The Indian Journal of Veterinary Sciences & Biotechnology ***(2018) Volume 13, Issue 4, 32-39***

ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT

10.21887/ijvsbt.v13i4.11555

Submitted : 15-01-2018 ***Accepted : 20-02-2018*** ***Published : 08-04-2018***

Therapeutic Efficacy of Febuxostat and Allopurinol on Gout Induced Model in Broiler Chicks

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Abstract

This work was conducted to evaluate the comparative therapeutic efficacy of febuxostat and allopurinol on gout induced model in broiler chicks. A total of 108, day-old broiler chicks of either sex were equally divided into six groups, each of 18 birds and numbered from I to VI. Group I was kept as control group, while group II received diclofenac @ 10 mg/kg b wt by oral gavage daily once for 21 days. Group III and IV received febuxostat and allopurinol @ 4 mg/kg and 25 mg/kg b wt daily once, while Group V and VI were treated with febuxostat and allopurinol along with diclofenac @10 mg/kg b.wt. daily once for 21 days. Broiler chicks in all six groups were fed with broiler pre-starter and starter feed *ad libitum*. The group II (diclofenac) showed clinical sings like dullness, depression and lethargy with shrunken eyes along with 27.77% mortality, and also showed decreased body weight during experiment. The birds from group V and VI also showed clinical signs like dullness, depression and sleepy appearance before death, with 5.55% and 11.11% mortality, respectively. The serum biochemical parameters, viz., Uric acid, Creatinine and BUN values were increased in the diclofenac treated group II which was lowered by the febuxostat and allopurinol treatment in the group V and VI. The birds which were sacrificed at weekly interval did not show any appreciable gross and histopathological lesions, whereas the birds died during experiment showed gross and microscopic lesions which were specific to visceral gout.

Key words: Broiler bird, Allopurinol, Diclofenac, Febuxostat, Visceral gout.

Introduction

Gout is commonly seen among broiler chicks during first two weeks of life and characterized by abnormal accumulation of uric acid and urate crystals on serosal surface of the pericardium, liver capsule, air sacs, and in the kidneys but may be found in any tissue (Phalen *et al*., 1990). The physiological susceptibility of gout in birds gets coupled with multiple etiological factors, *viz*., nutritional (Vitamin A deficiency), infectious (Infectious bronchitis virus), Astro (virus infection), drug (diclofenac sodium), toxic (Oosporein) and managemental (Water deprivation) factors may act together to add severity to the condition. In addition to these previous experimental toxicopathological studies on diclofenac drug, have proved it to cause visceral gout in broiler chicks.

The treatment of the gout affected chicken include removal of managemental causes like correction of diet, water management as well as by medication like probenecid, colchicine and allopurinol in broiler chicks. Protective effects of allopurinol on diclofenac induced toxicity in domestic chicken revealed that allopurinol can be used as the remedy for gout in broiler chicken (Aworh *et al.,* 2012). Febuxostat is a recent addition of drug which is used to treat long term gout and its prevention in human being, and it structurally differs from allopurinol. Febuxostat at a daily dose of 80 mg or 120 mg, was found more effective than allopurinol which is commonly used as fixed daily dose of 300 mg in lowering serum urate levels in human (Becker *et al.,* 2005). No information was available regarding febuxostat efficacy in treating visceral gout in broiler chicks. Keeping above perspective in mind, this study was carried out to compare therapeutic effect of febuxostat and allopurinol on gout induced model in broiler chicks.

Materials and Methods

Ethical Approval: The prior approval from the Institutional Animal Ethical Committee was obtained for use of the birds in this study.

