ORIGINAL ARTICLE

Comparative antidiarrheal and antiulcer effect of the aqueous and ethanolic stem bark extracts of *Tinospora cordifolia* in rats

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ABSTRACT

Tinospora cordifolia is indigenous to the tropical areas of India, Myanmar and Sri Lanka. The use of plant as remedy for diarrhea and ulcer is well-documented in Ayurvedic system of medicine. However, pharmacological evidence does not exist to substantiate its therapeutic efficacy for the same. The aim was to investigate the antidiarrheal and antiulcer activity of ethanolic and aqueous extracts of *T. cordifolia* in rats. The antidiarrheal activity of *T. cordifolia* extracts was evaluated by castor oil and magnesium sulfate-induced diarrhea using parameters such as onset of diarrhea, number of wet stools, total number of stool and weight of total number of stools. The antiulcer activity of extracts was investigated using ethanol and pylorus ligation-induced ulcer. Furthermore, tissue antioxidant parameters such as reduced glutathione, catalase activity and lipid peroxidation level were also investigated. *Tinospora cordifolia* extracts were more efficacious in reducing number of total stools in both the models of diarrhea and showed a dose-dependent antidiarrheal effect. The antiulcer activity of the extracts was confirmed by a reduction in ulcer index along with the decrease in gastric volume, total acidity, and an increase in pH of gastric content in both the models. The obtained results have established a pharmacological evidence for the folkloric use of the *T. cordifolia* as antidiarrhoeal and antiulcer agent.

Key words: Antidiarrheal, antiulcer, pylorus ligation, *Tinospora cordifolia*

INTRODUCTION

Diarrhea is a common worldwide problem responsible for considerable morbidity and mortality in infants, more specifically in the developing countries.[1,2] In a year, about 5 million people die of diarrhea, of which 2.5 millions are malnourished children of <5 years of age.[3] On the other hand, peptic ulcer is the most predominant of the gastrointestinal diseases. The etiological factors behind the disease are inadequate dietary habits, prolonged use of nonsteroidal anti-inflammatory drugs, stress, Helicobacter pylori infection and some genetic factors.[4]

Tinospora cordifolia - commonly known as Amrita or Guduchi- is a large, deciduous climbing shrub, indigenous to the tropical areas of India, Myanmar and Sri Lanka. It has been described in various classical texts of ayurvedic system of medicine as rasayanas- the rejuvenators with strong antioxidant activity, which reverse the disease process and prevent the re-occurrence.[5] In Bhava Prakash, the plant is considered as bitter tonic and curative against chronic diarrhea and dysentery.[6] Thus, present study was undertaken to evaluate the antidiarrhoeal and antiulcer activity of *T. cordifolia*.

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MATERIALS AND METHODS

Plant material and extraction

The plant was collected from local market and was authenticated at GNDU, Amritsar (reference no. 1045).

Dried stem bark of *T. cordifolia* was coarsely powdered and subjected to extraction in a soxhlet apparatus.[7]

Chemicals

Ranitidine was obtained from Axon Pharmaceuticals. Glutathione (GSH), malondialdehyde (MDA) and catalase were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai.

Experimental animals

Healthy Wistar rats (150-200 g) were procured from NIPER, Mohali. The animals were fed regularly with diet and water *ad libitum*. The protocol was approved by IAEC (954/ac/06/CPCSEA/11/09).

