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Hepatorenal toxicity of Paracetamol (Acetaminophen) in Broiler chicks at lethal dose

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Abstract

Study deals with involvement of hepatorenal damages in broiler chicks following paracetamol (acetaminophen) administration of lethal dose at 24 hr. Fourteen days old healthy, unsexed broiler chicks *Gallus gallus domesticus* were divided into two groups. Chicks of first group received single intramuscular injections of vehicle (Control-Gr.I) while chicks of another group received single i.m injections of paracetamol at 2gm/kg/bw; lethal dose at 24 hr. Next day freshly dissected liver and kidneys from both groups were washed, fixed, sectioned and stained for histological observations. Blood drawn directly from the heart was used for the estimations of the biochemical parameters. Observations of tissue slides revealed severe damage to hepatic and kidney tissues. Degenerative changes were main effects. Increased levels of transaminases (GOT & GPT), alkaline phosphatase, bilirubin , lowered protein and disturbed levels of electrolytes in the serum suggest impaired hepatorenal functions due to paracetamol toxicity. Observed results suggest that lethality of paracetamol in chicks also seems mainly due to severe oxidative damage to hepatorenal tissue as happens in human and experimental animals.

Key words: Broiler chicks, paracetamol, hepatorenal damage.

Diclofenac was held responsible for the extinction of Gyps Vultures hence it was banned in veterinary use and meloxicam emerged as safer one in 2006.[17,27] Some meloxicam brands also had paracetamol as second ingredient.[5] Pharmacokinetics and toxicity of paracetamol and role of NAPQI (N-acetyl benzo quinone imine) is fairly well known in experimental animals[4,14,19,26] and human[10] but little is known in poultry birds.[18,21] In spite of lesser known pharmacology of paracetamol, it is used as poultry medicine[6,20] and now it is feared that veterinary use of paracetamol shall increase in near future.[11] Interestingly paracetamol is also debated as friend or foe.[15] Recently very ground level

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information about paracetamol toxicity *i.e* LD_{50} and LD_{100} values and possible cause of death in broiler chicks were provided from our end. Among human beings principal adverse effect of acute overdose of acetaminophen is fatal hepatic necrosis[28] which may be associated with renal tubular necrosis.[10] In the present study an attempt was made to find out if similar hepatorenal damage occurs following administration of acetaminophen at lethal dose.

Material and experimental design :

Animal: One day old, healthy, unsexed, broiler chicks, *Gallus gallus domesticus* were procured from local poultry house. Chicks were acclimatized to departmental animal house for thirteen days. Food consisted of grinded wheat, corn, soybean and gram in equal ratio (1:1:1:1) wt/wt; also had 10% crushed small prawns. Tap water of known physiochemical properties[1] was supplied for drinking purpose. Food and water were provided *ad libitum.* Chicks were exposed to natural day night periods. On 14th day average body weight of chick was 260±20 gms.

Chemicals: Acetaminophen (Paracetamol) injections, trade name Fibrinil IM made by NLS Silmour HP were used for intramuscular administration. Each injection had 150 mg paracetamol/ml. Benzyl alcohol: CDH, Delhi.

Ethical Aspect: Study is a part of ongoing Ph.D. study of first author and is in the knowledge of university and departmental body. There is no ban on the use of chicks for experimentation, however, chicks were handled gently.

Experimental Design: Twenty chicks were randomly divided into two groups, each group had 10 chicks. Animals were starved for four hours before onset of experimentation on 14th day. Lethality was recorded among 10 chicks (Group II) which received 2gm/kg/bw at 24 hr dose as reported in our earlier publication[16]. Control chicks (Group I) received only vehicle *i.e* benzyl alcohol. Details of experimentation are given earlier.[16]

Tissue collection: After paracetamol injections chicks became dull first and unconscious later on, and died by 24 hr. Dying and unconscious chicks were dissected to obtain their livers and kidneys. Chicks of control group were administered mild chloroform anesthesia and unconscious ones were dissected. Organs were washed in 0.9% saline, cut into small pieces and were fixed in Bouin's fluid. Dehydrated material was embedded in wax for block making. Microtome sectioned material was fixed on slides and stained in haematoxyline eosine.

Haematoxylin : Ready to use Haematoxylin solution made by Ranbaxy Fine Chemicals Limited, New Delhi, was used for staining.

Eosin : Eosin (250 mg) blue powder [$C_{20}H_6Br_2N_2Na_2O_9$, M.W. 624.09] made by Central Drug House (P) Ltd. New Delhi, was dissolved in 100 ml of 90% alcohol to prepare eosine solution for staining.

Blood Collection: Blood was obtained from the ventricle of chicks using glass syringe and kept undisturbed. Separated serum was used for liver and kidney function tests. Biochemical parameters glutamate oxaloacetate transminase (GOT), glutamate pyruvate

transminase (GPT), alkaline phosphatase (AP), billirubin (BB) and total protein as liver function tests and urea, creatinine, electrolytes as kidney function tests were evaluated using ready to use available kits made by standard companies (*i.e* BEACON Diagnostics, Pvt.Ltd, ERBA diagnostics and Mannheim GmbH, AGAPPE Diagnostics Ltd.).

