

## LETHAL DOSES OF ACETAMINOPHEN (PARACETAMOL) FOR YOUNG BROILER CHICKS

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### ABSTRACT

LD<sub>100</sub> or LD<sub>50</sub> values of paracetamol for any variety of chicks are not on record hence these experiments were done. Healthy 14 days old weighing 260±20 gms, unsexed broiler chicks (*Gallus gallus domesticus*) were divided into eleven groups. Ten chicks were used in each group. Paracetamol injection was administered intramuscularly once to each chick. Concentrations administered were 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, 2500 mg/kg. For control, 10 chicks were injected with vehicle only. Changes in the behavior could be noticed after paracetamol administration. Mortality was recorded after 24 hrs. Experiments were repeated thrice. Values were plotted. 2 gm/kg dose is found lethal i.e LD<sub>100</sub> values at 24 hours and 1.8 gm/kg is calculated LD<sub>50</sub> at 24 hours for 14 days old chick. Possible explanation towards observed changes in the behavior and death of chicks is offered. Significance of findings is also discussed.

**Keywords:** Acetaminophen, Paracetamol, Chicks, LD<sub>100</sub> and LD<sub>50</sub>.

### INTRODUCTION

Paracetamol (Acetaminophen) was tried long back in poultry as antipyretic drug and as growth stimulator [1]. Paracetamol and aspirin are used as veterinary avian medicine [2-4]. Pharmacokinetics of paracetamol is studied following administration at 10 mg/kg [5] and at 100 mg/kg [6] in chicks. A careful survey of literature reveals scattered reports [7-13] on the toxicity of paracetamol in chicks, however no attempts was made to find out lethal doses i.e LD<sub>100</sub> or LD<sub>50</sub> exclusively for chicks of an age group. Present study tried to fill this lacuna.

### MATERIALS AND EXPERIMENTAL DESIGN

#### Avian Model

Broiler chick i.e *Gallus gallus domesticus* are used. 01 day old, healthy, unsexed chicks are obtained from local poultry. They were acclimatized to animal house for 13 days. On 14<sup>th</sup> day their body weight was recorded it was 260±20 gm. Chicks were fed on the same food which is used in the poultry. Its composition was: Grinded Wheat, Corn, Soyabean and Gram in equal quantity i.e (1:1:1:1 wt/wt) and 10 % per kg crushed prawns. Chicks were exposed to natural day night periods. Food was supplied in

plastic dishes. Tap water was supplied in plastic cups. Food and water were provided *ad-libitum*.

#### Paracetamol (Acetaminophen)

Febrinil IM injections made by Nitin Life Science, Simour (H.P.) and marketed by SVIZERA- H.P. are used for IM administration. Each ml of injection fluid contains 150 gm paracetamol in 2% v/v Benzyl alcohol and water qs.

**Benzyl alcohol:** CDH, Delhi.

**Ethical Aspect:** There is no ban on the use chicks, however, animals were handled gently. This study is in the knowledge of university and departmental body.

#### Experimental Design

Chicks were randomly divided into eleven groups, each consists of ten chicks. Animals were not provided food 4hr prior to experimentation on the 14<sup>th</sup> day i.e experimental day. Each chick was administered, calculated dose intramuscularly in pectoral muscle using fine 26 No. needle fitted on a sterilized, disposable syringe of 5 ml capacity.

For controls 10 chicks were injected with maximum volume 0.07 ml of vehicle (2% v/v Benzyl alcohol), it corresponded to the volume injected in highest dose of paracetamol. Experiment was conducted between 11-12 AM. Each group of ten chicks was kept separately in a cage. Next day i.e after 24 hrs, chicks were observed and mortality was recorded in each group. Percentage mortality (hence % survival too) was recorded at 24 hrs.

## RESULTS AND DISCUSSION

For convenience only findings are discussed under separate headings.

