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PATHOPHYSIOLOGICAL CHANGES IN THE GASTRIC MUCOSA AND LIVER AFTER TREATED WITH DICLOFENAC SODIUM IN MICE

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ABSTRACT

The present study was conducted to investigate the effect of diclofenac sodium on the stomach and liver under different conditions. A total of 45 Swiss Albino mice were randomly assigned into five equal groups (n=9) (A, B, C, D & E) and were kept under 12 hour light: 12 hour dark cycle with free access to food (mice pellet @ 25gm/mice/day) and water for 40 days. Four groups were treated with diclofenac sodium @ 3 mg/kg body weight (bwt) orally in empty or full stomach with or without vitamin B12 @ 10 µg/Kg body weight through intramuscular route while one group (A) was fed only basal diet and considered as control group. Total erythrocyte count (TEC), hemoglobin (Hb) % decreased significantly ($p<0.01$) in the treated mice. The highest PCV value was recorded in group E on the day 40 (42.10±0.15) and the lowest value was obtained from group B at day 40 (26.41±0.11). The highest SGPT value was recorded in group B on the day 40 (42.00±0.35). Grossly, all the treated mice showed slight to moderate congestion of the gastric mucosa and in the mice having decreased TEC and Hb possesses massive hemorrhage with ulceration in the stomach. In histopathology, the lesion includes infiltration of mononuclear cells between the gastric mucosa and in the liver, central vein congestion, sinusoidal congestion, and infiltration of mononuclear cells around central vein, slightly swollen hepatocytes and fatty changes characterized by minute vacuolation.

KEYWORDS: Stomach, Mice, Diclofenac sodium, TEC, Hb and SGPT.

INTRODUCTION

Diclofenac sodium is a non-steroidal anti-inflammatory drug designed by selection of appropriate physicochemical and steric properties. Its pharmacologic activity, specifically its effects in acute and sub-chronic inflammation and its analgesic activity have been assessed in animal models. The tolerability of the compound as judged by several parameters (i.e., ratio between the acute lethal dose or the dose inducing gastrointestinal blood loss and the desired pharmacologic activity) is favorable in comparison with other non-steroidal anti-inflammatory drugs. Diclofenac is used to relieve pain and inflammation in a wide range of conditions, including arthritis, gout, sprains, fractures, back pain and following minor surgery.

All the medicines in the NSAIDs group reduce inflammation caused by the body's own immune system and are effective painkillers, but must be taken with or after food to avoid stomach related side effects.[1-2] The pharmacologic effects of this drug include analgesia, antipyretics and control of inflammation. This drug induce undesirable and potentially life-threatening side effects including blood disorders, constipation, nausea, vomiting or abdominal pain, indigestion (dyspepsia), ulceration of the stomach or intestine, inflammation of the liver (hepatitis), loss of appetite, bleeding from the stomach or intestine , decreased kidney function and alteration in results of liver function tests.[3-4] Severe gastric disorder may result in vitamin B_{12} deficiency which in turn causes folic acid unavailable to operate several metabolic functions including erythropoiesis. Finally macrocytic normochromic anemia is developed due to vitamin B_{12} deficiency.[5] The detailed information on the adverse drug reactions of diclofenac sodium is almost lacking in most laboratory animals particularly in mice. And hence the research work was carried out to study the effect of diclofenac sodium on some organs such as stomach and liver.

MATERIALS AND METHODS

The research work was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh between July to August 2012 and the experiment was conducted for a period of 40 days. A total of 45 healthy Swiss albino mice were randomly divided into 5 equal groups (n=9) and kept group wise in 5 separate cages as group A, B, C, D, E. Out of five groups, one group of mice (Group A) was kept as control without giving any treatment. All groups were supplied with standard mice pellet (25gm/mice/day) with fresh drinking water (10 ml/mice/day) throughout the experimental period. Group B was treated with diclofenac sodium @ 3 mg/kg bwt orally in empty stomach whereas group C was treated with diclofenac sodium @ 3 mg/kg bwt orally in full stomach. In group D, animals were treated with diclofenac sodium @ 3 mg/kg bwt orally plus vitamin B_{12} @ 10 µg/Kg bwt intramuscularly (i/m) in empty stomach. The fifth group was designated as E and animals were treated with and diclofenac sodium @ 3 mg/kg bwt orally plus vitamin B_{12} @ 10 µg/Kg bwt intramuscularly (i/m) in full stomach.

