# Effect of ambroxol on experimentally induced acute oxidative stress in the heart, kidney, and intestine in rats

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**Background**
Intestinal ischemia–reperfusion (IR) is a frequently occurring phenomenon during abdominal and thoracic vascular surgery, small bowel transplantation, hemorrhagic shock, and surgery using cardiopulmonary bypass, carrying high morbidity and mortality. Ambroxol hydrochloride is an active *N*-desmethyl metabolite of bromhexine hydrochloride. Ambroxol is indicated as 'secretolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport'.

**Aim**
The present study was designed to evaluate the effect of ambroxol on experimentally induced acute organ oxidative stress in the form of remote organ injury including kidney and heart after intestinal IR.

**Materials and methods**
Thirty animals were classified into five groups. First group: normal control group, second group: nontreated intestinal IR group (intestinal IR was induced by mesenteric artery ligation), third group: ambroxol pretreated intestinal IR group (35 mg/kg), fourth group: ambroxol pretreated intestinal IR group (70 mg/kg), fifth group: ambroxol pretreated intestinal IR group (140 mg/kg).

**Results**
Ambroxol significantly reduced the malondialdehyde level in the heart and the kidney when compared with intestinal IR with no medication group. It also improved cardiac troponin and, kidney functions (urea and creatinine) and histopathological affection when compared with intestinal IR with no medication group. Conclusively, in this study ambroxol could have a protective effect against intestinal IR through its antioxidative and anti-inflammatory effect.

**Keywords:**
ambroxol, intestinal ischemia–reperfusion, malondialdehyde and cardiac troponin 1, oxidative stress

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## Introduction

Intestinal ischemia is a life-threatening condition associated with a broad range of clinical conditions including atherosclerosis, thrombosis, hypotension, necrotizing enterocolitis, bowel transplantation, trauma, and chronic inflammation [1]. The majority of ischemic episodes seen in clinics in the western world are due to thromboembolic or artherothrombotic vaso-occlusive disease. The major risk factors for such events are advancing age, male sex, and hereditary factors. However, other important risk factors can be modified or controlled, including tobacco smoking, hyperlipidemia, hypertension, physical inactivity, obesity, metabolic syndrome, and diabetes mellitus. Ethanol intake at high levels (3–4 or more drinks), either in acute or chronic (daily) settings, also increases the risk for myocardial infarction and ischemic stroke [2].

The ischemia and reperfusion (IR) syndrome performs a fundamental role in the pathophysiology of several clinical–surgical conditions and may be caused by intestinal intussusception, acute mesenteric arterial occlusion, transplants of the small intestine, and hemodynamic shock [3]. Ambroxol has important pharmacological properties as surfactant stimulatory, anti-inflammatory, antioxidant, and local anesthetic effects in addition to the mucokinetic and mucociliary effects. Recognition of the surfactant stimulatory and anti-inflammatory properties of the drug has led to the importance of the drug in the management of obstructive airway disorders [4]. The present study was designed to evaluate the effect of ambroxol on experimentally induced acute organ oxidative stress in remote organs such as kidney and heart after intestinal IR in adult rats.



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## Materials and methods

### Materials

(1) Animals: 30 adult male albino rats weighing between 150 and 250 g (at the beginning of the study) were used. They have been acclimatized for 1 week and were caged in fully ventilated rooms (at room temperature). Rats were allowed free access to food (balanced diet) and water in the Pharmacology Department, Benha Faculty of Medicine. Rats were randomly subdivided into five groups (six rats each).
(2) Drugs and chemicals:
   (a) Ambroxol (powder): October Pharma, Giza, Egypt.
   (b) Hematoxylin and eosin: (E. Merck, Darmstadt, Indiana, USA).
   (c) Urethane (ethyl carbamat): (white crystals) (Prolabo, Paris, France). White crystals 0.6 g/100 ml of 25% freshly prepared solution.
   (d) Urea kits: Diamond Diagnostic, Egypt.
   (e) Creatinine kits: Diamond Diagnostic.
   (f) Phenobarbital sodium.

Ambroxol was dissolved in distilled water and was given to rats by intravenous injection in intestinal IR model. The procedures were reviewed and approved by the research ethical committee of animal care and use of Benha faculty of medicine, Benha university.

### Methods

To investigate the effect of ambroxol on intestinal IR, 30 adult male albino rats are used and divided into five groups. Group 1: control normal group (sham operated). Adult rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg), and a cannula was inserted into the tail vein. A midline laparotomy was performed [5].

Group 2: nontreated intestinal IR group. Adult rats were operated by sham operation and collateral vessels from the caudal mesenteric artery was ligated with silk ties, and the superior mesenteric artery was occluded with a microvascular clip for half an hour and was reperfused for 2 h [6].

Group 3: ambroxol pretreated intestinal IR group (35 mg ambroxol was given into the rat vein 5 min before the surgical operation) [7].