### Behavior of Chicks

No effect could be observed upto 1250 mg/kg bw. At 1500 and 1750 mg/kg bw, four hours after paracetamol injections toxic effect could be noticed. Chicks become dull and could not keep on standing posture which is their normal posture. Later on they become unconscious and finally died. At 2000 mg/kg bw toxic effect could be seen after an hour past paracetamol injection. Birds became dull and finally unconscious but were seen sitting with eyes closed. They opened eyes when disturbed, just at the time of death they lost balance and fell on the floor of cages. Chicks did not consume water after paracetamol injections. No other symptoms could be seen. Neirinckx et al [5] injected paracetamol solution into wing vein at 10 mg/kg to 5 week old female broiler chicken. Maximum plasma concentration was achieved in less than 1 hour time. Clearance indicated a fast biotransformation and excretion of paracetamol. Pharmacokinetics of paracetamol following single intraperitoneal dose at 100 mg/kg bw have also been reported [6]. These reports indicated that paracetamol is well distributed after 10 minutes of administration in the body of chicks and eliminated too. Severe damage to liver and brain of chick can make them unconscious, in the present case.

### Lethality

Percentage lethality i.e death of chicks after each dose administered is shown in Table 1. Data were plotted on a graph and LD<sub>50</sub> is calculated (Graph-1). Standard procedure suggested for LD<sub>100</sub> and LD<sub>50</sub> determination is followed [14]. LD<sub>100</sub> for 14 days old *Gallus gallus domesticus* (Broiler chick) is found to be 2000 mg i.e 2 gm/kg bw and LD<sub>50</sub> value determined is 1800 mg i.e 1.8 gm/kg bw. No earlier report revealed LD<sub>100</sub> or LD<sub>50</sub> of paracetamol for particular variety of chickens hence present findings cannot be compared with any values of LD<sub>100</sub> and LD<sub>50</sub>. Looking to toxic doses tried by earlier workers present values are not very unexpected ones.

### Possible cause of death of chicks

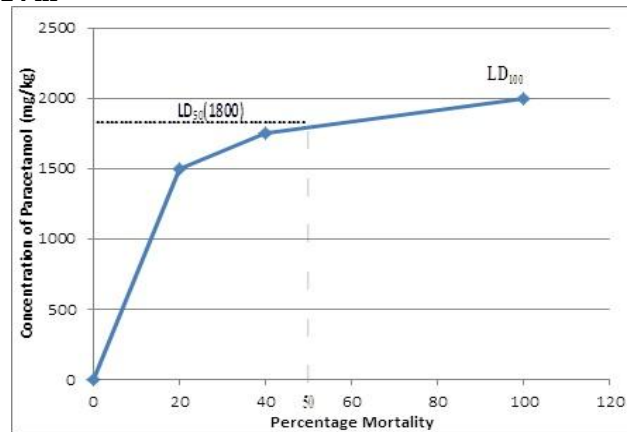
In the present experimentations observed death of chicks is a result of paracetamol injections as no chick died in control group. Also, usual mortality rate of broiler was

only 3.8% in 2011 [15]. Tap water provided as drinking water had quite usual physicochemical characteristics and it has not cause any cyto or genotoxic effects in *Allium cepa* bulbs grown in it for controls as reported in our publication [16].

Toxicity metabolism of paracetamol and role of NAPQI (N-acetylbenzoquinone imine) is fairly well known in human [17] and experimental animals [18-21]. On the other hand metabolism of paracetamol in chickens (Birds) is lesser known [5,6] but role of N-acetylbenzoquinone imine (NAPQI) in birds is at all not known, however, paracetamol induced decline in GSH and increased lipid peroxidation in the liver of chicken is known [8]. Extra hepatic lesions induced by overdose of paracetamol in mice are known [22] but additionally only brain damage in chickens on record [11]. Possibility of extra hepatic organ/tissue damage in chicks too exists.

Exact mechanism of toxic damage caused by paracetamol in chicks (Birds) is not fully known. Lacuna in the information prevents to give clear cut reason for paracetamol induced death of chicks. It is of interest to mention here that hepatic Cytochrome P450 and drug metabolizing enzymes have been shown to play role in detoxification in 4-6 weeks old broiler chicks in response to different bedding material [23] but unfortunately no such investigations have been undertaken to find detailed degradation pathway of paracetamol in birds. Death of chicks following administration massive i.e lethal dose of paracetamol in the present case may be attributed possibly due to severe oxidative damage to liver and extra hepatic tissues.

