



## ACETAMINOPHEN-INDUCED PROVENTRICULAR DAMAGE IN BROILER CHICK

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### ABSTRACT

Recently lethality of paracetamol and damage towards both, target and non target organs came into light. Proventriculus is important organ in broiler chicks hence it is selected in this study. One group, controls, 14 days old broiler chicken were administered only vehicle by i.m. injections, while another experimental group received intra muscular injection of paracetamol at lethal dose. Treatment induced unexpected results as mucosal layer revealed hyperplasia but submucosal proventricular glands revealed damage and disorganization. It is suggested that a safe dose and duration must be researched for treating chicken with paracetamol. Fast growth of broiler chickens is due to better food utilization due to better digestion by enzyme and HCl secreted by proventriculus. Significance of results is discussed.

**Key Words:-** Acetaminophen, Proventriculus, Histopathology, Broiler Chicken, Hyperplasia.

### INTRODUCTION

Broiler chickens grow faster in comparison to other indigeneous fowls on account of better digestive system which in turn results in higher FCR=Food Conversion Ratio (Verdal HD *et al.*, 2010; Nasrin M *et al.*, 2012). Stomach in chicken has two parts; proventriculus as glandular stomach and gizzard as muscular stomach (Liman N *et al.*, 2010). Proventriculus is susceptible to toxins (Kumar P *et al.*, 2009) and many NSAID i.e. non steroidal anti inflammatory drugs (Mohan K, 2010). Little is known about toxicity of acetaminophen (paracetamol) which is expected to be used intensively in poultry in future due to ban on the veterinary use of diclofenac (MOEF, 2006; Swan G *et al.*, 2006; Cuthbert R *et al.*, 2011; Dama M, 2014). Of late, lethal dose of paracetamol and its toxic effects towards target and non target organs in broiler chicken have been reported (Marmat S *et al.*, 2015; Marmat S and Rathore HS, 2015a & b; Marmat S

and Rathore HS, 2016). In this communication acetaminophen-induced histopathological changes in the proventriculus of broiler chicken are reported.

### MATERIALS AND METHODS

#### Animal Model

Twenty apparently healthy, unsexed broiler chicks i.e. *Gallus gallus domesticus*, aged one day old were procured from commercial poultry farm. All birds were housed in the experimental house for 13 days for acclimatization. Body weight was ranging 260±20 gms on 14<sup>th</sup> day. Food provided to birds contained of gram, corn, soyabean and grinded wheat in equal proportion i.e. (1:1:1:1) also having 10% per kg crushed prawns mixed with it. Food and water were fed *ad libitum*. In plastic cups tap water was supplied to chicks.

#### Paracetamol (Acetaminophen)

Fibrinil IM manufactured by NLS Silmour HP (India) are used for intramuscular (i.m.) injections. In each ml injection fluid consists of 150 mg paracetamol in 2%

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v/v Benzyle alcohol and water qs. Benzyl alcohol used as a vehicle in control group is made by CDH, Delhi.

### Ethical Aspect

Animals were treated gently however, there is no ban on the use of chicks. Work is a part of ongoing study of first author for her Ph.D degree and this fact is in the knowledge of departmental ethical body.

### Experimental Design

Randomly divided twenty chicks in to two groups i.e. control and treated, each group contains 10 chicks. Animals were not provided food 4 hr prior to experimentation on experiment day i.e. 14<sup>th</sup> day. Benzyl alcohol i.e. vehicle is injected intramuscularly to chicks of group 1 (control) while lethal dose of paracetamol (2 gm/kg/bw) was administered by i.m. injections to chicks of group 2 (treated). All details of experimentation are reported in earlier publications (Marmat S *et al*, 2015).

### Tissue Collection

Chicks became inactive and unconscious later on due to paracetamol treatment and finally died within 24 hrs. Dull and dying chicks were dissected to obtain proventriculus. Chicks of control group were dissected under mild chloroform anesthesia. Dissected organ were wash out in 0.9 saline, cut in to pieces and then fixed in

Bouin's fluid. Fixed tissues were then processed via routine paraffin embedding process and sectioned at 4 micron and stained with haemotoxylin and eosine (H&E).

### Haemotoxylin

Ready to use haemotoxylin stain manufactured by Ranbaxy Fine Chemicals Limited, New Delhi is used.

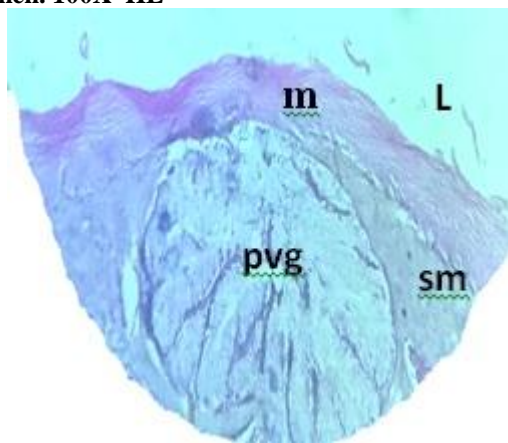
### Eosine

Ready to use eosine stain manufactured by Central Drug House (Pvt.) Ltd, New Delhi is used.