Group 4: ambroxol pretreated intestinal IR group (70 mg ambroxol was given into the rat vein 5 min before the surgical operation).

Group 5: ambroxol pretreated intestinal IR group (140 mg ambroxol was given into the rat vein 5 min before the surgical operation) [8].

### Biochemical parameters

The blood samples will be collected from retrobulbar vessels [9]. Samples were centrifuged at 2000 rpm for 15 min for separation of serum and stored at −20°C for biochemical analysis.

#### *Measurement of blood urea*

Urea in the sample is hydrolyzed enzymatically into ammonia and carbon dioxide. Ammonia ions formed reacts with salicylate and hypochlorite (NaClO) in the presence of the catalyst nitroprusside, to form green indophenols. The intensity of the color formed is proportional to the urea concentration in the sample [10].

#### *Measurement of blood creatinine*

The assay is based on the reaction of creatinine with sodium picrate.

Creatinine reacts with alkaline picrate forming a red complex.

The time interval chosen for measurement avoids interference from other serum constituents. The intensity of the color formed is proportional to creatinine concentration in the plasma [11].

#### *Measurement of serum troponin-I activity*

Serum troponin-I activity was determined according to the method used by Bodor [12].

#### *Measurement of malondialdehyde in the heart and kidney*

Thiobarbituric acid reacts with malondialdehyde (MDA) in acidic medium at a temperature of 90°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm [13].

#### *Histopathology of the heart, kidney, and intestine*

After functional studies were completed, heart, kidney, and intestine were removed and put into a buffered 4% formaline fixation solution and processed with paraffin wax for histopathological examination. Sections (5 μm) were stained with hematoxylin and eosin [14].

### Statistical analysis

In the statistical comparison between the different groups, the significance of difference was tested using analysis of variance test ($F$ value): used to compare the mean of more than two groups of quantitative data using multiple comparison post-hoc test (least significant difference). A $P$ value less than 0.05 was considered statistically significant, whereas

more than 0.05 was statistically insignificant. *P* value less than 0.01 was considered highly significant.

## Results

### Serum level of cardiac troponin-I changes

In intestinal IR nontreated group there was a statistically significant increase ($P<0.05$) in the serum troponin-I level compared with the control group. Pretreatment of intestinal IR group with ambroxol resulted in significant reduction ($P<0.05$) in serum troponin-I level compared with intestinal the IR nontreated group but it was still at a significant higher level ($P<0.05$) if compared with the control group (Fig. 1).

### Serum level of urea changes

In intestinal IR nontreated group, there was a statistically significant increase ($P<0.05$) in the serum urea level compared with the control group. Pretreatment of intestinal IR group with ambroxol resulted in a significant reduction ($P<0.05$) in serum urea level compared with intestinal IR nontreated group, but it was still at a significant higher level ($P<0.05$) if compared with the control group (Fig. 2).

### Serum level of creatinine changes

In intestinal IR nontreated group there was a statistically significant increase ($P<0.05$) in serum creatinine level compared with the control group. Pretreatment of intestinal IR group with ambroxol resulted in significant reduction ($P<0.05$) in serum urea level compared with intestinal IR nontreated group, but it was still at a significant higher level ($P<0.05$) if compared with the control group (Fig. 3).

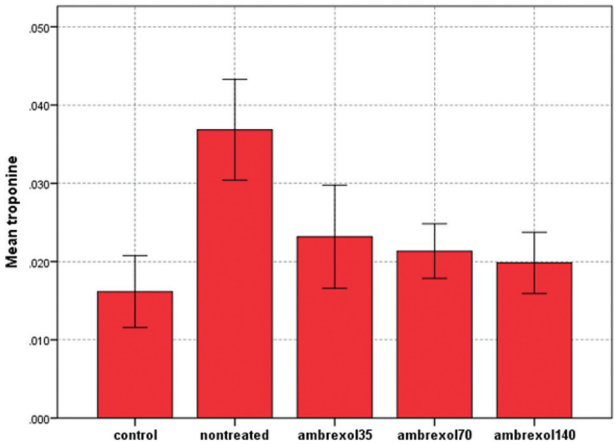
### Level of malondialdehyde changes in the heart

In the intestinal IR nontreated group, there was a statistically significant increase ($P<0.05$) in the MDA level in the heart compared with the control group. Pretreatment of intestinal IR group with ambroxol resulted in a significant reduction ($P<0.05$) in the MDA level in the heart compared with the intestinal IR nontreated group, but it was still at a significant higher level ($P<0.05$) if compared with the control group (Fig. 4).