Statistics: Experiments were done thrice and only consistent data were considered and were subjected to't' test for significance. In each case number of observations were six (n=6).

Histological: Liver of control group of chicks revealed quite normal histoarchitecture (Fig.1). Lethal dose (2 gm/kg/bw) of paracetamol damaged hepatocytes and blood vessels (Fig. 2). Kidney in control group of chicks revealed normal structure (Fig. 3) but paracetamol damaged both tubules and glomeruli badly (Fig. 4).

Biochemical (LFT and KFT): Single administration of paracetamol at lethal dose altered hepatorenal functioning (Table 1). Histological observations very well corroborate physiological *i.e* liver and kidney functioning. Damaged tissue is not expected to work properly hence deviated values from control values for biochemical parameters related to liver and kidney of chicks are obtained. Serum electrolytes, both sodium and potassium increased while chloride remained unaffected. Serum proteins also showed alterations. In nutshell, paracetamol at lethal dose badly damaged hepatorenal tissue which in turn revealed disturbed biochemical functioning.

Few reports exist solely on the toxicity of paracetamol in birds while in others liver was damaged by paracetamol to study protection with herbals. Paracetamol (acetaminophen) induced damage toward bird's liver and kidney are on record. In 28 days old broiler chickens (bw 0.8 to 0.9 kg). paracetamol i.m. at 250 mg/kg/bw (six daily doses) altered biochemical parameters of liver function tests, caused degeneration of hepatocytes, infilteration of mononuclear cells and proliferation of bile ducts. Paracetamol induced damage is held responsible due to free radical induced peroxidation of cellular membranes in hepatic tissue.[13] Cockerel birds receiving single oral dose of paracetamol at 20 and 40 mg/kg/bw revealed degenerative changes in their liver and kidneys.[7] Rangnathan[24] observed cytopathological changes and enhanced levels of enzymes of liver function test following oral administration of paracetamol at 2 gm/kg bw for 23 days. Toxicity of paracetamol was studied in one day old broiler chicks (bw 41-42gm) at 250 mg/kg/bw i.p in 50% ethanol single dose. Decreased GSH and increased lipid peroxidation in degenerative liver tissue could be seen.[3]

Paracetamol induced damage in the liver of Bovans-Hybrid chicks is reported by Bakhiet and Mohammad[2] following feeding 500 mg/kg paracetamol in basal diet for two weeks to 14 days old chicks. Damage, necrosis, fatty vacuolization of centrilobular hepatocytes could be seen. Biochemical parameters were also found altered. In our earlier study paracetamol administration at lethal dose (2gm/kg/bw single IM) exerted dullness in broiler chicks[16] after an hour. Severe damaged liver and kidney tissue cannot work properly. Probably toxic metabolite(s) of paracetamol like NAPQI (N-acetyl benzo quinone imine) are expected to

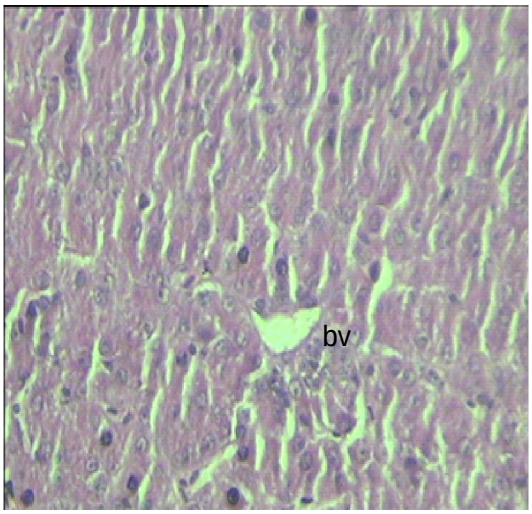

Fig. 1. Showing normal histology of liver of control group of chick, well organized hepatocytes are seen around blood vessel (bv). HE 400X

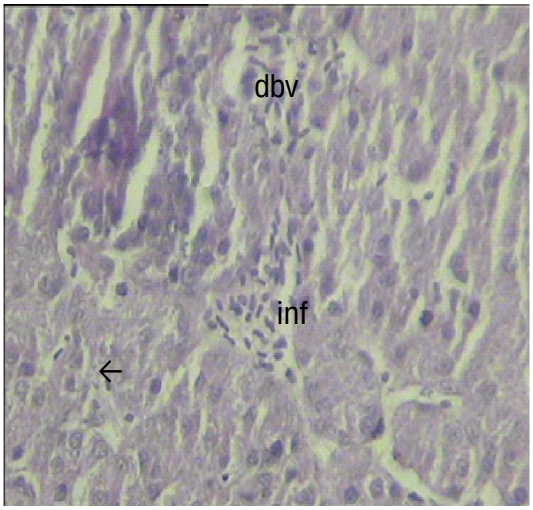

Fig2. Showing severe disorganization of hepatic tissue due to paracetamol. Damaged blood vessel (dbv) and in filteration (inf) evident. Dying hepatocytes are seen (←) HE 400X

