

# “TOXICOPATHOLOGICAL STUDIES OF ACETYL SALICYLIC ACID (ASA) IN BROILER CHICKS”

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## ABSTRACT

*Study was undertaken to assess the effects of Acetyl salicylic acid (ASA) administration in 80 day old Cobb broiler chicks which were divided into 4 groups. ASA was administered at the dose rate of 400, 1600 and 4000 ppm orally (Group II to IV) through feed for 21 consecutive days along with distilled water as vehicle in Group I. Birds of group III and IV were slightly dull, depressed and anorectic. Slow growth rate and blood mixed faecal droppings were observed in few birds of group IV with maximum mortality of 5%. A dose dependent reduction in body weight and feed consumption and dose dependent significant ( $p < 0.05$ ) increase in kidney: body weight ratio was observed in group III and IV as compared to control. There was significant decrease in the values of AKP, PCV, TEC and Hb in group IV. Histopathological section revealed damage to the intestine, kidney and lungs. It can be concluded that ASA at very high doses i.e. 4000ppm for longer duration can cause bone damage and anemia which can lead to mortality.*

**Keywords:** Alkaline Phosphatase, Acetyl salicylic acid, Hyperplasia and Necrosis.

## INTRODUCTION

Veterinary medicine faces the unique challenge of having to treat different types of domestic animal species, including mammals, birds and fishes. The main challenge for veterinarians is not to select a drug but to determine, for the selected agent, a rational dosing regimen because the dosage regimen for a drug in a given species may depend on its anatomy, biochemistry, physiology and behaviour as well as on the nature and causes of the condition requiring treatment (Toutain, 2010).

ASA (aspirin) is a non-steroidal anti-inflammatory drug (NSAID) commonly used in veterinary practice [Livingston, 2010; Miller and

Richerdson, 2011) with some uses in the avian medicine (Machin, 2005; Hawkins and Paul Murphy, 2011). ASA as sodium salt had been administered orally in pigs, calves and chicken @ 5-300 mg/kg/day for not exceeding 10 days. Dose and time dependent residues are found in skin, fat, muscle, liver and kidney.

Beneficial effect of NSAID like aspirin on ascites, growth and egg production of chickens during heat stress were appreciated (Galil, 2004; Mohammed, 2012). Aspirin is used frequently in ducks, when a reovirus infection with tenosynovitis occurs. Other uses are for treating locomotor disorders, stimulation of egg production and improving

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eggshell thickness, as well as for respiratory and digestive problems (Boulianne and Hunter, 1990; Jouglar and Bernard, 1992; Balog et al. 2000). In poultry, aspirin may be used as an anti-stress agent (Mc Daniel and Parker, 2004).

In food-producing birds as well as in companion birds, anti-inflammatory drugs are seldom prescribed due to scarcity of basic knowledge and scientific research on pharmacokinetic data of veterinary relevant NSAIDs. There have been several studies focused on the pharmacokinetics of Sodium salicylate in poultry (Baert and De Backer, 2002; Baert et al. 2004) and its efficacy in treating inflammation in birds (Baert et al. 2005) but limited information is available on the toxicopathological effects of acetyl salicylic acid in young chicks. Keeping in view, the beneficial effects of ASA and its frequent usage, it was felt necessary to study the toxic effect of ASA at different dose level in broiler chicks.

## MATERIALS AND METHODS

Study was conducted on 80 day old cobb broiler chicks which were procured from a local commercial hatchery (Shakti Hatcheries Pvt. Ltd., Sarsa, Anand, Gujarat, India) and were maintained under standard managemental conditions. The chicks were kept in cages and were housed in Experimental unit, Department of Veterinary Pathology, College of Veterinary science and animal husbandry, Anand. All procedures performed in this biological trial involving animals were in accordance with the Institutional Animal Ethics Committee (IAEC) of Veterinary College Anand Agriculture University.

All the 80 chicks were randomly divided into four equal groups (group I to IV) consisting of 20 chicks each. The group I served as control and received only vehicle (Distilled water), while group II, III and IV received acetyl salicylic acid at doses of 400, 1600 and 4000 ppm through feed consecutively for 21 days. At the end of 21 days

i.e. 22nd day of experiment, all the chicks were sacrificed and blood was collected from right jugular vein for hematological and serum biochemical analysis. The postmortem examination of each broiler bird of different groups was performed and gross lesions if any, were recorded. Tissue samples from liver, kidney, lung, spleen, heart and intestine were collected and preserved in 10% neutral buffer formalin for further histopathological examination.