### Level of malondialdehyde changes in the kidney

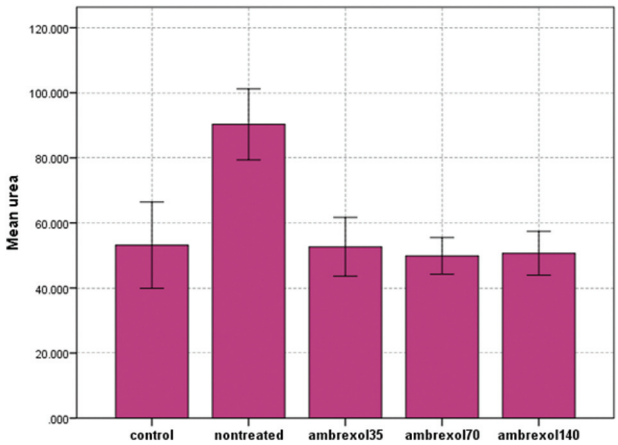
In intestinal IR nontreated group there was a statistically significant increase ($P<0.05$) in the MDA level in the kidney compared with the control group. Pretreatment of intestinal IR group with ambroxol resulted in a significant reduction ($P<0.05$) in the MDA level in the kidney compared with the intestinal IR nontreated group, but it was still at a significant higher level ($P<0.05$) if compared with the control group (Fig. 5 and Table 1).

**Figure 1**



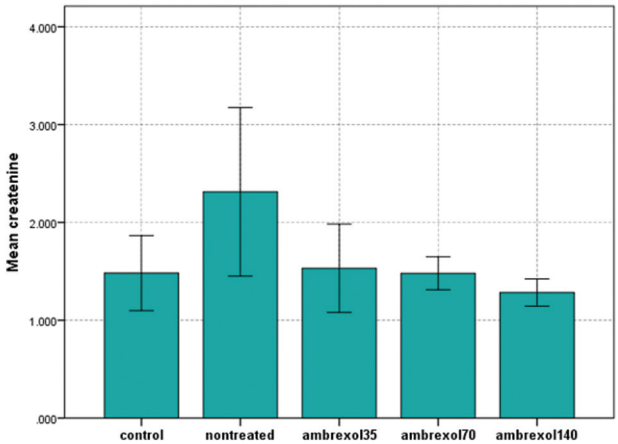

Effect of ambroxol on troponine.

**Figure 2**



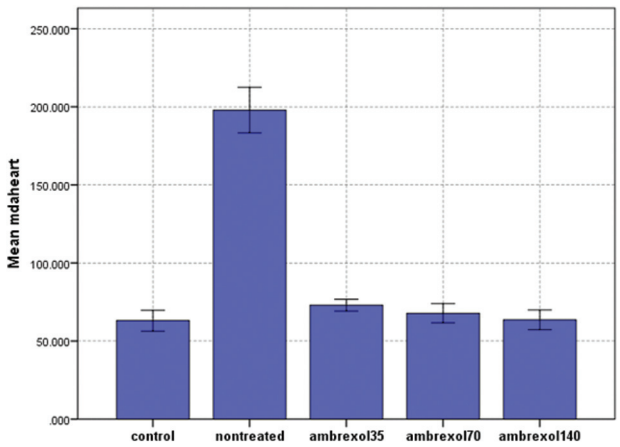

Effect of ambroxol on urea.

**Figure 3**



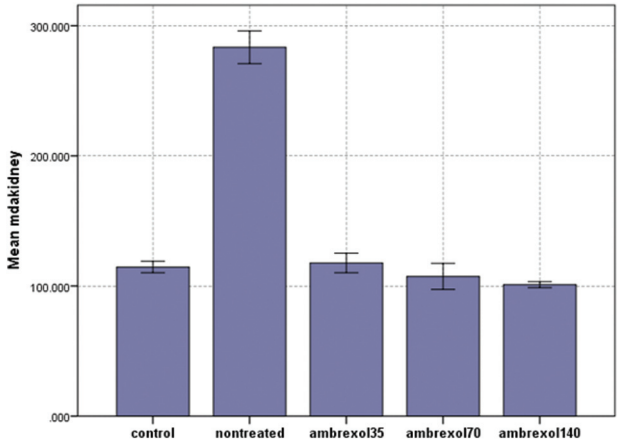

Effect of ambroxol on creatinine.

**Figure 4**




Effect of ambroxol on MDA heart.

**Figure 5**




Effect of ambroxol on MDA kidney.

### Histopathological examination

*Heart*

Control group: showed normal cardiac architecture composed of cardiac muscle fibers and normal blood vessels (healthy myocardium) (Fig. 6).

Intestinal IR nontreated group: showed acute inflammatory cell infiltration and congestion (Fig. 7).

Ambroxol-treated group (35 mg/kg): showed a decrease in inflammatory cells and congestion (Fig. 8).

Ambroxol-treated group (70 mg/kg): showed a decrease in inflammatory cells and congestion in degree more than the ambroxol-treated group (35 mg/kg) (Fig. 9).