Experimental design: The study was conducted on broiler chicks for 21 days. A total of 108, day-old chicks were procured from the Shakti Hatcheries, Sarsa, Anand, Gujarat and were maintained under standard managemental condition. Diclofenac drug was used for induction of the visceral gout in broiler chicks, whereas allopurinol and febuxostat drugs were used for the prevention of the visceral gout. All the chicks were equally divided in to six groups and numbered from I to VI, each group had 18 day-old chicks. Group I was kept as control group, while group II received diclofenac @ 10 mg/kg body weight by oral gavage daily once for 21 days. Group III and IV received febuxostat and allopurinol @ 4 mg/kg and 25 mg/kg body weight daily once by oral gavage for 21 days. Group V and VI were treated with febuxostat (4 mg/kg) and allopurinol (25 mg/kg), respectively, along with diclofenac (10 mg/kg) daily once for 21 days. Broiler chicks in all six groups were fed with pre-starter and starter feed *ad libitum*. The chicks were observed daily for abnormal clinical signs, if any. Weighing of chicks was carried out daily for 21 days. Mortalities and morbidities were recorded throughout the experiment and feed intake was determined by measuring the total amount of feed that each group had consumed taking leftover into consideration during the course of experiment. Six birds from each group were sacrificed at the end of every week and blood was collected prior to sacrifice for serum biochemical analysis following standard methods in use. A detailed post-mortem examination was carried out on birds which died during the study and sacrificed at the end of the every week and gross lesions were recorded, if any. The internal organs, viz., kidney, liver, heart and lung, proventriculus and spleen were collected and preserved in 10% formalin for further histopathological examination following routine methods.

Statistical analysis: The data obtained were subjected to statistical analysis by using one way ANOVA and Duncan's Multiple Range Test was used for showing significance between different groups.

Results and Discussion

Clinical Signs

The clinical signs were seen only in those birds which died during of the study period. The group II diclofenac treated birds showed varying degree of clinical signs like dehydration, depression, lethargic with shrunken eyes and no response to external stimuli. They also exhibited a tendency to remain standing at one place with apathy, unthriftiness with ruffled feathers, dullness and drooping of the wings. Similar clinical signs were also observed by the previous workers (Agarwal and Prajapati, 2008; Aworh *et al.,* 2012; Sharma *et al.,* 2012; Ghodasara *et al.,* 2014). Chicks from groups V and VI, which died during the experiment showed clinical signs like dullness, depression, ruffled feathers, and appeared to be sleepy and eventually died. In absence of the available literature regarding febuxostat toxicity in the poultry birds and its correlation with mortality with clinical signs

it can be stated that febuxostat was better in comparison to allopurinol at respective dose level.

Mortality

No mortality was observed in birds of the groups I, III and IV whereas, birds of group II treated with diclofenac showed (5/18) 27.77% mortality from day 6 to 12 of the experiment. This was in agreement with findings of earlier researchers (Agarwal and Prajapati, 2008; Sharma *et al.,* 2012; Ghodasara *et al.,* 2014). Gajera (2006) found maximum mortality during second week of age and stated that birds in second week of age are most vulnerable for the visceral gout. However groups V and VI showed mortality of one and two birds, respectively, between 10 to 12 days of experiment which was lower as compared to birds of group II. Group VI had more mortality as compared to group V. So it indicates that both febuxostat and allopurinol reduced mortality caused by diclofenac, and febuxostat was more efficacious as compared to allopurinol.

Effect on Body Weight

Reduction in body weight was observed only in diclofenac treated group II as compared to control group at the end of 1st, 2nd and 3rd week during the experiment (Table 1)**.** Similar observations were made by Agarwal and Prajapati (2008), Irtaza *et al.* (2008) and Ghodasara *et al.* (2014). There was no significant difference in body weight of birds of groups III, IV, V and VI as compared to control group I at the end of 1st, 2nd and 3rd week of experiment, so it indicate that febuxostat and allopurinol are safer to use in birds without side effect and to reduce toxicity caused by diclofenac in terms of reduction in body weight. Patel (2009) observed that febuxostat up to 50 mg/kg did not reveal any change in the body weight in Sprague Dawley rat.

Table 1: Weekly mean (±SE) body weight in broiler chicks of different experimental groups

Experimental groups	Body weight at day			
	0	7	14	21
Group I (Control)	51.90^{a} ±0.01	173.66^{b} ±1.08	421.33^{b} ±0.35	752.59^{bc} ±0.12
Group II (Diclofenac)	51.30^{a} ±0.20	142.10^{a} ±0.39	309.00^{a} ±0.31	508.00^{a} ±0.57
Group III (Febuxostat)	51.45^{a} ±0.23	173.75^{b} ±1.07	421.11^{b} ±0.25	752.73^{bc} ±0.35
Group IV (Allopurinol)	51.32^{a} ±0.16	173.10^{b} ±0.19	421.08^{b} ±0.58	753.71^{c} ±0.98
Group V (Febuxostat + Diclofenac)	51.20^{a} ±0.18	174.25^{b} ±0.79	421.21^{b} ±0.26	751.44^{b} ±0.83
Group VI (Allopurinol+ Diclofenac	51.77^{a} ±0.04	172.58^{b} ±0.38	422.26^{b} ±0.57	752.84^{bc} ±0.25

Means with different superscripts within the column differ significantly (P<0.05).