The different parameters like clinical signs, mortality, body weight, kidney:body weight ratio, feed consumption, feed conversion ratio (FCR) were studied. Biochemical estimations were carried out by Semi-automatic analyzer NOVA-2021 manufactured by Analytical Technologies Ltd. with the diagnostic kits (Crest Biosystems; a division of Coral Clinical Systems) for AST, ALT, AKP, Serum creatinine, BUN and Uric acid. The hematological parameters viz. TEC, PCV and Hb were analyzed by Automatic Blood Analyzer BS 120 manufactured by Mindray Technologies Ltd. The data obtained was subjected to one way ANOVA test, where ( $p < 0.05$ ) was considered as statistically significant and ( $p < 0.01$ ) as highly significant.

## RESULTS AND DISCUSSION

All the birds belonging to group I to IV were observed daily for any abnormal physical or behavioral changes throughout the period of 21 days of experiment. Chicks under groups I and II appeared clinically healthy and did not reveal any remarkable clinical signs. However, birds in the group III and IV were slightly dull, depressed and anorectic. Slow growth rate and blood mixed faecal droppings were observed in few birds of group IV. Clinical signs observed were in agreement with Mohan et al. (2012) who found hypoactivity, disinclination to move and watery droppings accompanied with blood mixed mucous as clinical signs in birds administered with ASA at the dose rate of 10 mg/kg/day, per os daily at 24 h interval for five consecutive days.

Reduction in body weight gain was observed in ASA treated group III and IV but the significant reduction in body weight ( $p < 0.05$ ) was observed in group IV at the end of 1st, 2nd and 3rd week as compared to control group (Table I). The observations were in close association with the previous studies on ASA by Glick (1962), Proudfoot and Hulan (1983) and Pozniak *et al.* (2012) in broilers. Birds in Group IV alone showed 5% mortality during the second week.

Group III and IV revealed significant ( $p < 0.05$ ) increase in kidney: body weight ratio as compared to control (Table I). Increase in the ratio was mainly due to reduced body weight and increased kidney weight which is in agreement to the findings of Pozniak *et al.* (2010, 2012). In ASA treated group, there is functional hypertrophy of the kidney involved in salicylate elimination. Pozniak *et al.* (2012) reported functional hypertrophy by the negative correlation between duration of treatment and plasma minimal salicylate concentration. This correlation was more significant at higher doses of drug and was related to the kidney size. Higher doses of salicylate lead to accumulation as it conjugated with glycine in humans or with ornithine in galliform birds (Baert *et al.* 2004).

The present study revealed that there was dose dependent decrease in feed consumption in group III and IV when compared to control (Table II) during entire period of experiment which was in support of observations made by Murai *et al.* (1994) who studied the effect of dietary aspirin on laying performance and egg yolk fatty acid composition in Japanese quail. The average FCR was calculated at the end of experiment. It was 2.18 in group I (Table I). Group IV showed dose dependent marginal increase in FCR due to decrease in average body weight gain as compared to normal birds.

Group IV revealed significant change ( $p < 0.05$ ) in the value of PCV, TEC and Hb as compared to control. Group II and III revealed non significant decrease in the values as compared

to control (Table II). Hematological alterations indicates anemia which may be due to loss of blood because of internal hemorrhages in visceral organs which were in agreement with results of Merchant and Modi (2004) and Johnson and Manuwa (2014) who found reduced RBC count, hemoglobin and other red cell indices and increased WBC counts as compared to control in mice and rat. Mohan *et al.* (2012) found significant decrease in serum total protein concentration, total erythrocyte count, hemoglobin concentration, PCV and platelet after oral administration of ASA (10 mg/kg) daily for a period of five days.

There was no significant difference observed in any of the following serum biochemical parameters except AKP, studied for all the four groups *i.e.* Uric acid, Creatinine, Blood urea nitrogen (BUN), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). Birds of group IV showed significant decrease in AKP ( $p < 0.05$ ) as compared to the control (Table III). Sudden decrease in the AKP values might be due to the bone damage in agreement with the results obtained by Derakhshanfar *et al.* (2012) who found decrease in AKP level in the serum of broiler chicks given ASA at the dose rate of 400mg/L for 21 days. The results obtained show that long term usage of ASA at a high dose can affect the mineral density of bone. Aspirin has been shown to decrease bone mineral density at multiple skeletal sites, suggesting a role of COX-2/acetyl salicylic acid in osteoporosis and tibial dyschondroplasia in poultry birds.