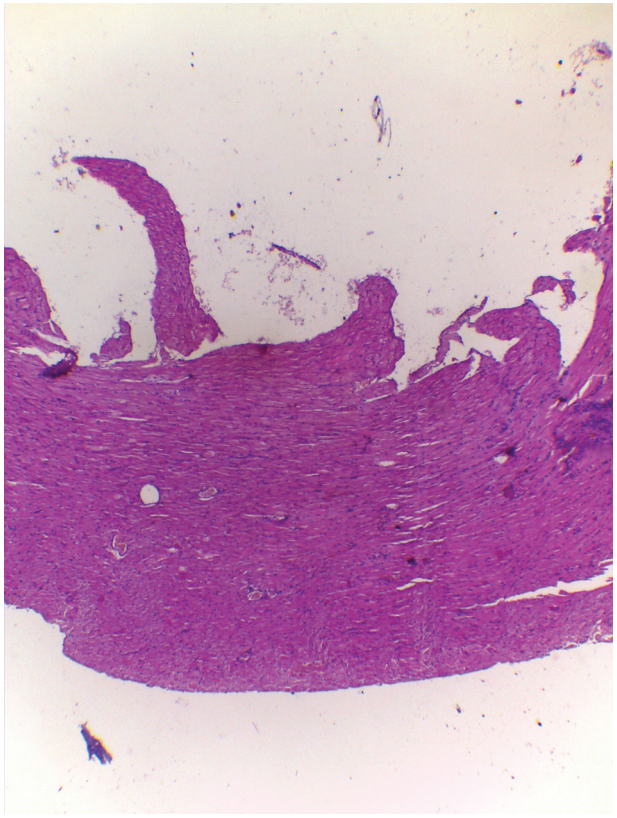
Ambroxol-treated group (140 mg/kg): showed a decrease in inflammatory cells and congestion in degree more than the ambroxol-treated group (70 mg/kg) (Fig. 10).

**Table 1 Effect of prophylactic therapy with multiple doses of ambroxol 5 min before experimentally induced intestinal ischemia–reperfusion on different parameters among the studied groups**

| | Control group (*n*=6) | | N-treated (*n*=6) | | Ambroxol 35 mg (*n*=6) | | Ambroxol 70 mg (*n*=6) | | Ambroxol 140 mg (*n*=6) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean±S.D | Range | Mean±S.D | Range | Mean±S.D | Range | Mean±S.D | Range | Mean±S.D | Range |
| Troponine | 0.017±0.006 | 0.01–0.026 | 0.04±0.007 | 0.028–0.050 | 0.023±0.008 | 0.01–0.031 | 0.02±0.004 | 0.02–0.03 | 0.02±0.004 | 0.015–0.03 |
| Urea | 53.2±16.2 | 40–73.6 | 90.3±13.4 | 72.7–100 | 52.7±11.1 | 42–73 | 49.9±6.9 | 40.9–56.3 | 50.7±8.3 | 40–56 |
| Creatnine | 1.5±0.47 | 0.76–2 | 2.3±10.05 | 1.4–4.4 | 1.5±0.55 | 1.2–2.7 | 1.5±0.21 | 1.1–1.6 | 1.3±0.17 | 0.6–1.4 |
| MDA in heart | 63.1±8.3 | 55.5–77.9 | 197.8±17.9 | 179.4–219.5 | 72.9±4.6 | 67.1–76.9 | 67.8±7.6 | 55.6–76.9 | 63.6±7.8 | 55–74 |
| MDA in kidney | 114.8±5.4 | 107.7–119 | 283.4±5.4 | 268–300 | 117.7±9 | 110–134 | 107±12.2 | 98–130 | 101±2.8 | 98–106 |

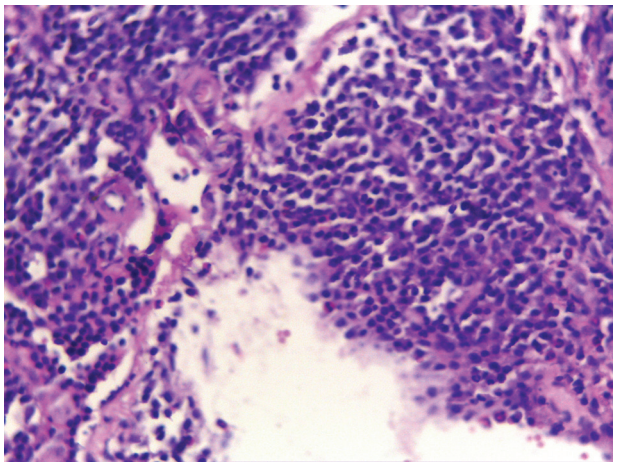
MDA, malondialdehyde. [a]Significant results. [*]*P*=0.001 versus nontreated. [**]*P*=0.001 versus nontreated. [***]*P*=0.001 versus nontreated. [****]*P*=0.001 versus nontreated.