Serum Biochemical Parameters

The mean values of various serum biochemical parameters are present in Table 2. The mean values of the serum uric acid in the group II at the end of 1st, 2nd and 3rd week was significantly increased as compared to other groups. Increased serum uric acid in diclofenac treated group II attributed to kidney damage and similar findings have been also reported by Agarwal and Prajapati (2008), Sharma *et al.* (2012), Ghodasara *et al.* (2014) and Ramzan *et al.* (2015) during their study in diclofenac treated groups. The values of uric acid were decreased to basel values in groups V and VI as compared to group II at the end of 1st, 2nd and 3rd week, so it indicate that allopurinol and febuxostat have helped in elimination of uric acid, and also at end of 3rd week group V showed decreased uric acid as compared to control group, which further indicated that febuxostat had better efficacy as compared to allopurinol in terms of reduction in serum uric acid. Similar observations were also made in earlier studies (Aworh *et al.,* 2012; Schumacher *et al.,* 2008; Becker *et al.,* 2010; Chohan

Table 2: Mean (±SE) values of serum uric acid, creatinine and BUN in broiler chicks of different experimental groups

Experimental Groups	Uric acid (mg/dl)			Creatinine (mg/dl)			BUN (mg/dl)		
	First Week	Second Week	Third Week	First Week	Second Week	Third Week	First Week	Second Week	Third Week
Group – I	6.61^{a} ±0.14	6.59^{a} ±0.16	6.60^{b} ±0.14	0.18^{a} ±0.01	0.18^{a} ±0.00	0.18^{a} ±0.00	2.06^{a} ±0.17	2.07^{a} ±0.13	2.06^{a} ±0.19
Group - II	16.87^{b} ±0.96	17.13^{b} ±1.06	16.07^{c} ±0.77	0.27^{b} ±0.01	0.27^{b} ±0.00	0.26^{b} ±0.03	3.59^{b} ±0.07	7.03^{d} ±0.48	2.73^{b} ±0.21
Group - III	6.61^{a} ±0.14	6.61^{a} ±0.16	6.60^{b} ±0.13	0.17^{a} ±0.00	0.17^{a} ±0.01	0.17^{a} ±0.00	2.06^{a} ±0.41	1.50^{ab} ±0.55	2.08^{a} ±0.13
Group - IV	6.58^{a} ±0.15	6.60^{a} ±0.14	6.62^{b} ±0.13	0.16^{a} ±0.02	0.16^{a} ±0.03	0.17^{a} ±0.01	2.02^{ab} ±0.27	2.04^{a} ±0.07	2.04^{a} ±0.22
Group – V	6.06^{a} ±0.23	6.93^{a} ±0.25	5.01^{a} ±0.44	0.16^{a} ±0.03	0.16^{a} ±0.07	0.15^{a} ±0.01	1.39^{a} ±0.266	1.17^{a} ±0.195	1.98^{a} ±0.315
Group – VI	7.10^{a} ±0.18	6.98^{a} ±0.21	7.27^{b} ±0.22	0.17^{a} ±0.04	0.18^{a} ±0.01	0.18^{a} ±0.01	2.65^{ab} ±1.20	1.70^{bc} ±0.18	1.85^{a} ±0.19

Means with different superscripts within the column differ significantly (P<0.05).

et al., 2012; Shah *et al.,* 2014) during their febuxostat and allopurinol comparative efficacy study.