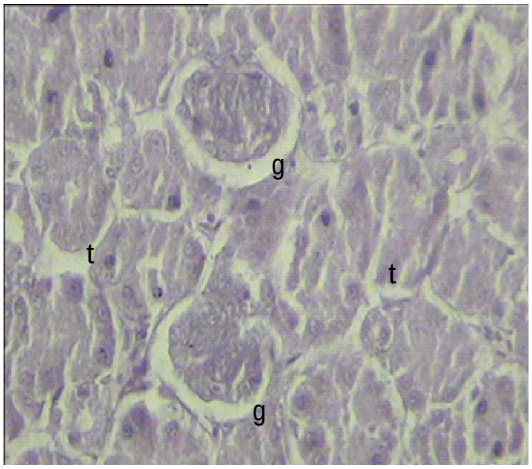

Fig 3. Showing normal histology of kidney of control chick, well organized glomeruli (g) and tubules (t). HE 400X

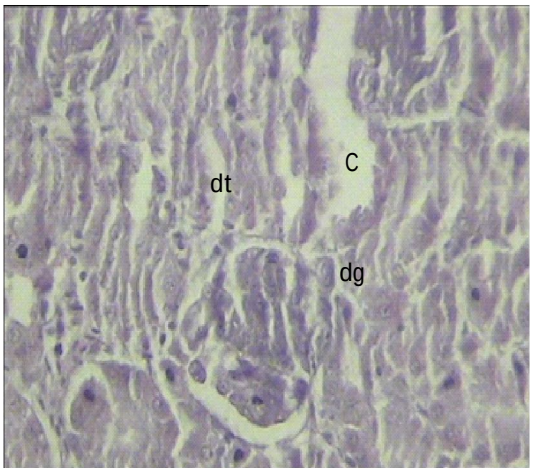

Fig 4. Showing severe disorganization due to paracetamol. Damaged glomeruli (dg) and dialated tubules (dt). Dead tubules are seen as cast (C). HE 400X

Table 1. Effects of Paracetamol on liver function tests in Broiler chicks (n=6).

S.No.	Parameters	Control(M±SEM)	Treated(M±SEM)	% Change
1.	S. Bilirubin(mgms%)	0.25±0.02	1.20*±0.21	↑380%
2.	S.SGPT(U/L)	136.50±21.3	422.00*±1.95	↑209.15%
3.	S.SGOT(U/L)	658.30±69.9	1097*±2.28	↑66.69%
4.	S.Alk. Phosphatase(IU/L)	4339±27.70	13396*±217.11	↑208.73%
5.	S. ProteinsTotal(Gms%)	3.90±0.06	5.30*±0.02	↑35.89%
6.	Albumin(Gms %)	1.20±0.03	1.30*±0.02	↑8.33%
7.	Globulin(Gms%)	2.70±0.04	4.00*±0.02	↑48.19%
8.	A/G Ratio	0.32±0.009	0.45*±0.007	↑40.62%

*Statistically significant based on 't' test at 5% level of significance (p<2.201).

↑ = Increase

Table 2. Effects of Paracetamol on renal function Tests in Broiler chicks. (n=6).

S.No.	Parameters	ControlM±SEM	TreatedM±SEM	% change
1.	Urea(mgms%)	11.6±0.57	19.05*±1.20	↑64.22%
2.	Creatinine(mgms %)	0.73±0.05	0.90*±0.01	↑23.28%
3.	S.Electrolytes (a) S.Sodium(mmol/L)	132.40±1.34	149.00*±1.06	↑12.53%
4.	(b) S. Potassium(mmol/L)	6.30±0.08	15.70*±0.32	↑149.20%
5.	(c) S. Chloride(mmol/L)	100.90±0.47	101.50±0.41	NS

*Statistically significant based on 't' test at 5% level of significance (p<2.201).

↑ = Increase, NS= Non Significant

deplete stored GSH in the liver and extra hepatic tissues too, because paracetamol is known to decline GSH level and to enhance lipid peroxidation[3] in the liver of chicks. This sudden sharp decline in GSH and increased lipid peroxidation of lipids, proteins and DNA[25,22,23] along with accumulation of nitrogenous wastes and electrolytes seems due to failure of kidneys.[8,9,12] All these expected toxic effects can result in a shock to treated chicks making them progressive dull, unconsciousness and dead. Possibility of toxicity of paracetamol at lethal dose towards non target organs also cannot be ruled out. It is concluded from this investigation that paracetamol- induced death in broiler chicks can be attributed mainly due to the serious adverse hepatorenal effects.

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