**Figure 6**



Cut section in heart control group.

**Figure 7**



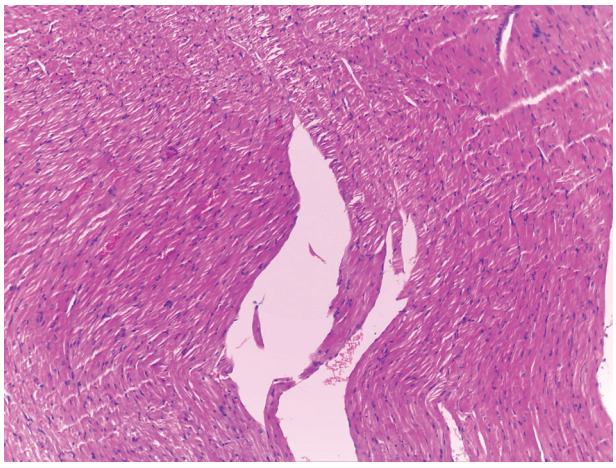
Cut section in heart non treated group.

*Kidney*

Control group: showed regular morphology of renal parenchyma with well-designated glomeruli and tubules with no infiltration of inflammatory cells (Fig. 11).

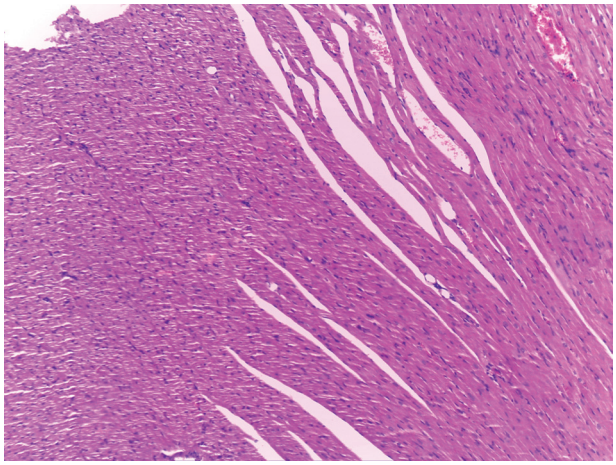
Intestinal IR nontreated group: showed a marked degree of inflammatory cells infiltration and tubular cell vacuolation (Fig. 12).

**Figure 8**



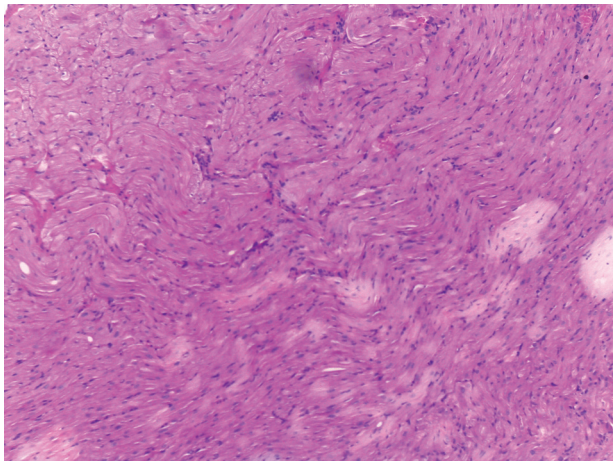
Cut section in heart ambroxol treated group 35 mglKG.

**Figure 9**



Cut section in heart ambroxol treated group 70 mglKG.

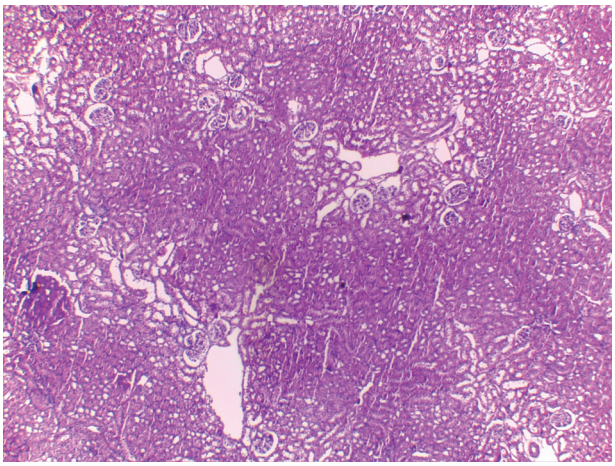
**Figure 10**



Cut section in heart ambroxol treated group 140 mglKG.