The blood was collected from the tail to check the TEC, Hb, PCV and SGPT on day 1 and day 20. At the end of experimental period (day 40) the mice were kept fasting overnight. A set of sterile test tubes containing anticoagulant (Double oxalate salt) at a ratio of 1:10 were taken. The blood was collected directly from heart at this time. The blood was kept in refrigerator till examination was done. Hematological studies were performed within two hours of blood collection. On this day, the mice of all groups were sacrificed using anaesthetic, diethyl ether (overdose) and the following parameters were studied as per routine method.

- Gross pathological changes in the liver and stomach.
- Histopatholigical changes in the liver and stomach after preserving sample in 10% buffered formalin

Statistical analysis

The data were analyzed between the control and treated values by using SPSS version 18.0 for windows (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA). Differences in mean values were tested using ANOVA, followed by a multiple pairwise comparison using a student *t*-test. In all cases, $P \leq 0.05$ was considered significant and the graphical presentation was done by the Sigma Plot version 12.

RESULTS

Total erythrocyte count (TEC) values presented in table 1 and fig 1. Among these values, the highest value was recorded in full stomach animal on the day 40 (7.15±0.08) which was treated with diclofenac sodium plus vitamin B_{12} and the lowest value was recorded in empty stomached animal which was treated only with diclofenac sodium on the day 40 (5.78±0.2) and also in empty stomached animal which was treated with diclofenac sodium plus vitamin B_{12} on the day 40 (5.68±0.26). The highest and lowest value recorded had a gross fluctuation and they were statistically significant ($P<0.01$).

Total hemoglobin content (gm/dl) is presented in table 2 and fig 2. Among these values, the highest value was recorded in full stomached animal on the day 40 (13.04±0.19) which was treated with diclofenac sodium plus vitamin B_{12}. The lowest value was recorded in empty stomached animal which was treated with only diclofenac sodium on the day 40 (8.98±0.21). The obtained highest and lowest value showed a gross fluctuation and they were statistically significant ($P<0.01$).

Packed cell volume (%) is presented in table 3 and fig. 3. Among these values, the highest value was recorded in group E on the day 40 (42.10±0.15)**.** Such findings was also agreed by Dilov *et al.* (1983), and reported that cyanocobalamin containing biofer showed a better anti-anemic effect in anemic albino rats. The lowest value was obtained from group B at day 40 (26.41±0.11) it might be for oral administration of diclofenac sodium on fasting condition. Sharma *et al*. 1993, Bhaumik *et al*. 1993, Durgan *et al* 1998 and Ramesh *et al.* 2001 depicted almost similar result in their experiment on Swiss albino mice. The decreased in PCV may be due to decreased on TEC. The obtained highest and lowest value showed a gross fluctuation and they were statistically significant ($P<0.01$).

Serum glutamate pyruvate transaminase (SGPT), total SGPT count is presented in Table 4 and Fig. 4. Among these values, the highest value was recorded in group B on the day 40 (42.00±0.35). This increase level of SGPT was due to effect of Diclofenac sodium on liver cell. Elevated levels indicate that there is impairment of the liver function. Any deviation of liver function is reflected in the serum glutamate pyruvate transaminase (SGPT) activities, most of the metabolism of all the nutrients take place first in the liver and then to the other organs. Such findings were also agreed by Deneke *et al. (*1985).