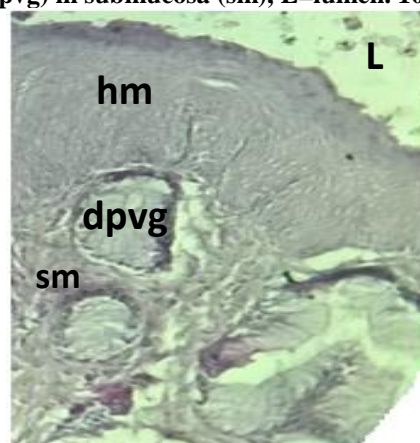
### RESULTS

Among controls cross section of proventriculus of broiler chickens reveals normal histoarchitecture. Innermost i.e lumen facing innermost mucosal layer is well organized and underlying submucosa lodges proventricular glands arranged in lobules (Fig 1). Such histology has been reported earlier (Aitken RNC, 1958; Nasrin M *et al.*, 2012; Ventura A *et al.*, 2013). Figures do not show any pathological sign. Lethal dose of acetaminophen thickened mucosal layer due to hyperplasia and debris of fully damaged proventricular glands are seen (Fig 2). This cellular damage seems to be due to direct toxicity of acetaminophen overdose. No other significant histological changes could be seen in muscle layers and serosa hence only luminal face (L) is shown in fig 1 and 2.

**Fig 1. Control showing usual mucosa (m) and well organised proventricular gland(pvg) in sub mucosa (sm), L=lumen. 100X HE**



**Fig 2. Paracetamol treated showing thick i.e hyperplastic mucosa (hm) and damaged- collapsed proventricular glands (dpvg) in submucosa (sm), L=lumen. 100X HE**



### DISCUSSION

Results clearly indicate that paracetamol at its lethal dose (2 gm/kg/bw) resulted in the destruction of vital part of proventriculus which is must for its function i.e production of the HCl and digestive enzymes (Liman N

*et al.*, 2010). High dose of paracetamol may induce gastro-duodenal ulcers specially under hyperacidity and chronic inflammation in human and rat (Rainsford KD and Whitehouse MW, 2006). Paracetamol-induced villus degeneration and infiltration in proventriculus and

hyperplastic intestinal villi were noticed in five weeks old broiler chicks after 05 days of post oral administration at 10 mg/kg/bw (Mohan K, 2010). Epithelial hyperplasia and hyperkeratosis of the nonglandular mucosa of the stomach induced by acetaminophen in rat is also observed following (400 mg/kg/day) intravenous exposure for 14 days (McKee JS and Gass JH, 2011). In this report McKee and Gass were first to indicate proliferative injury i.e lesions due to i.v administration of paracetamol but they could not explain exact mechanism and/or cause of such effect of paracetamol. It is known that abnormal endocrine stimulates both physiological and pathological hyperplasia (Wheater P *et al.*, 1990). Recently mild analgesics like paracetamol, aspirin and indomethacin are found to cause endocrine disturbances in adult human testis *in vitro* (Albert O *et al.*, 2013) Present finding partially in conformity with the findings of Mohan (2010). In the present case hyperplasia is seen in the mucosal area of proventriculus but Mohan (2010) found so in the intestine only.

At this lethal dose both target organs (liver and kidney) and non target organs (left ventricle, lung, spleen and blood) were damaged and direct toxic effects of paracetamol metabolite NAPQI i.e N-acetyl-benzo quinone imine are suggested for these organs (Marmat S and Rathore HS, 2015 a & b). It is difficult to understood how NAPQI induced hyperplasia in the mucosal layer but damaged proventricular glands. Toxicity and metabolism of paracetamol and role of NAPQI (N-acetyl benzo quinone imine) is well known in human (Insel PA, 1996) as well as in experimental animals (Boyd EM and Bereczkey GM, 1966; Mohandas J *et al.*, 1981 Smolarek TA *et al.*, 1990; Lara JP *et al.*, 2003). On the contrary paracetamol metabolism in chickens and other birds is

lesser known (Neirinckx E *et al.*, 2010; Mohammad FK *et al.*, 2012) but role of N-acetylbenzoquinone imine (NAPQI) in birds is at all not known. Paracetamol induced decline in GSH and increased lipid peroxidation in the liver of chicken is known (Bhar MK *et al.*, 2005). An Infact detail of exact actual pharmaceutical dose of paracetamol in poultry birds is not on record. Wherever paracetamol in future is to be used as a routine NSAID drug in poultry care must be taken to select safe dose and duration too as gut damage shall hamper their growth too. It is of interest to mention here that one syndrome in broiler chickens was observed which involved lesion of proventriculus and gizzard with their enlargement (Stuart BP *et al.*, 1986) and due to this poor feed conversion and late marketing of broiler chicken was a matter of concern.

### CONCLUSION

Paracetamol (acetaminophen) administration at lethal dose (2gm/kg/bw, i.m) once to 14 days old broiler chicks induced hyperplasia of mucosal layer but caused severe disorganization at cellular levels in the vital submucosal layer lodging proventricular glands. Digestive enzymes and HCl secretion by these glands is of great importance for better food utilization and faster growth of broiler chickens.

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### CONFLICT OF INTEREST

No interest

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