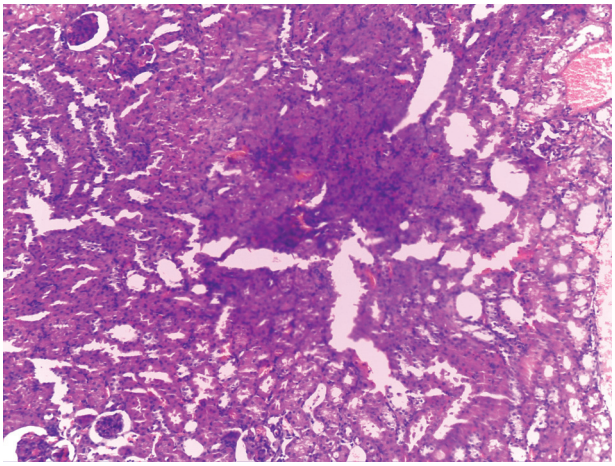
Intestinal IR ambroxol-treated group (35 mg/kg): showed a decrease in inflammatory cells and congestion (Fig. 13).

**Figure 11**



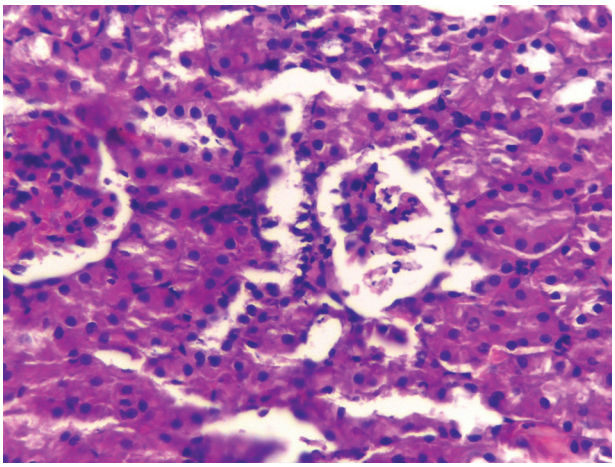
Cut section in kidney control group.

**Figure 12**



Cut section in kidney non treated group.

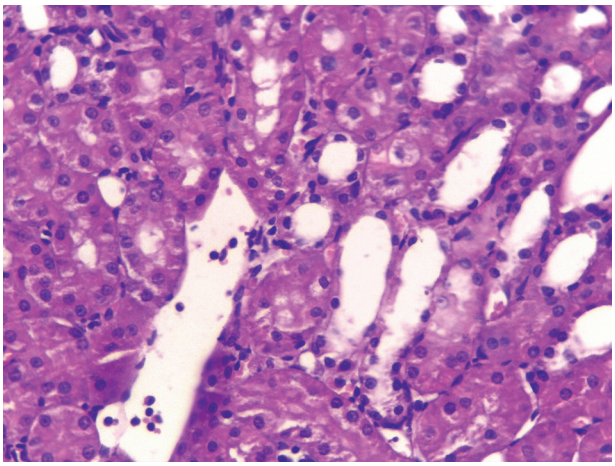
**Figure 13**



Cut section in kidney ambroxol treated group 35 mglKG.

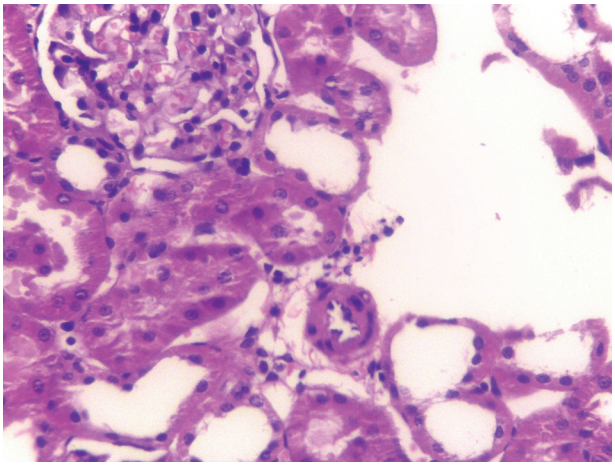
Intestinal IR ambroxol-treated group (70 mg/kg): showed decrease in inflammatory cells and congestion in degree more than the ambroxol-treated group (35 mg/kg) (Fig. 14).

**Figure 14**



Cut section in kidney ambroxol treated group 70 mglKG.

**Figure 15**



Cut section in kidney ambroxol treated group 140 mglKG.

Intestinal IR ambroxol-treated group (140 mg/kg): showed a decrease in inflammatory cells and congestion in degree more than the ambroxol-treated group (70 mg/kg)(Fig. 15).