**Graph 1. LD<sub>50</sub> determination in 14 days old Broiler chick following single paracetamol administration after 24 hr**



### Need of standard LD<sub>100</sub> and LD<sub>50</sub> for paracetamol

Today there is an urgent need to find out lethal and effective doses of paracetamol for poultry birds because of following reasons. It is an established fact that diclofenac toxicity is responsible for the decline in Gyps vultures in India hence government of India, Nepal and

**Table 1. Paracetamol (Acetaminophen) induced mortality in Broiler Chicks at 24 hr**

S.No.	Number of Chicks	Concentration of Paracetamol mg/kg	No. of dead chicks	% Mortality
1	10	0.00 Control	NIL	0.00
2	10	250	NIL	0.00
3	10	500	NIL	0.00
4	10	750	NIL	0.00
5	10	1000	NIL	0.00
6	10	1250	NIL	0.00
7	10	1500	02	20%
8	10	1750	04	40%
9	10	2000	10	100%
10	10	2250	10	100%
11	10	2500	10	100%

(Constant values obtained in experiments done thrice)

Pakistan has banned manufacture and import of veterinary diclofenac. Meloxicam was found safer than other compounds hence in 2006 it was announced safe alternative to diclofenac [24,25]. In a national level study on veterinary drugs, 50% of the meloxicam brands had paracetamol as second ingredient [26]. Paracetamol is already in use as avian medicine [4,27]. It is felt that now veterinarians will use more paracetamol for the treatment of birds [12]. As overdose of paracetamol is hepatorenal toxic in human beings investigations have already begun to find out similar threats in birds as evident by pharmacokinetic studies on paracetamol (and aspirin) in chickens [5,6]. Chickens are also used in toxicological models [28-30]. Paracetamol induced histophysiological changes in liver, kidney and brain in cockrail chicken are on record [11].

During last few years many herbal compounds have been tested for their ability to reduce or nullify acetaminophen (paracetamol) induced hepato and hepatorenal in experimental animals and a review deals with these aspects [31]. Now such attempts have begun to reduce toxicity of acetaminophen in birds too [8-10]. LD<sub>100</sub> and LD<sub>50</sub> for common poultry birds i.e chickens, turkey and ducks must be known for future research works. It is needless to say that present study has provided useful data.

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#### REFERENCES

- Dikstein S, Zor U, Ruah D, Sulman FG. Stimulatory effect of paracetamol on chicken growth. *Poul Sci*, 45, 1966, 744-746.
- Machin KL. Controlling avian pain. Compendium on continuing education for the practicing veterinarian. 27, 2005, 299-308.
- Livingston A. Pain and analgesia in domestic animals. Handbook of Experimental Pharmacology, 199, 2010, 159-189.
- Hawkins MG, Paul Murphy J. Avian analgesia. *Veterinary clinics of North America, Exotic Animal Practise*, 14, 2011, 61-80.
- Neirinckx E, Veraet C, De Boever S, Remon JP, Gommeron K, Daminet S. et al. Species comparison of oral bioavailability, first pass metabolism and pharmacokinetics of acetaminophen. *Research in Veterinary Science*, 89, 2010, 113-119.
- Mohammad FK, Mansoor AS, Al-Zubaidy MHI. Comparative single intraperitoneal dose pharmacokinetics of aspirin and acetaminophen in chicks. *Veterinari Medicina*, 57(3), 2012, 121-124.
- Lindenthal J, Sinclair JF, Howell S, Cargill I, Sinclair PR, Tailor T. Toxicity of paracetamol in cultured chick hepatocytes treated with methotrexate. *European Journal of Pharmacology*, 228, 1993, 289-298.
- Bhar MK, Das SK, Chakraborty AK, Mandal TK, Roy S. Hepatoprotective effect of Enliv® on paracetamol-induced liver damage in broiler chicks. *Int J Pharmacol*, 37(4), 2005, 257-258.
- Bakhiet AO, Mohammad A. Response of bovans-hybrid chicks to *Allium cepa*, paracetamol or their mixture. *Journal of Veterinary Science Animal Husbandary*, 47, 2008, 1-2.
- Kumar P, Prasad R, Singh KK, Roy BK. Hepatoprotective effect of *Centella asiatica* against paracetamol induced liver damage in broiler chicken. *Indian Journal of Poultry Science*, 44(1), 2009, 101-104.
- Hedau M, Bhandarkar AG. Histopathological changes in experimental paracetamol toxicity in poultry. *Royal Veterinary Journal of India*, 6(1), 2010, 23-25.