In vivo antidiarrhoeal activity

Castor oil-induced diarrhea

Rats of either sex were fasted for 18 h and divided into six groups. Rats in Groups I and II received vehicle and loperamide (3 mg/kg p.o.) respectively, Groups III and IV received the ethanolic extract (250 and 500 mg/kg p.o. respectively), and Groups V and VI received the aqueous extract (250 and 500 mg/kg p.o. respectively). After 30 min, diarrhea was induced by oral administration of castor oil (1 ml) to each rat. Rats were observed for a period of 4 h during which the total number of fecal outputs and the number of diarrheic feces excreted were recorded.[3,8,9]

Magnesium sulfate-induced diarrhea

A similar protocol as for castor oil-induced diarrhea was followed. Diarrhea was induced by oral administration of magnesium sulfate (2 mg/kg) to the animals.[8]

In vivo antiulcer activity

Pylorus ligation-induced ulcer

Rats were divided into seven groups ($n = 6$). Group I received no treatment, Group II received vehicle, Group III received ranitidine (50 mg/kg), Groups IV and V received ethanolic extract (250 and 500 mg/kg p.o. respectively) and Groups VI and VII received aqueous extract (250 and 500 mg/kg p.o. respectively). One hour posttreatment, animals were anesthetized and their pylorus was ligated.[5] After 2 h, rats were sacrificed and their stomach was removed. The gastric content was collected to determine its volume, pH, total acidity and free acidity using protocols of Bharti *et al*.[10] The mucosal surface of the stomach was scored in terms of ulcer index. Protein and hexosamine content in gastric juice was also estimated.[11]

Ethanol-induced ulcer

Rats were divided into seven groups as in the previous model. After 30 min of respective treatment, ulcer was induced by oral administration of ethanol (1 ml) except in Group I. One hour post ethanol administration, animals were sacrificed and their stomach was removed to examine extent of mucosal damage in terms of ulcer index.[12] The gastric tissue was used for the estimation of catalase, GSH, and lipid peroxidation.[13]

Histological studies

The sections of freshly excised gastric tissue were stained with hematoxylin and eosin for histopathological evaluation at magnification ×40.[14]

Statistical analysis

The experimental results have been expressed as mean ± standard error of the mean. One-way ANOVA followed by Dunnett's test was used to analyze the data.

RESULTS

Castor oil-induced diarrhea

Rats in control group produced copious diarrhea after castor oil administration. However, rats pretreated with ethanolic extract of *T. cordifolia* (ETETC) and Aqueous extract of *T. cordifolia* (AQETC) showed a significant delay in onset of diarrhea, decrease in the frequency of wet and total stools as well as reduction in the weight of wet stool and total weight of stools [Table 1].

Magnesium sulfate-induced diarrhea

As in the previous model, ETETC, AQETC and standard drug significantly ($P < 0.01$) prolonged the time for diarrheal induction dose-dependently [Table 1]. Furthermore, the number of wet stools and total number of stools were also found to be decreased in the extract as well as standard groups.

Pylorus ligation-induced ulcer

When compared to the normal rats, pylorus ligation caused gastric damage with high ulcer index in the experimental control rats. Ranitidine, ETETC and AQETC were found to produce significant reduction in the ulcer index dose-dependently [Table 2]. All the aggressive factors (e.g. gastric volume, total acidity, and free acidity) were decreased and gastric pH was increased in the ranitidine and extract treated groups.

The increased hexosamine content and decreased protein content in ETETC and AQETC treated groups, when compared to experimental group, also supported the result [Table 3].

The pylorus ligation increased the lipid peroxidation and reduced catalase and GSH in the experimental control group. Treatment with ranitidine, ETETC and AQETC significantly reduced lipid peroxidation and increased the activity of antioxidant enzymes [Table 3].

Ethanol-induced ulcer

Oral administration of alcohol produced severe gastric lesions in the experimental control group. Pretreatment with ranitidine, ETETC and AQETC caused a significant decrease in

Table 1: Effect of ETETC and AQETC on castor oil and magnesium sulfate-induced diarrhea