Gross lesion on the liver and stomach (fig. 5)

Liver became enlarged, pale, friable and congested with hemorrhage and ulceration found in the gastric mucosa after observing gross changes in empty stomached animal. In all the groups, liver and stomach samples were examined for the detection of gross and pathological lesions if any. In case of empty stomached animal, those treated with diclofenac sodium or diclofenac sodium plus Vitamin $B_{12,}$ the lesion includes infiltration of mononuclear cells between the gastric mucosal layer and in the liver, central vein congestion, sinusoidal congestion and infiltration of mononuclear cells around central vein, slightly swollen hepatocytes and fatty changes characterized by minute vacuolation.

Histopathology of liver and stomach (fig 6-9).

Liver and stomach samples in all groups were examined for the detection of pathological lesion if any. In group B and D the lesion includes central vein congestion, sinusoidal congestion, infiltration of mononuclear cells around central vein, slightly swollen hepatocytes and fatty changes characterized by minute vacuolation found in liver. The centrilobular necrosis with congestion observed in this present study may be due to anoxic condition resulting from fall of systemic blood pressure by recurrent hemorrhage from gastric mucosa. Lipotrophic factors are necessary for removal of fat from liver brought to it by the blood. Phospholipids are essential for the transport of fat and as choline promote the formation of phospholipids. This food factors absorption was less due to ulceration leads to the rapid accumulation of fat in liver. There was also found infiltration of mononuclear cells between the gastric mucosal layer in group B and D. It thought to be occurred by harmful effect of diclofenac sodium in empty stomach.

No such histopathological changes were observed in control group.

Table 1. Effect of oral administration of diclofenac sodium on total erythrocyte count (X 10^6/mm^3).

Group	Treatment	Days		
		Pre treatment	Post treatment	
		Day 1	Day 20	Day 40
A	Control	6.54^{bcd} ±0.1	6.85^{abc} ±0.2	6.38^{cd} ±0.16
B	Diclofenac Sodium before meal	6.87^{abc} ±0.2	6.29^{d} ±0.11	5.78^{e} ±0.25**
C	Diclofenac Sodium after meal	6.84^{abc} ±0.11	6.17^{de} ±0.38	6.17^{de} ±0.38
D	Diclofenac Sodium plus Vitamin B_{12} before meal	6.35^{cd} ±0.25	5.73^{e} ±0.20**	5.68^{e} ±0.26**
E	Diclofenac Sodium plus Vitamin B_{12} after meal	6.59^{cd}±0.48b	7.01^{ab} ±0.87	7.15^{a} ±0.08**

The values given above represent the mean ± standard deviation (SD) of 9 mice (n=9).
** = Significant at p<0.01. *= Significant at p<0.05.

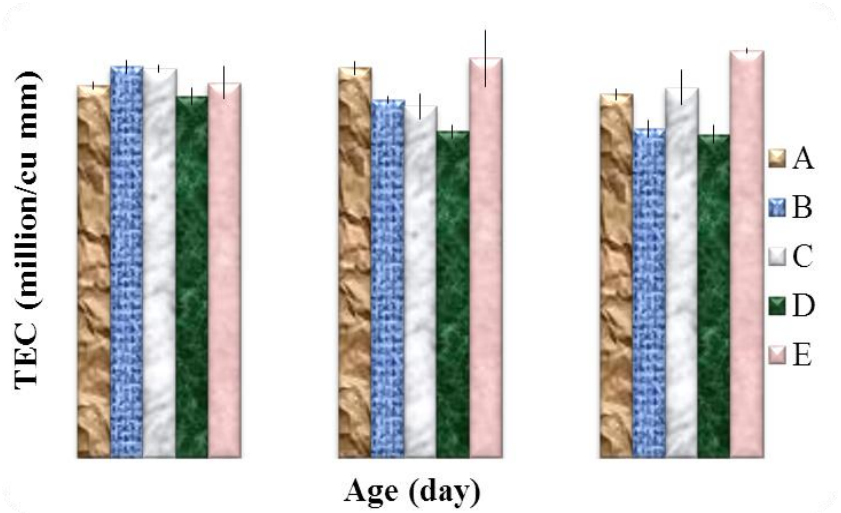


Fig 1: Effect of oral administration of Diclofenac sodium on total erythrocyte count (X 10^6/mm^3) in different mice.

