for a long period. Some globulins are produced in the liver, while others are made by the immune system. It is one of the major blood proteins involved in body defense through humoral immunity. If the globulin levels fall below the normal range, it can be a sign of several serious health conditions. Renal disease, hepatic dysfunction, acute hemolytic anemia, and hypogamma-globulinemia can cause the globulin levels to drop. This is also a sign that proteins taken in by the digestive system are not being broken down or absorbed properly.

In our findings the variations in level of urea, creatinine and globulin falls within the normal range hence, it appears that allopurinol might have some effect on kidneys but not significant enough to cause any overt deleterious effect or clinical manifestation.

Table 2: Showing effect of subacute allopurinol toxicity on biochemical parameters in experimental broilers:

Parameters	C	T
Urea(mg/dl)	13.63± 1.08[a]	14.01 ± 0.49 [a]
Creatinine (mg/dl)	0.35±0.02[a]	0.32±0.02 [a]
Globulin(mg/dl)	2.71±0.12[a]	2.58±0.35 [a]

Significant ($p < 0.05$)

The findings of haematological studies have been shown in Table 3. The findings showed that there was no significant difference in the values of total leucocyte count and total erythrocyte count in between the birds of control group and treated allopurinol group. However, the value of TEC was slightly less and value of TLC was slightly more than control group. There was significant increase in the number of heterophil count, suggesting mild irritating nature of drug in higher dose. However, there was significant decrease in number of lymphocyte and no significant effect was observed on monocyte and eosinophil count. However, the variations are within the normal range showing no any adverse effect.

Table 3: Showing effect of subacute allopurinol toxicity on haematological parameters in experimental broilers:

Parameters	C	T
TEC(m/mm^3)	3.18±0.05 [a]	3.15±0.09 [a]
TLC (/mm^3)	10383.33±218.20 [a]	11016.67±175.9 [a]
Differential leucocyte count (%)		
Heterophil	37.83±2.06 [a]	47.16±1.72 [b]
Lymphocyte	54.17±1.74 [b]	45.00±2.08 [a]
Monocyte	3.00±0.37 [a]	2.83±0.31 [a]
Eosinophil	5.00±0.45 [a]	5.00±0.52 [a]

Significant ($p < 0.05$)

Boyer, T.D. *et al*. (1977) also reported that allopurinol is a safe and useful drug with few side effects in humans. J. Poffers *et al.* (2002) [5] studied the effects of allopurinol in a Red-tailed Hawks hyperuricaemic model at three different doses i.e. 100, 50 and 25mg/kg. It was observed that allopurinol at 100 and 50 mg/kg dose was toxic; however, at dose of 25 mg/kg allopurinol was safe to administer.

The active metabolite of allopurinol is oxipurinol, which is also an inhibitor of xanthine oxidase. Allopurinol is almost completely metabolized to oxipurinol within two hours of oral administration, followed by slow excretion of oxipurinol by kidney over 18–30 hours. For this reason, oxipurinol is believed responsible for the majority of allopurinol's effect. Being an isomer of hypoxanthine it competitively inhibits the function of xanthine oxidase which is needed for conversion of hypoxanthine into xanthine and thereafter into uric acid (Brunton LL, *et al.* 2011) [6]. Besides its reducing effect on uric acid in poultry blood, there are direct and indirect evidences of antioxidant effect of allopurinol. It was shown to scavenge superoxide anion and hydroxyl radicals during *in vitro* experimental studies (Pacher P, *et al.* 2006) [7].

In our experiment we were able to find that allopurinol when administered to birds in significantly higher doses as compared to earlier reports did not cause any overt deleterious effect and seem to be quite safe for use in broiler at therapeutic dose.

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