Groups	Onset of diarrhea (min)	Number of wet stools	Weight of wet stools (g)	Total number of stools	Weight of total number of stools (g)
Castor oil-induced diarrhea					
Control	36.16±2.27	9.83±0.30	1.85±0.03	11.33±0.42	1.93±0.04
Positive control	307.50±2.75**	0.71±0.18**	0.43±0.01**	1.00±0.25**	0.52±0.03**
ETETC-250	151.00±2.59**	2.66±0.33**	0.76±0.02**	2.83±0.30**	0.80±0.01**
ETETC-500	186.50±2.74**	1.66±0.21**	0.57±0.01**	2.00±0.25**	0.67±0.01**
AQETC-250	105.66±2.17**	5.33±0.33**	0.91±0.02**	6.83±0.40**	1.10±0.03**
AQETC-500	126.16±2.52**	3.83±0.40**	0.86±0.02**	4.00±0.36**	0.97±0.03**
Magnesium sulfate-induced diarrhea					
Control	50.16±2.70	6.66±0.30	1.43±0.04	8.66±0.33	1.55±0.05
Positive control	310.83±2.83**	0.57±0.20**	0.25±0.02**	0.83±0.30**	0.32±0.03**
ETETC-250	186.16±2.24**	1.83±0.30**	0.56±0.04**	3.00±0.25**	0.62±0.06**
ETETC-500	250.83±2.41**	1.00±0.44**	0.34±0.05**	2.00±0.25**	0.49±0.07**
AQETC-250	132.00±2.89**	4.16±0.30**	1.12±0.06**	6.00±0.36**	1.18±0.08**
AQETC-500	167.83±2.21**	3.66±0.33**	0.90±0.05**	4.50±0.22**	0.94±0.06**

Results are expressed as mean±SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's test. **P<0.01 versus control. ETETC: Ethanolic extract of *Tinospora cordifolia*, AQETC: Aqueous extract of *Tinospora cordifolia*, SEM: Standard error of the mean

Table 2: Effect of ETETC and AQETC on different parameters in pylorus ligation-induced ulcer

Groups	Ulcer index (% protection)	pH	Gastric volume (ml)	Free acidity (mEq/L)	Total acidity (mEq/L)
Control	-	4.33±0.08	1.01±0.06	15.93±0.15	33.4±0.51
Experimental control	4.33±0.54	2.08±0.06	7.91±0.21	45.16±0.60	72.83±0.47
Standard (ranitidine)	0.50±0.12** (88.5)	3.68±0.15**	3.76±0.10**	18.50±0.71**	34.16±0.47**
ETETC-250	1.00±0.18** (76.9)	3.06±0.13**	5.15±0.11**	28.83±0.60**	48.83±0.74**
ETETC-500	0.83±0.24** (80.8)	3.18±0.15**	4.75±0.25**	23.50±0.50**	40.33±0.76**
AQETC-250	1.50±0.56** (65.4)	2.66±0.11**	6.38±0.13**	37.50±0.61**	59.00±0.68**
AQETC-500	1.25±0.40** (71.1)	2.75±0.08**	5.95±0.20**	32.50±0.67**	50.50±0.56**

Results are expressed as mean±SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's test. **P<0.01 versus experimental control. ETETC: Ethanolic extract of *Tinospora cordifolia*, AQETC: Aqueous extract of *Tinospora cordifolia*, SEM: Standard error of the mean

Table 3: Effect of ETETC and AQETC on hexosamine, total protein content, CAT, GSH, and MDA in pylorus ligation-induced ulcer

Groups	Hexosamine (μg/ml)	Total proteins (μg/ml)	CAT (μmol H_2O_2/ min/100 mg protein)	GSH (μmol/100 mg)	MDA (nmol/mg)
Control	-	-	36.06±0.11	347.84±7.98	3.88±0.07
Experimental control	223.37±1.60	426.48±6.11	19.01±0.06	117.57±5.85	11.98±0.58
Standard (ranitidine)	329.07±2.59**	164.72±5.26**	33.95±0.65**	225.51±1.16**	4.16±0.15**
ETETC-250	284.17±5.34**	277.70±6.05**	28.54±0.08**	178.00±1.58**	5.50±0.05**
ETETC-500	309.97±1.76**	213.15±6.74**	30.71±0.06**	191.60±1.58**	4.94±0.05**
AQETC-250	237.34±2.36**	367.34±8.92**	23.07±0.77**	159.41±2.48**	8.93±0.06**
AQETC-500	266.97±2.00**	339.11±9.21**	25.95±0.44**	167.80±1.63**	7.62±0.14**