Table 2. Effect of oral administration of diclofenac sodium on hemoglobin content (gm/dl).

Group	Treatment	Days		
		Pre treatment	Post treatment	
		Day 1	Day 20	Day 40
A	Control	12.60^{cd} ±0.32	12.71^{c} ±0.14	12.76^{bc} ±0.06
B	Diclofenac Sodium before meal	12.39^{d} ±0.37	10.67^{g} ±0.44	8.98^{h} ±0.21**
C	Diclofenac Sodium after meal	12.96^{ab} ±0.45	13.01^{a} ±0.24**	12.15^{e} ± 0.09
D	Diclofenac Sodium plus Vitamin B_{12} before meal	12.05^{e} ±0.13	11.5^{f} ±0.15	10.72^{g} ± 0.09
E	Diclofenac Sodium plus Vitamin B_{12} after meal	12.44^{d} ± 0.37	12.09^{e} ± 0.29	13.04^{a} ± 0.19**

The values given above represent the mean ± standard deviation (SD) of 9 mice (n=9).
** = Significant at p<0.01. * = Significant at p<0.05.

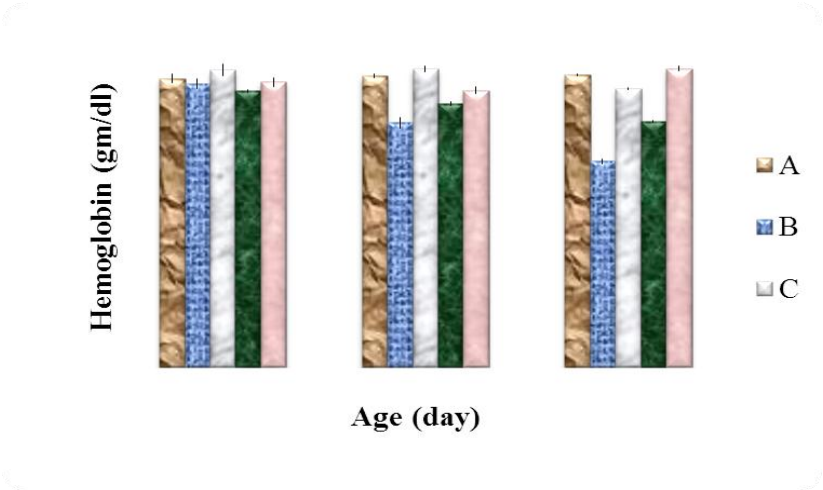


Fig 2: Effect of oral administration of Diclofenac sodium on hemoglobin content (gm/dl) in different mice

Table 3. Effect of oral administration of Diclofenac sodium on packed cell volume (%).

Group	Treatment	Days		
		Pre treatment	Post treatment	
		Day 1	Day 20	Day 40
A	Control	$34.00^{f} \pm 0.88$	$38.00^{c} \pm 0.67$	$39.00^{b} \pm 0.74$
B	Diclofenac Sodium before meal	$35.00^{e} \pm 0.63$	$30.29^{i} \pm 0.24$	$26.40^{j} \pm 0.11^{**}$
C	Diclofenac Sodium after meal	$34.45^{g} \pm 1.59$	$34.35^{f} \pm 0.35$	$32.05^{h} \pm 0.2$
D	Diclofenac Sodium plus Vitamin B_{12} before meal	$36.09^{d} \pm 0.2$	$34.20^{f} \pm 0.17$	$32.22^{h} \pm 0.09$
E	Diclofenac Sodium plus Vitamin B_{12} after meal	$37.69^{c} \pm 0.35$	$39.43^{b} \pm 0.13$	$42.10^{a} \pm 0.15^{**}$
Values given above represent the mean ± standard deviation (SD) of 3 mice (n=3)				
** = Significant at p<0.01 and * = Significant at p<0.05				