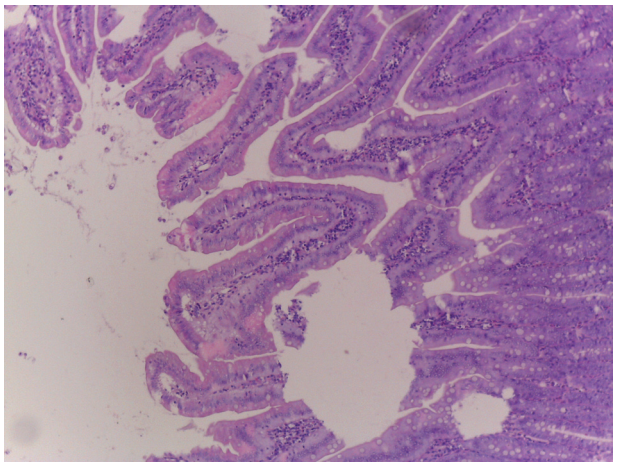
*Intestine*

Control group: showed normal intestinal architecture composed of normal intestinal villi and goblet cells (Fig. 16).

Intestinal IR nontreated group: showed loss of villi, presence of hemorrhage, edema in the submucosa, and inflammatory infiltrate (Fig. 17).

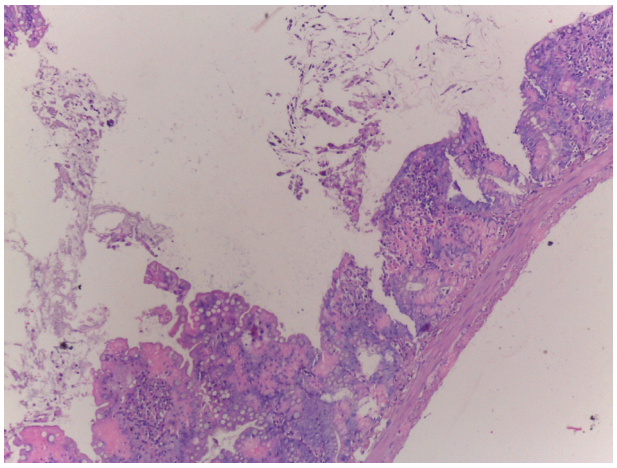
Ambroxol-treated group (35 mg/kg): showed loss of villi, presence of hemorrhage, edema in the submucosa,

**Figure 16**



Cut section in intestine control group.

**Figure 17**



Cut section in intestine non treated group.

and inflammatory infiltrate (no improvement) (Fig. 18).

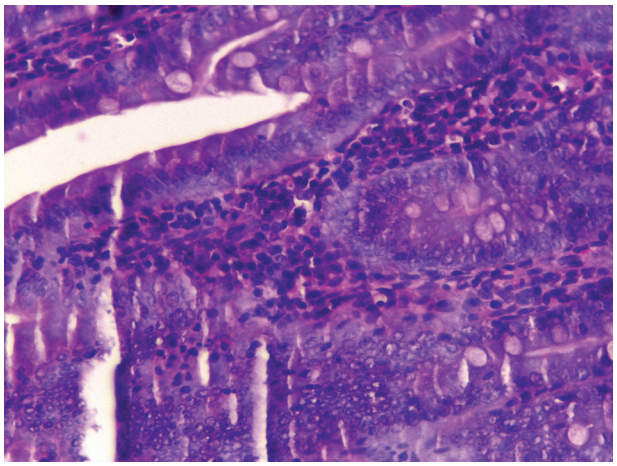
Ambroxol-treated group (70 mg/kg): showed loss of villi, presence of hemorrhage, edema in the submucosa, and inflammatory infiltrate (no improvement) (Fig. 19).

Ambroxol-treated group (140 mg/kg): showed loss of villi, presence of hemorrhage, edema in the submucosa, and inflammatory infiltrate (no improvement) (Fig. 20).

## Discussion

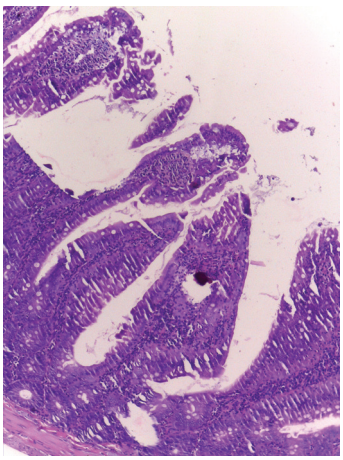
Oxidative stress in organisms appears when the balance between oxidants and antioxidants is disrupted due to either inadequate antioxidant activity or excessive accumulation of the reactive oxygen species, or both, leading to oxidative damage [15].

**Figure 18**



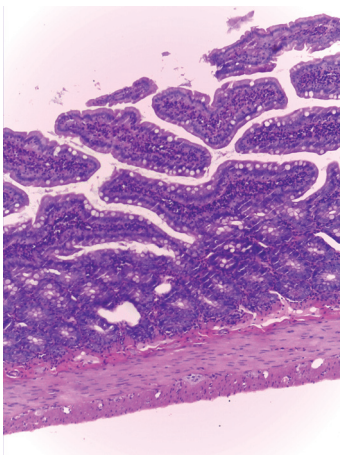
Cut section in intestine ambroxol treated group 35 mglKG.