Results are expressed as mean±SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's test, **P<0.01 versus experimental control. ETETC: Ethanolic extract of *Tinospora cordifolia*, AQETC: Aqueous extract of *Tinospora cordifolia*, SEM: Standard error of the mean, CAT: Catalase, GSH: Glutathione, MDA: Malon-dialdehyde

the gastric damage represented by a reduction in ulcer index as compared to the experimental control group [Table 4].

Ethanol administration in the experimental control animals increased the lipid peroxidation and decreased catalase and GSH levels as compared to the normal animals. Pretreatment with ETETC and AQETC significantly decreased the lipid peroxidation and increased the levels of catalase and GSH [Table 4].

Morphological study

In normal group, stomach integrity was maintained and appeared normal [Figure 1]. In experimental control group, severe bleeding, perforation, spot ulcer, streaks were observed. The gastric mucosa was appeared intact in the stomach of ranitidine treated animals. The gastric mucosa was seen to be slightly damaged with red coloration, spot ulcers and a few hemorrhagic streaks in the stomach of ETETC and AQETC (250 mg/kg) treated animals. However,

in the stomach of the ETETC and AQETC (500 mg/kg) treated animals gastric mucosal damage was negligible with a continuous epithelial surface.

Histological study

As depicted in Figure 2, mucosal organization was normal in normal control animals. In experimental control group, gastric mucosa showed many pit-shaped ulcers, mucosal and submucosal congestion, along with surface erosion at several places. In ranitidine treated group, gastric mucosa revealed mild superficial erosions with intact submucosa and deeper mucosa. In ETETC and AQETC (250 mg/kg) groups, superficial erosions and a few ulcers with mild disorganization of mucosa were observed. However, the ETETC and AQETC (500 mg/kg) group revealed mild superficial erosions, very slight disorganization of the mucosa with no appreciable inflammation.

DISCUSSION

Tinospora cordifolia is a large, deciduous climbing shrub of family Menispermaceae. The major phytoconstituents isolated from the stem of *T. cordifolia* are sesquiterpene tinocordifolin, sesquiterpene glycoside tinocordifolioside and tinocordiside, arabinogalactan, phytoecdysones viz., ecdysterone and makisterone, alkaloids viz., berberine, palmatine and magnoflorine.[15,16] All these active compounds have physiological roles of different types, thereby demonstrating the diverse versatility of the plant.

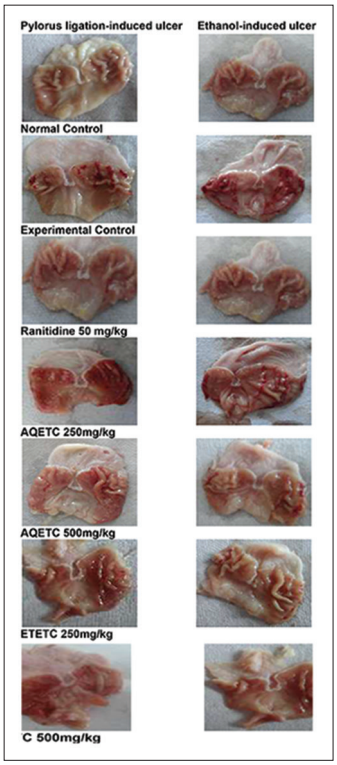

Figure 1: Morphological features of stomach in pylorus ligation and ethanol-induced ulcer