The mean values of serum creatinine and BUN were significantly increased in diclofenac treated group II at the end of 1st, 2nd and 3rd week as compared to control group as well as with other groups, as reported in earlier studies (Sharma *et al.,* 2012; Agarwal and Prajapati, 2008; Irtaza *et al.,* 2008; Jain *et al.,* 2009), whereas the groups treated with febuxostat and allopurinol alone or along with diclofenac did not reveal any change in creatinine values. It can be stated that both these drugs prevented kidneys from toxic effect of diclofenac. However, efficacy of febuxostat appeared better in comparison to allopurinol up to 2nd week of age in terms of reduction of BUN (Table 2)**.**

Gross and Histopathological Changes

The gross and histopathological changes were mainly observed in chicks that died during the experiment from treatment groups II, V and VI. The chicks which were sacrificed at the end of 1st, 2nd and 3rd week from different experimental groups did not show specific gross lesions suggestive of visceral gout. The gross lesions in kidneys of birds died from group II showed more enlarged and frosted chalky white urate deposition and pinpoint haemorrhages on the surface along with dilatation of ureter filled with urate [Fig. 1] and kidneys of dead chicks from groups V and VI showed marked swelling, uratic deposition along with pinpoint haemorrhages and paleness of both the kidneys [Fig. 2]. Similar gross lesions were also reported by various workers (Sharma *et al.,* 2012; Irtaza *et al.,* 2008; Ramzan *et al.,* 2015; Akhter and Sarker 2015). The chicks died from diclofenac treated group II showed slightly enlarged, soft, friable and congested liver and varying degree of chalky white urate deposits on the capsular surface of liver, and heart also showed chalky white urate deposition over pericardium [Fig. 3]. These chalky urate deposits were firmly adhered with parenchyma. Similar lesions were also noticed on liver and heart of those chicks died from experimental groups V and VI. Similar gross lesions were also noticed by Gajera (2006), Agarwal and Prajapati (2008), Hedau *et al.* (2008) and Ghodasara *et al.* (2014). Some birds from diclofenac treated group also showed deposition of urate over proventriculus, and spleen and lungs were congested in most of the cases. The presence of urate crystals over proventriculus and spleen in birds died from the group II were similar to those noticed by Agarwal and Prajapati (2008), Ramzan *et al.* (2012) and Ghodasara *et al.* (2014).

The birds died from groups II, V and VI showed varying degree of histological lesions in the kidneys,

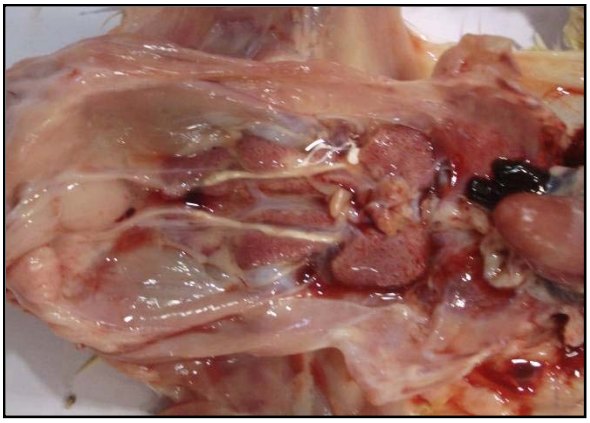

Fig.1. Bird died from group II showing enlarged kidney and urate deposition with pin haemorrhage and ureter filled with urate. (H & E) × 240.

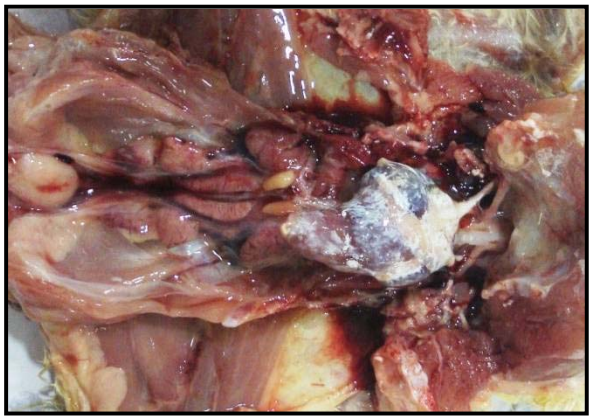

Fig.2. Bird died from group VI showing enlarged pale, swelled kidney with and urate deposition on the pericardium. (H & E) × 240.