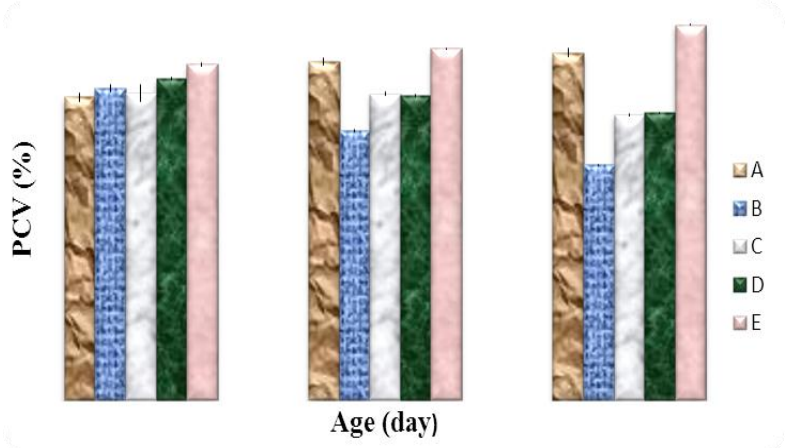


Fig 3: Effect of oral administration of Diclofenac sodium on packed cell volume (%) in different mice.

Table 4. Effect of oral administration of Diclofenac sodium on serum glutamate pyruvate transaminase (SGPT) count (U/L).

Group	Treatment	Days		
		Pre treatment	Post treatment	
		Day 1	Day 20	Day 40
A	Control	$30.00^{h} \pm 0.47$	$32.00^{g} \pm 0.51$	$34.33^{g} \pm 4.61$
B	Diclofenac Sodium before meal	$32.00^{g} \pm 0.52$	$37.00^{c} \pm 0.40$	$42.00^{a} \pm 0.35^{**}$
C	Diclofenac Sodium after meal	$32.00^{g} \pm 0.96$	$34.00^{e} \pm 0.87$	$34.99^{d} \pm 0.73$
D	Diclofenac Sodium plus Vitamin B_{12} before meal	$29.00^{i} \pm 0.46$	$33.00^{f} \pm 0.61$	$37.99^{b} \pm 0.45$
E	Diclofenac Sodium plus Vitamin B_{12} after meal	$33.33^{f} \pm 0.59$	$30.00^{h} \pm 0.91$	$28.00^{j} \pm 0.77^{**}$
Values given above represent the mean ± Standard Deviation (SD) of 3 mice (n=3)				
** = Significant at p<0.01 and * = Significant at p<0.05				

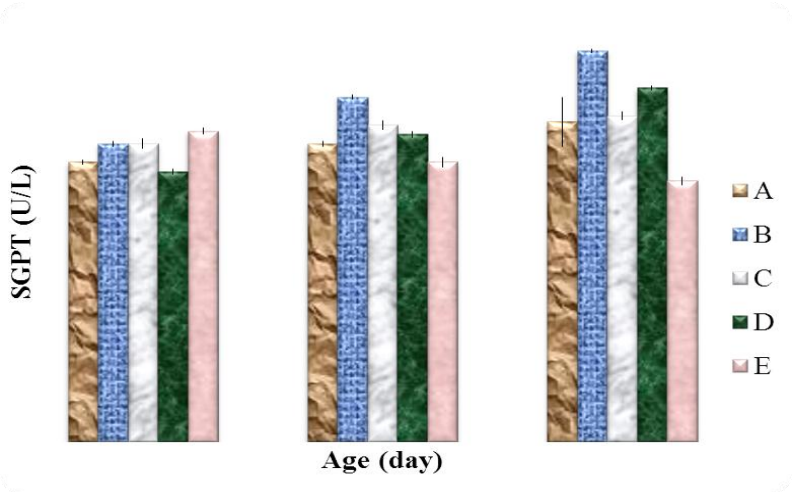


Fig 4: Effect of oral administration of Diclofenac sodium on serum glutamate pyruvate transaminase count (U/L) in different mice.