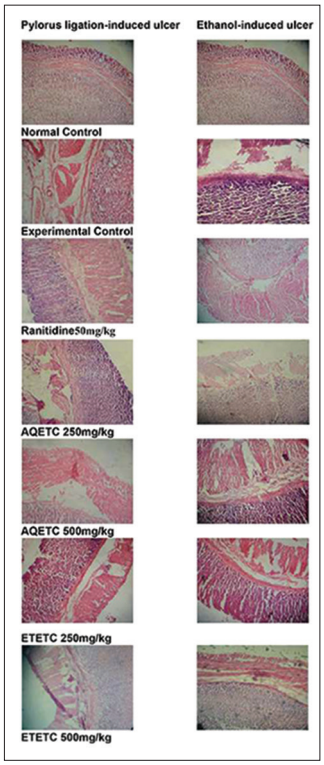

Figure 2: Histology of stomach in pylorus ligation and ethanol-induced ulcer

Table 4: Effect of ETETC and AQETC on different parameters in ethanol induced ulcer

Groups	Ulcer index (% protection)	CAT (μmol H_2O_2/ min/100 mg protein)	GSH (μmol/100 mg)	MDA (nmol/mg)
Control	-	36.06±0.11	347.84±7.98	3.88±0.07
Experimental control	14.58±0.94	18.28±0.07	115.41±4.78	9.23±0.32
Standard (ranitidine)	2.33±0.84** (84.0)	32.87±0.62**	193.19±6.88**	4.03±0.15**
ETETC-250	4.16±0.47** (71.4)	27.08±0.09**	166.66±1.39**	5.47±0.03**
ETETC-500	3.75±0.46** (74.3)	29.18±0.54**	181.85±7.24**	4.69±0.22**
AQETC-250	6.66±0.44** (54.3)	21.85±0.12**	147.05±2.21**	7.45±0.04**
AQETC-500	5.50±0.46** (62.3)	24.85±0.15**	152.26±2.30**	6.30s±0.25**

Results are expressed as mean±SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's test. **P<0.01 versus experimental control. ETETC: Ethanolic extract of *Tinospora cordifolia*, AQETC: Aqueous extract of *Tinospora cordifolia*, CAT: Catalase, GSH: Glutathione, MDA: Malon-dialdehyde, SEM: Standard error of the mean

In the present study, an attempt has been made to investigate the therapeutic success of the stem bark extracts to be a potential antidiarrheal and antiulcer agent.

The *in vivo* antidiarrheal activity of extracts was assessed using castor oil and magnesium sulfate-induced diarrhea by means of evaluating onset of diarrhea, frequency of wet and total stools, weight of wet stool and total weight of stools. Castor oil (hydrolytic metabolite, i.e. ricinoleic acid) induces diarrhea by releasing nitric oxide (NO), stimulating prostaglandin synthesis and increasing peristalsis.[2,17] While, magnesium sulfate prevents reabsorption of water and promotes cholecystokinin (CCK) release from the duodenal mucosa [Figure 3]. Since, pretreatment with extracts provide significant protection against castor oil and magnesium sulfate-induced diarrhea, the extracts may presumed to have antisecretory and preventive action towards CCK release.

The *in vivo* antiulcer activity of the plant extracts was investigated using pylorus ligation and ethanol-induced ulcer. Pylorus ligation cause accumulation of acid and pepsin leading to auto digestion of gastric mucosa and ulceration.[10] Ethanol cause depletion of gastric mucus content and increased expression of inflammatory mediators and vascular permeability [Figure 4].[18]

In both the models, extracts provide a significant protection against ulcer as indicated by a decrease in the ulcer index, acidity and volume of gastric content and increase in pH of gastric content. The pathogenesis of pylorus ligation and ethanol-induced ulcer also involves the generation of reactive oxygen species (ROS) [Figure 4]. *T. cordifolia* has been previously reported of its strong free radical scavenging properties against superoxide anion, hydroxyl radicals, NO radical, and peroxynitrite anion.[19] In this study, extracts significantly increased the level of antioxidant enzymes like catalase and GSH providing the first line defense system against ROS induced gastric mucosal damage in both the ulcer models.

