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Inflammatory Mediator and Antioxidant Role in Gastroprotective of *Tinospora crispa* against Ethanol Induced Gastric Ulcer

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Abstract

This research performed to determine the potential protective effects of Tinosporacrispa stems to the rat gastric mucosal injury of induced by ethanol. As well as clarify the role of gastrin, pepsin, Prostaglandin E2 (PGE2), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) and cytokines (TGFB1 and TNF-α). Seven groups of rats were orally pre-treated with Tween 20 as vehicle control group, Tween 20 as ulcer group, 20 mg/kg of omeprazole as reference drug group, 100 mg/kg, 200 mg/kg, 500 mg/kg and 1000 mg/kg of extract as the experimental groups. An hour later, induction ulcer by given 95% ethanol orally except vehicle control group. The results have been showed significant ulcer protective effects by reduction the ulcer area and increase the ulcer inhibition grossly and histology. As well as significant elevate the gastric juice pH and increasing the production of mucus. In addition, significant elevated of inflammatory mediators PGE2, increased the activity of SOD and CAT have shown in gastric mucosa and significant elevated of in TGFB1. On the other hand, observed reduction the serum level of gastrin and pepsin, and decreasing the level of MDA and TNF-α. In conclusion, our results proof that *T. crispa* pretreatment has protective effects in ethanol-induced gastric ulcers in rats. Moreover, these results provide evidence that these protective effects of *T. crispa* by stimulation of some inflammatory mediators as PGE2, gastrin, TGFB1 and TNF-α. Moreover, important antioxidant enzymes such as SOD and CAT which are scavengers of ROS and therefore prevent gastric injury induced by them.

Keywords: *Tinospora crispa*; Gastroprotective; Prostaglandin E2; TGFB1; TNF-α; Antioxidants enzyme

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Introduction

Gastric ulcer has been composed the highest percentage among the diseases in the world due to rising the stress that faces the human, consumable alcohol, taking many drugs that in the long term of use will cause gastric ulcer as non-steroidal anti-inflammatory drugs as (aspirin and indomethacin) and nutritional deficiencies [1].

Many medications used to treat gastric ulcer. H2-antagonists, Omeprazole, Ranitidin and Famotidine work the present medications of gastric ulcer as secretion inhibitor of the gastric acid. Though, the one problem in gastric ulcer treated is that, in spite of the rate of healing between (80% to 100%) through (4 to 8) weeks when used the proton pump inhibitors and H2-antagonists, but the ulcer returning when stopped therapy in the rate 40% to 80% [2]. Besides, more of these medications have side effects for long use [3]. Therefore, we are looking for the new therapeutically anti- ulcer agents.

Gastrin is a polypeptide hormone synthesized in gastrin cells and play an important role in modulates a variety functions in the gastrointestinal tract, include cell proliferation, motility and acid secretion. These actions have been mediated by gastrin/CCKB receptors [4]. Prostaglandins and growth factors have important roles to maintain gastrointestinal mucosal integrity, reform of gastrointestinal mucosal injury, and ulcer healing [5]. Variety of mediators and cytokines as PGE2, TGFB1 and TNF-α are coordinate in the inflammatory responses. That liberate from many cells in the lamina propria response to infection, injury and exposure to antigens. The effect of these mediators directly on the mucosal integrity through vascular permeability, modulation epithelial and influence on blood flow [6]. Superoxide radical anion and hydroxyl radical now considered are Reactive Oxygen Species (ROS) the major causative factors for mucosal lesions. Endogenous

antioxidant enzymes like Superoxide Dismutase (SOD) and Catalase (CAT) in tissues, which scavenge these ROS and therefore prevent LPO and tissue damage. In gastric ulceration, ROS may be produce in excess due to delicate balance between ROS and endogenous antioxidants or antioxidant enzymes [7].

In the recent year, the people trained to use the traditional medicine for treatment of the diseases. *T. crispa* one of traditional drugs that use in the treatment inflammation, cancer, diabetic, anti-Hypercholesterolemia [8]. In this work, we used *T. crispa* stems extract as anti-inflammatory and anti-oxidant drug to investigate the gastro protective and conducted to investigate the mechanisms responsible for the gastroprotective.

Materials and Methods

Material

T. crispa dried stems were collected at Selangor housing area voucher specimen (KLU 45568) at Herbarium of IBS, UPM. Tween 20 (Sigma-Aldrich, Germany), omeprazole (TROGE medical GMBH, Hamburg), ethanol (Fisher Scientific, UK), xylazine and ketamine (ilium, Australia), Alcian blue (ACROS, USA), D (+) sucrose (Fisher Scientific, UK), Sodium acetate and Magnesium Chloride (Sigma-Aldrich, Germany), ethyl ether (Anala R, England) and PBS (DulbeccoA, Oxoid, England).