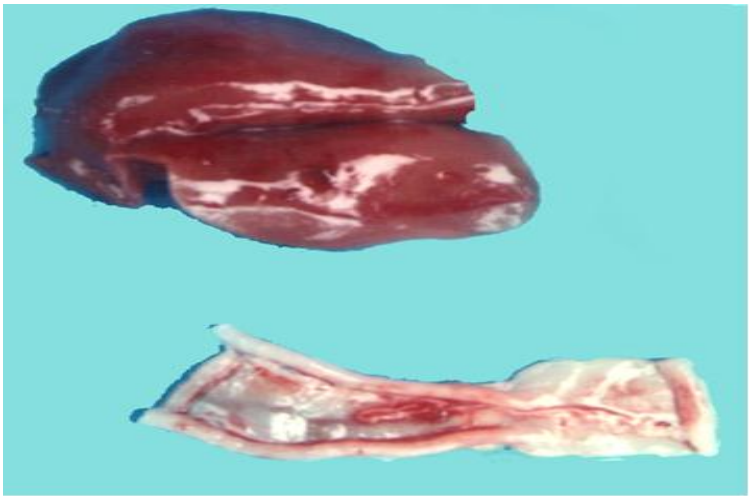
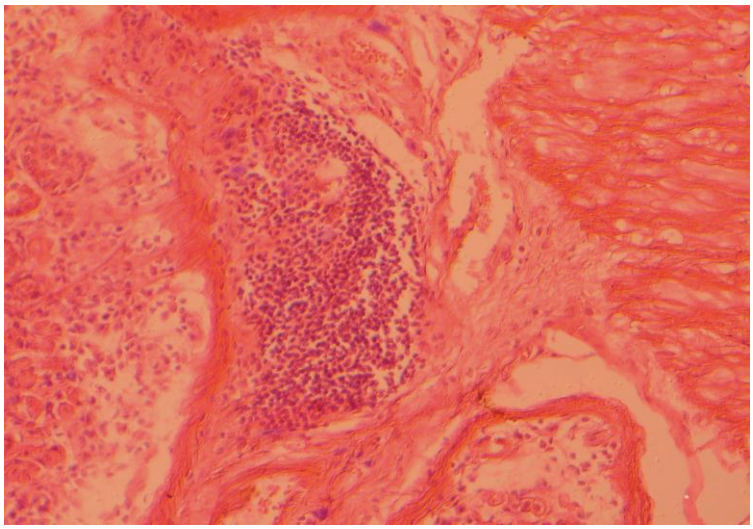


Fig. 5. Congested liver and ulcerative lesion with hemorrhagic spot in stomach of mice



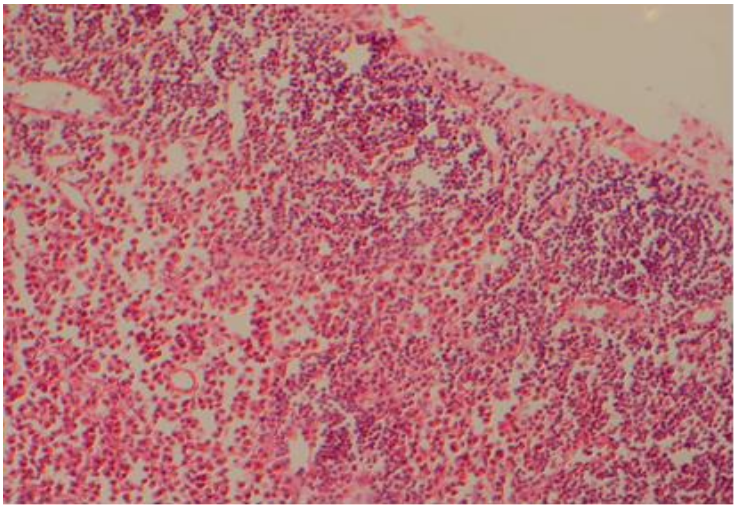
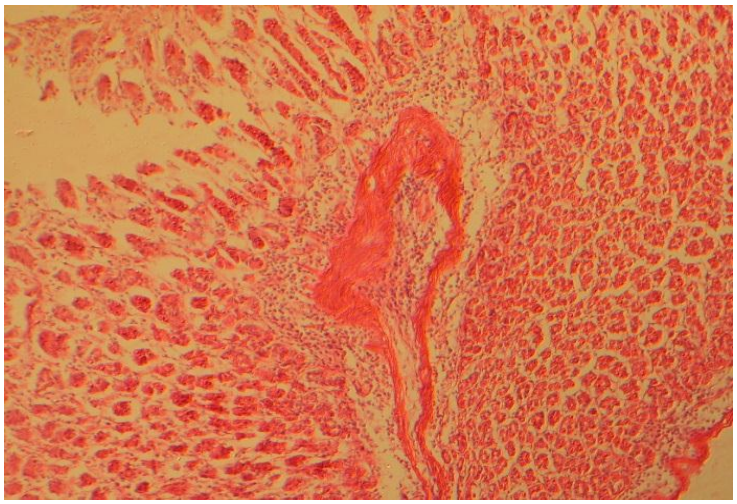