The obtained results have established a pharmacological evidence for the folkloric use of the *T. cordifolia* as antidiarrheal and antiulcer agent. The future scope of the research remains in exploiting the signaling pathways of the active components of *Tinospora* thus, enabling effective disease targeting.

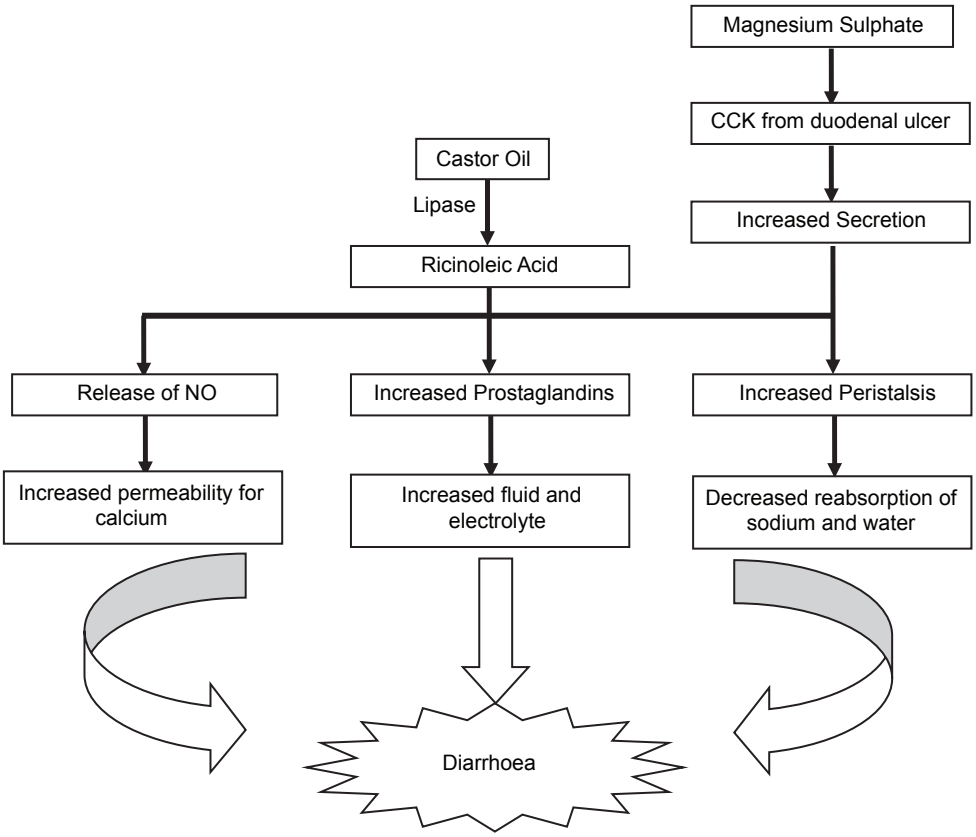

Figure 3: Mechanism of castor oil and magnesium sulfate-induced diarrhea. Castor oil-induces diarrhea by: (1) Releasing nitric oxide and thereby increasing permeability of gastrointestinal membrane for calcium; (2) stimulating prostaglandin synthesis and thereby increasing fluid and electrolytes into the lumen of the bowel; and (3) increasing peristalsis. On the other hand, magnesium sulfate induces diarrhea by promoting cholecytokinin release from the duodenal mucosa preventing the reabsorption of sodium chloride and water from the lumen