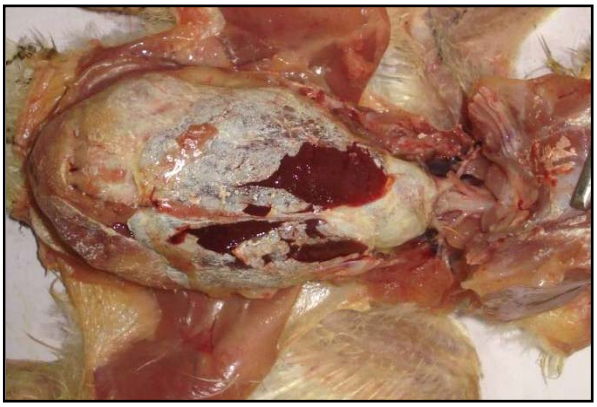

Fig. 3. Bird died from group II showing urate deposition over pericardium and capsular surface of the liver. (H & E) ×

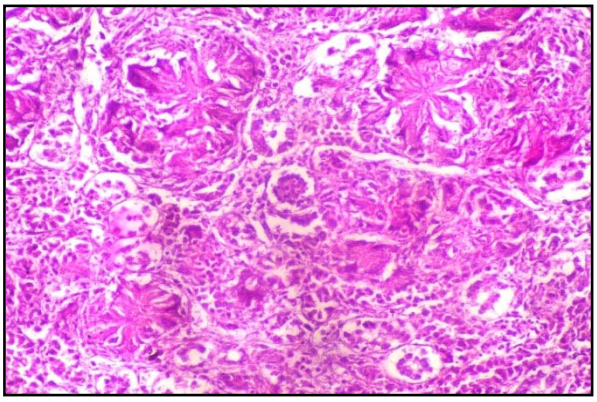

Fig. 4. Kidney from bird died in group II showing deposition of uric acid crystals in radiating pattern along with degeneration and necrosis of tubular epithelium (H & E) × 240.

liver, heart, spleen and proventriculus. The histopathological sections of the kidneys from birds died showed congestion, haemorrhages, degenerative changes along with varied severity of the urate deposition in the kidney tubules [Fig. 4]. Kidneys also showed mild to moderate congestion, intertubular haemorrhages, cystic dilatation of the tubules [Fig. 5, 6] and De'galantha's stained section of kidney showed marked presence of the urate crystals in the radiating pattern [Fig. 7]. The histopathological lesions in the kidneys were similar to the observations of earlier workers (Agarwal and Prajapati, 2008; Irtaza *et al.,* 2008; Ghodasara *et al.,* 2014; Akhter and Sarker, 2015). The sections of the liver from the birds died in groups II, V and VI showed congestion, haemorrhages and degeneration with deposition of the uratic tophi surrounded by the inflammatory cells [Fig. 8, 9]. These changes were similar to the observations of above workers. The section of the heart from the birds from groups II, V and VI showed congestion and haemorrhages between muscles. The urate crystals were dissolved out from cardiac tissue during tissue processing. Pericardium showed marked thickening with urate deposition as the homogenous material in group II [Fig. 10, as noticed by Gajera (2006), Agarwal and Prajapati (2008), Irtaza *et al.* (2008), Jana *et al.* (2009) and

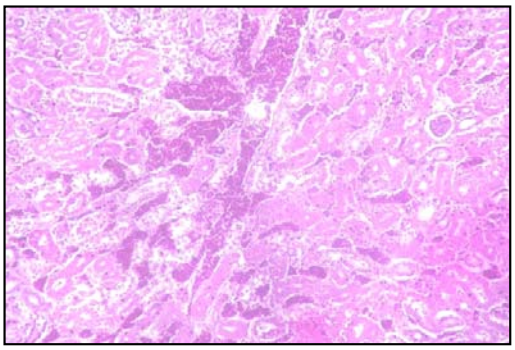

Fig. 5. Bird died from group II showing marked tubular degeneration and intertubular hemorrhages in renal parenchyma (H & E) × 120.

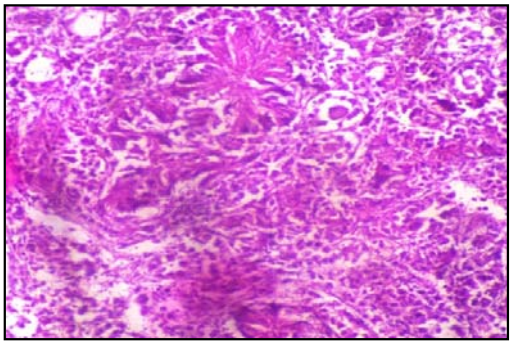

Fig. 6. Bird died from group VI showing uric acid crystals in radiating pattern and tubular degeneration and necrosis along with mild mononuclear cells infiltration (H & E) × 240.