Fig.6 (A & B). Histopathological section of stomach showing infiltration of mononuclear cell between the gastric mucosal layers (H&E x100)



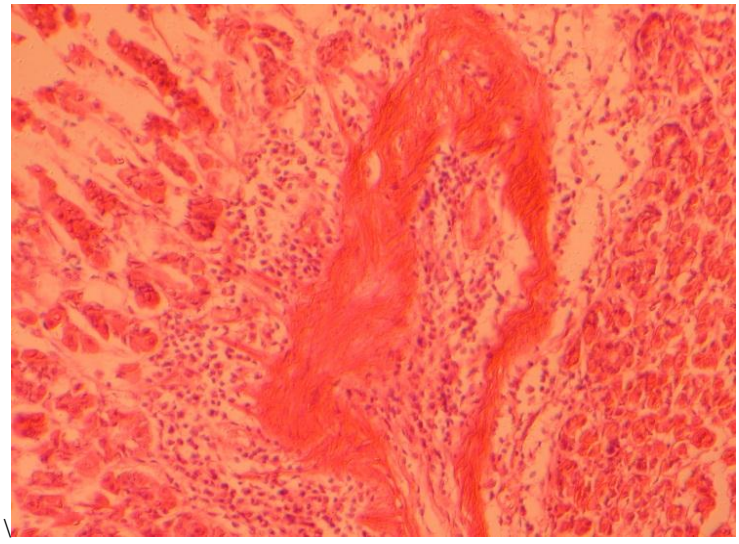


Fig.7(A & B). Histopathological section of stomach showing infiltration of mononuclear cell with ulcerative congestion between the gastric mucosal layers (H&E x100)