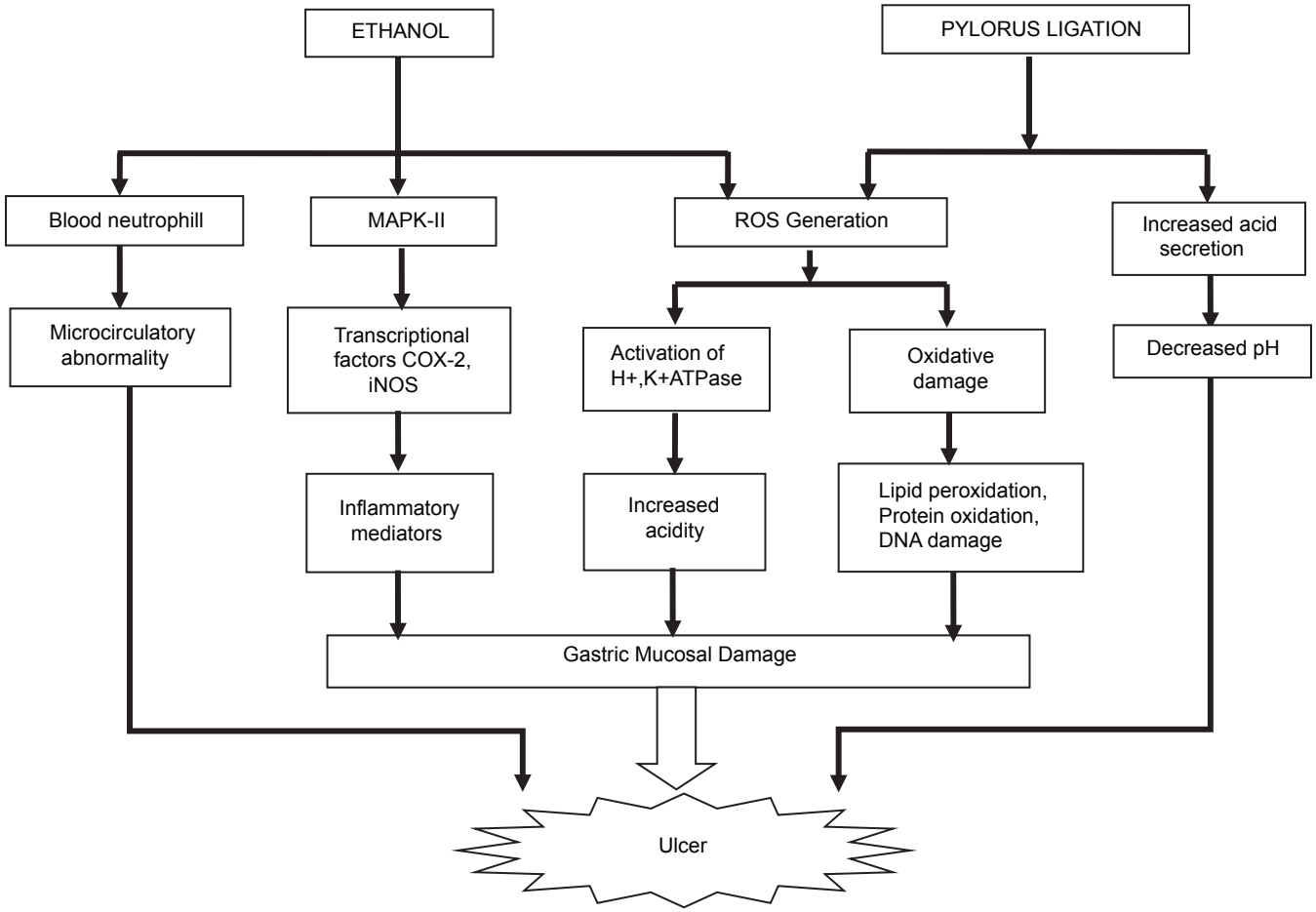

Figure 4: Mechanism of pylorus ligation and ethanol induced ulcer. Pylorus ligation causes accumulation of acid and pepsin, which leads to auto digestion of gastric mucosa and ulceration. On the other hand, ulcer due to ethanol is the result of reactive oxygen species generation, microvascular injury and release of inflammatory mediators, which leads to increased vascular permeability, edema formation, and epithelial lifting resulting in necrotic lesions in the gastric mucosa

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