12. Jayakumar K, Mohan K, Narayanaswamy HD, Shridhar NB, Bayer MD. Study of nephrotoxic potential of acetaminophen in birds. *Toxicology International*, 17(2), 2010, 86-89.
13. Ranganathan V, Punnamurthy D, Ahamad B, Kumar MS. Hepatoprotective activity of *Indigofera aspalathoides* in chicken. Agricultural Research Communication Centre, 2013.
14. Gupta PK. Introduction and Brief History in Modern Toxicology, Edited by Gupta PK and Salunke, Metropolitan Book Co. Pvt. Ltd. New Delhi-2, 1985, 55-56.
15. National Chicken Council. "U.S. Broiler Performance", 2012.
16. Ahmed Z, Rathore H, Marmat S, Qureshi TN and Shrivastava S. Biological activities of *Dalbergia sissoo* leaves in different models. *International Journal of Green and Herbal Chemistry*, 3(4), 2014, 1705-1713.
17. Insel PA. Analgesic-antipyretic acid anti-inflammatory agents and drug employed in the treatment of gout. In Goodman & Gilman's, The pharmacological basis of therapeutics. 9th Edn. Eds. P.B. Molinoff and RW Ruddon, McGraw Hill International, Texas, 1996, 631-632.
18. Lara JP, Mc Cullough SS, Lamps LW and Hinson JA. Effect of N-acetylcysteine on acetaminophen toxicity in mice, Relationship to reactive nitrogen and cytokine formation. *Toxicological Sciences*, 75(2), 2003, 458-467.
19. Boyd EM, Bereczkey GM. Liver necrosis from paracetamol. *Brit J Pharmacol*, 26, 1966, 606-614.
20. Mohandas J, Duggin GG, Horvath JS, Tiller DJ. Metabolic oxidation of acetaminophen (paracetamol) mediated by Cytochrome P-450 mixed function oxidase and prostaglandin endoperoxidase synthetase in rabbit kidney. *Toxicology and Applied Pharmacology*, 61(2), 1981, 252-259.
21. Smolarek TA, Higgins CV and Amacher DE. Metabolism and cytotoxicity of acetaminophen in hepatocytes cultures from rat, rabbit, dog and monkey. *Drug metabolism and disposition*, 18(5), 1990, 654-663.
22. Placke ME, Wyand DS, Cohen SD. Extrahepatic lesions induced by acetaminophen in the mouse. *Toxicologic Pathology*. 15(4), 1987, 381-387.
23. Kawalek JC, Myers MJ, Howard KD, Farrell DE, Shaikh B. Hepatic CYP isoforms and drug metabolizing enzyme activities in broiler chicks. *International Journal of Poultry Science*, 5(2), 2006, 104-111.
24. MoEF (Ministry of Environment and Forests). Proceedings of the International Conference on Vulture Conservation, New Delhi, Government of India, 2006.
25. Swan G, Naido V, Cuthbert R, Green RE, Pain DJ, Swarup D. Removing the threat of diclofenac to critically endangered Asian Vultures. *Public Library of Science Biology*, 2006, 4.
26. Cuthbert R, Dave R, Chakraborty SS, Kumar S, Prakash S, Rande SP, Prakash V. Assessing the ongoing threat from veterinary non-steroidal anti-inflammatory drugs to critically endangered Gyps vulture in India. *Fauna and Flora International, Oryx*, 45(3), 2011, 420-426.
27. Muchadevi FC, Sibanda S, Kusina NT, Kusina J, Makuza S. The village chicken production system in Rushinga District of Zimbabwe. *Livestock research for rural development*, 2004, 16(6).
28. Lambert PR, Palmer PE, Rubel EW. The interaction of noise and aspirin in the chick basilar papilla. Noise and aspirin toxicity. *Archives of otolaryngology-Head and Neck Surgery*, 112, 1986, 1043-1049.
29. Murai A, Furuse M, Okumura J. Aspirin toxicity in chicks given diets deficient in linoleic acid. *Pharmacology, biochemistry and behaviour*, 48, 1994, 1047-1051.
30. Mohan K, Jayakumar K, Narayanaswamy HD, Shridhar NB, Bayer MD, Manafi M. Hepatotoxicity of acetaminophen in chickens. *Journal of veterinary Pharmacology and Toxicology*, 7, 2008, 48-49.
31. Sharma A, Makwana M, Rathore HS. Can herbal paracetamol combination drug prevent both liver and kidney disease? Results and possibilities. *Ethanobotanical Leaflets*, USA, 12, 2008, 286-298.