Birds died and sacrificed revealed hemorrhage in intestine and lungs, ulceration in intestine and pale enlarged kidneys due to ASA high dose. Histopathological section of intestine of Group IV revealed submucosal hemorrhage, infiltration of mononuclear cells and desquamation of villi, section of lung revealed congestion and mild to severe hemorrhages which may also responsible for fall in hematological parameters. Section of kidneys revealed varying degree of congestion,

hemorrhages, degenerative and necrotic changes, tubular swelling along with hyperplasia and hypertrophy which were in agreement with the findings of Pozniak et al. (2010) who reported an increase in kidney size in chickens treated with ASA at the dose of 200 mg/kg. Pozniak et al. (2012) observed kidney parenchymal degeneration with lipid accumulation and tubular swelling in individuals from the salicylate treated ASA 400 and SS 400 groups in poultry.

The birds treated with ASA at the dose rate of 400 and 1600 in feed did not reveal any patho-

morphological, hematological and biochemical alterations. In group IV, ASA was given at the dose rate of 4000 ppm for 21 days which is equal to 500 mg/kg body weight, which is far more than the therapeutic dose of ASA and at this dose it causes intestinal hemorrhage leading to anemia and fall in AKP may be due to bone damage. So it can be concluded that judicious use of ASA is acceptable in broiler chicks, keeping in view its beneficial effects (up to 1600 ppm dose) as no early signs of adverse effects were found but at very high doses it causes mortality due to anemia and bone damage.

**Table I**

**Mean values of feed conversion ratio (FCR), feed consumption (g), body weight (g) and Kidney: Body Weight Ratio ( $\times 10^{-3}$ ) in three week old broiler chicks of different treatment groups.**

Experimental Group	Feed consumption (g)	Body weight (g)	FCR	Kidney: Body Weight Ratio ( $\times 10^{-3}$ ) Mean $\pm$ SE
Group - I	1455.60	668	2.18	9.24 $\pm$ 0.256
Group - II	1452.90	688	2.11	9.38 $\pm$ 0.248
Group - III	1448.00	612	2.36	10.16 $\pm$ 0.461*
Group - IV	1433.75	588	2.44	10.40 $\pm$ 0.478*

\* Significant ( $p < 0.05$ )

**Table II**

**Mean values of PCV (%), TEC (x106/uL) and Hb (g/dL) in three week old broiler chicks of different groups**

Experimental Groups	PCV (%)	TEC (x106/uL)	Hb (g/dL)
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Group - I	36.52 $\pm$ 0.413	2.62 $\pm$ 0.040	12.20 $\pm$ 0.064
Group - II	34.80 $\pm$ 0.378	2.51 $\pm$ 0.032	11.42 $\pm$ 0.046
Group - III	34.42 $\pm$ 0.275	2.49 $\pm$ 0.077	11.38 $\pm$ 0.044
Group - IV	33.98 $\pm$ 0.375*	2.42 $\pm$ 0.031*	11.22 $\pm$ 0.038*

\* Significant ( $p < 0.05$ )

**Table III**

**Mean values of serum uric acid (mg/dl), creatinine (mg/dl), BUN (mg/dl), ALT (U/L), AST (U/L) and AKP (U/L) in three week old broiler chicks of different groups**

Experimental Groups	Uric acid (mg/dl)	Creatinin (mg/dl)	BUN (mg/dl)	ALT (U/L)	AST (U/L)	AKP (U/L)
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Group – I	6.58 ± 0.221	0.182 ± 0.010	2.06 ± 0.178	23.44 ± 0.442	178.10 ± 1.65	485.98 ± 1.09
Group – II	6.44 ± 0.314	0.178 ± 0.012	2.02 ± 0.069	23.09 ± 0.566	165.10 ± 1.72	485.28 ± 1.43
Group – III	6.86 ± 0.497	0.182 ± 0.009	2.10 ± 0.083	23.20 ± 0.868	180.20 ± 3.40	486.27 ± 1.47
Group – IV	7.99 ± 0.067	0.188 ± 0.013	2.10 ± 0.083	23.36 ± 1.037	186.80 ± 5.28	480.97 ± 1.22*

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