**Figure 19**



Cut section in intestine ambroxol treated group 70 mglKG.

**Figure 20**



Cut section in intestine ambroxol treated group 140 mglKG.

The data of the present work has shown that intestinal ischemia for half an hour and perfusion for 2 h caused

significant elevation in the serum level of cardiac troponin 1 urea, creatinine, and significant elevation in the MDA level in cardiac, renal tissues.

Histopathological examination has shown cardiac, renal, and intestinal alteration in the form of inflammatory cells infiltration and congestion.

These results are in agreement with He *et al*. [16] who reported that oxidative stress is involved in local and systemic lesions in intestinal IR, because the accumulation of reactive oxygen species can lead to protein oxidation, lipid peroxidation, and DNA damage.

Results obtained have shown that intestinal IR caused significant affection in cardiac functions in the form of elevation of cardiac troponin 1 in serum; this is consistent with Horton and White [17] who reported that intestinal IR is an oxidative stress process leading to cardiac contractile injury in the form of fall in the left ventricular pressure and a rightward shift in the left ventricular function curves, and a decreased responsiveness to perfusate $Ca_2^+$.

Results obtained have shown that intestinal IR caused a significant elevation in the serum levels of urea and creatinine; this is consistent with Alexandropoulos *et al*. [18], who reported that intestinal IR lead to renal tissue injury with an elevation of serum levels of urea and creatinine and histopathological alteration in the renal tissue.

Results obtained have shown that intestinal IR caused significant elevation in remote tissue MDA as the heart and kidney; this is consistent with Kazantzidou *et al*. [19], who reported that intestinal IR caused significant oxidative injury in rat renal parenchyma which consisted of severe alterations observed in the subcellular renal structures associated with a significant increase in renal MDA levels.

Histopathological examination of the heart has shown that intestinal IR resulted in cardiac tissue injury in the form of multiple congested capillaries between fibers and mononuclear infiltrate among the muscle fibers; this is consistent with Zickri *et al*. [20], who reported that intestinal IR induced cardiac muscle degenerative changes.

Histopathological examination of the kidney has shown that intestinal IR resulted in renal tissue injury in the form of marked degree of inflammatory cells infiltration and tubular cell vacuolation; this is consistent with Stringa *et al*. [21], who reported that intestinal IR is an inflammatory process leading to local and remote organ injury in the form of histological alteration in the liver (sinusoidal congestion and hepatic vacuolization) and the kidney (acute tubular necrosis and hydropic degeneration).

Histopathological examination of the intestine has shown that intestinal IR resulted in intestinal tissue injury in the form of loss of villi, presence of hemorrhage, edema in the submucosal layer, and inflammatory infiltrate; this is in agreement with Ben Shahar *et al*. [22], who reported that intestinal IR causes intestinal apoptotic cell death and mucosal cell damage.

Results obtained have shown that the use of ambroxol in three different doses in intestinal IR lead to an improvement in the abnormal levels of cardiac function (cardiac troponine 1); this is in agreement with Czech *et al*. [23], who showed that treatment with ambroxol reduces the doxorubicin-induced oxidative stress in hearts and lungs of mice and significantly attenuated the DOX-elicited rise in the end products of lipid peroxidation and $H_2O_2$ activity in hearts and lungs.

Results obtained have shown that the use of ambroxol in three different doses in intestinal IR lead to an improvement in the abnormal levels of urea and creatinine (kidney function) in the serum; this is in agreement with Liu *et al*. [24], who showed that ambroxol is effective in reducing cardiopulmonary bypass-induced renal injury in children undergoing repair of ventricular septal defect through normalization of abnormal kidney function (serum level of urea).

Results obtained have shown that the use of ambroxol in three different doses (3 570 140 mg/kg) in intestinal IR lead to an improvement in the abnormal MDA level in remote tissues (heart and kidney); this is in agreement with Tian *et al*. [25], who reported that ambroxol could influence the status of oxidative stress in the lung and alleviate lung injury induced by paraquat and improve abnormal levels of MDA in blood.

Results obtained have shown that the use of ambroxol in three different doses in intestinal IR lead to improvement of remote tissue (kidney and heart) inflammatory and oxidative stress affection as seen in histopathological examination; this is consistent with Ottonello *et al*. [26], who proved that ambroxol can limit the excessive recruitment of neutrophils in

inflamed bronchopulmonary tissue and inhibit the production of cytokines.

Results obtained have shown that the antioxidant and anti-inflammatory effect of ambroxol increased by increasing the dose; this is consistent with Jiang *et al*. [8], who proved that ambroxol alleviates hepatic IR injury in dose–effect relationship.

Results obtained have shown that the use of ambroxol in three different doses in intestinal IR cannot improve the abnormalities in intestinal tissue histology.

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Nil.

**Conflicts of interest**
There are no conflicts of interest.

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