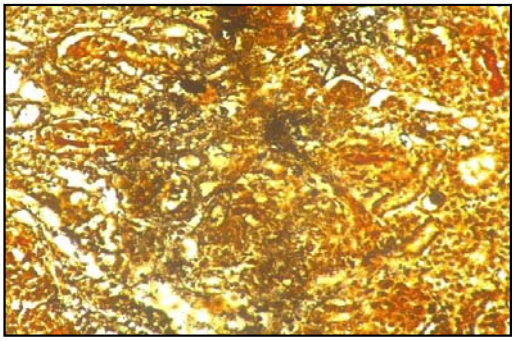

Fig. 7. Section of kidney showing black color uric acid crystal in radiating pattern in renal interstitium, De Galantha's stain × 240.

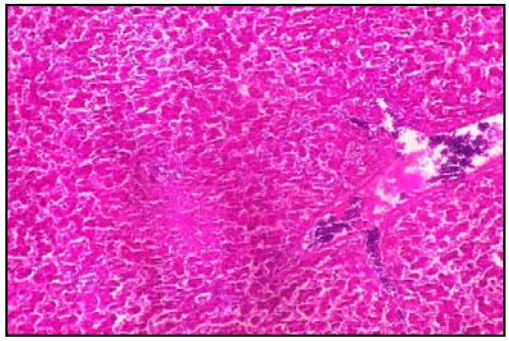

Fig. 8. Section of liver from bird died in group II showing deposition of urate tophi along with mild to moderate congestion in hepatic parenchyma (H&E) × 240.

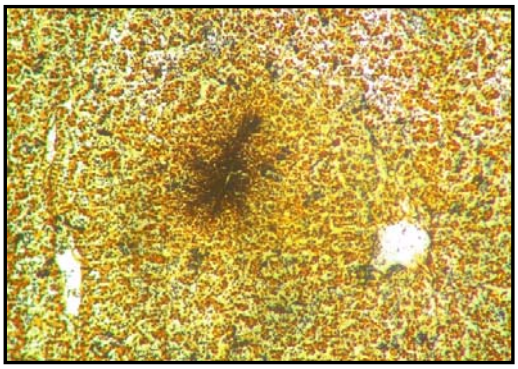

Fig. 9. Section of liver from bird died in group V showing black color needle shape uric acid crystal in the hepatic parenchyma, De galantha's × 240.

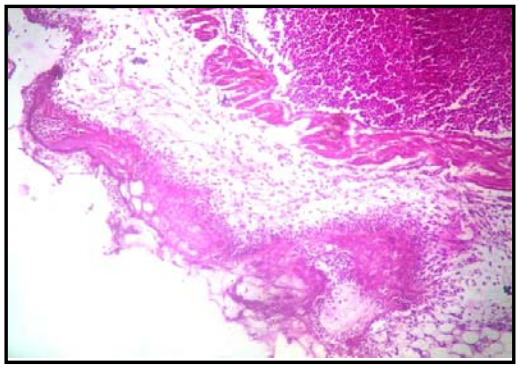

Fig.10. Section of the heart showing marked thickening of pericardium with urate deposition as homogenous amorphous material along with mononuclear cells infiltration (arrow)

Ghodasara *et al.* (2014). Histopathological sections of birds died from group II also showed deposition of the urate crystals in glandular parenchyma.

The birds which were sacrificed from the different groups at the end of 1st, 2nd and 3rd week did not show any specific gross or microscopic lesions in the visceral organs. The lesions were specific to the visceral gout and observed in the birds died during the experiment, hence it can be stated that diclofenac @ 10 mg/kg body weight oral causes the visceral gout in the birds. As the mortality was low in diclofenac +febuxostat treated group V as compared to diclofenac + allopurinol treatment group VI, it can be stated that the febuxostat was better in comparison to allopurinol in terms of the reduction on visceral gout. However further studies are required with large number of data for its confirmation.

Acknowledgements

Authors are thankful to Dean of the College and Staff of Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Anand AAU, Anand for their support and help.

Conflict of Interest: All authors declare no conflict of interest.

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