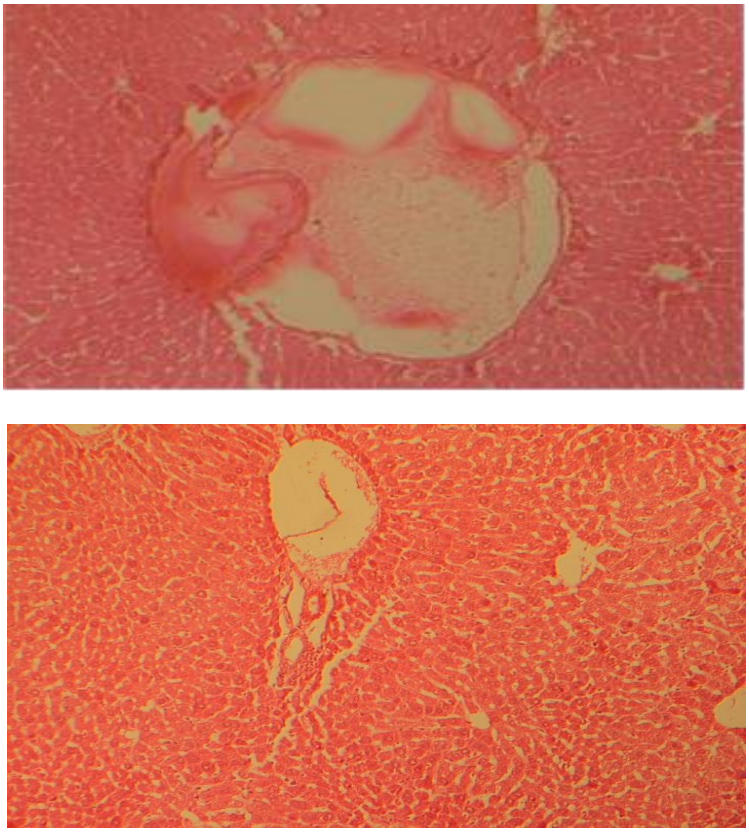


Fig. 8(A & B). Histopathological Section of Liver showing fatty changes characterized by minute vacuolation (H&E x100)

DISCUSSION

The increased total erythrocyte count and total hemoglobin content (gm/dl) among the animals treated by diclofenac sodium and vitamin B_{12} might be due to liver invigorating effect of Vitamin B_{12} and failure of the drugs to cause harm to gastric mucosa when it is filled with feed. Such findings were agreed by Dilov[8] who reported that cyanocobalamin containing biofer showed a better anti-anemic effect in anemic albino rats. This result indicates that vitamin B_{12} has no positive effect unless if administered in full stomach. Here vitamin B_{12} is assumed to activate folic acid in this process.[10] On the other hand the decreased TEC and Hb in the animals treated by diclofenac sodium in empty stomach might be a case of faulty absorption of Vitamin $B_{12.}$ It might be the effect of oral administration of diclofenac sodium on fasting condition. Similar findings were also reported by Richard[6], Ramesh[7], Sharma[9] and Bhaumik.[9]

Gross pathological changes might be the consequence of diclofenac sodium in empty stomach. The centrilobular necrosis with congestion observed in this present study may be due to anoxic condition resulting from fall of systemic blood pressure by recurrent hemorrhage from gastric mucosa in empty stomached animal treated with diclofenac sodium or diclofenac sodium plus Vitamin $B_{12.}$ There was also infiltration of mononuclear cells between the gastric mucosal layer in empty stomached animal treated with diclofenac sodium or diclofenac sodium plus Vitamin $B_{12.}$ No such histopathological changes were observed in in the animals treated with diclofenac sodium or diclofenac sodium plus Vitamin B_{12} in full stomach.

CONCLUSION

The oral administration of Diclofenac sodium significantly ($p<0.01$) decreased the total erythrocyte count followed by PCV % and hemoglobin content in animals with empty stomach regardless of vitamin B_{12} supplementation. The increase level of SGPT was due to the effect of Diclofenac sodium on the liver which indicates the impairment of the liver function. Any deviation of liver function is reflected in the SGPT activities. Congested liver and hemorrhage with ulcer was found in animals with empty stomach. The lesion includes central vein congestion, sinusoidal congestion, and infiltration of mononuclear cells around central vein, slightly swollen hepatocytes and fatty changes characterized by minute vacuolation. There was also infiltration of mononuclear cells between the gastric mucosal layer in empty stomach animal treated with only diclofenac sodium and also in empty stomach animal which was treated with diclofenac sodium plus vitamin $B_{12.}$ Our data demonstrated the harmful effect of diclofenac sodium on erythropoiesis has been counteracted by vitamin B_{12}. Further study is needed to investigate the molecular mechanism of the effect of diclofenac sodium on the stomach and liver.

Conflict of interest statement
The authors have no competing interests.

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Author's contribution
M. M. R. Chowdhury designed the experiment and Fahmida Afrin performed the experiment, S. S. Shaha helps in histopathology of the samples, M. A. Asgar analyze the data, M. N. Hoque perform the hematological studies and Prof. M. K. Islam supervises and check the manuscript.

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