Plants extraction

Dried stems of *T. crispa* crushed. The powder (100 g) is soaked in 1000 ml of 95% ethanol in conical flask, for three days at room temperature (30°C ± 2°C). The suspension is shook from time to time to allow the stems powder completely dissolve in the ethanol and changed the color to dark brown. After three days, the mixture filtered by using a filter paper (Whitman, 185 mm) and distilled under reduced pressure in rotary evaporator (Buchi, Switzerland). The outcome of extract is saved at -20°C till be use [9].

Acute toxicity test

Forty-eight rats (24 males and 24 females), weight between (160 g to 180 g) are randomly separated in four groups, each group has six males and six females, each rat is caged alone, fasted prior to dosing (food but not water should be withheld overnight). The *T. crispa* stems extract is dissolved in Tween 20 (5% v/v) and administered at a single dose of 50, 500 and 2000 mg/kg body weight per rat (5 ml/kg). The control group A received the same volume of Tween 20 (5% v/v)), for both treated and control groups respectively, are administered a single dose orally by using oral gavage tube to each rat. After *T. crispa* stems extract has been administered, food withheld for a further (3 h to 4 h). Toxicity symptoms and mortality are observed, after dosing once during the first 30 min, recurrently during the first 24 h, with special awareness given during the first four hours and daily then, for a total of fourteen days, the body weight is gained each 7 days. After 14 days the animals from each group are weighed, anesthetized with 0.01 ml/kg xylazine and 0.09 ml/kg ketamine, blood sample is collected to analyze the biochemical tests, kidney and liver is taken for histopathological assessment.

Induction of gastric ulcer by ethanol

Healthy female *Sprague Dawley* rats, weight between (200 g to 250 g). The animals randomly divided in to seven groups (6 rats/groups) the water is removed one hour before the experiment. First group (vehicle control group), in this group the rats are given vehicle Tween 20 (5% v/v) (5 ml/kg). Second group (ulcer control group) the rats in this group received Tween 20 (5% v/v) (5 ml/kg). Third group (reference drug group) in this group the rats are treated with omeprazole 20 mg/kg solution (5 ml/kg). Fourth groups the rats are treated with (100 mg/kg, 200 mg/kg, 500 mg/kg, and 1000 mg/kg) from *T. crispa* stems extract respectively. After the treatment at one hour, all rats are received 95% Ethanol (EtOH) at (5 ml/kg) orally to induce gastric ulcer (except the first group). After one hour later all the rats are sacrificed, collect the blood, and removed the stomach and opened over the greater curvature [10].

Measurement of ulcer index

The ulcers have been located in the gastric mucosa; seem as hemorrhagic extended bands of lesions parallel to the long axis of the stomach. The gastric mucosa ulcer is calculated by measured the sum of the all lesions areas for each stomach is used in the calculation of the Ulcer Area (UA) is equal to (the sum of small squares × 4 × 1.8 = UA mm2). The Ulcer Inhibition Percentage (UI %) is calculated as described by [11].

$$(\text{UI }\%) = [(\text{UA control} - \text{UA treated}) \div \text{UA control}] \times 100\%$$

Measurement of gastric juice PH

After opened the stomach, the gastric juice collected and measured for the acidity by using digital PH meter [12].

Histological evaluation of gastric lesions

A piece of glandular portion of each stomach is fixed in 10% formalin and then dehydration by alcohol using ascending grades, after that embedded the processes tissue in paraffin to prepare the tissue for sectioning by using microtome to cut the tissue in sections with five micrometers in order to be ready for stained with hematoxylin-eosin solutions [13].

Determination of gastric wall mucus content

The gastric mucus contents are determined according the assay was described by [14]. Adult female *Sprague Dawley* rats, that weight between (200 g to 250 g) is fasted for 24 h, the animals randomly divided in to four groups (6 rats/groups) the water is removed one hour before the experiment. First group (vehicle control group) in this group the rats are given vehicle Tween 20 (5% v/v) (5 ml/kg). Second group (ulcer control group) the rats in this group received Tween 20 (5% v/v) (5 ml/kg). Third group (reference drug group) in this group the rats are treated with omeprazole 20 mg/kg solution (5 ml/kg), while at the fourth group the rats are treated with (250 mg/kg) from *T. crispa* stems extract. After the treatment at one hour, all rats are received 98% ethanol at (5 ml/kg) orally to induce gastric ulcer (except the first group). After one hour later all the rats sacrificed by euthanasia, the stomach is removed. Each glandular part weighed and submerged into 1% Alcian blue solution (0.16 M sucrose/0.05 M sodium acetate, pH 5.8). Then submerge for 2 h, the excess stain is rinsed with (10 mL of sucrose at 0.25 M) two consecutive washes, the first time for 15 min and the second for 45 min. Then the stomachs are transmitted to the tubes containing 10 ml of the magnesium chloride at 0.5 M for 30 min. When the 30 min finished, mixed 4 mL of the mixture with 4 mL of ethyl ether and then shack the mixture for 2 min. The final emulsion is centrifuged for (10 min at 3000 rpm) and discarded the supernatant. Read the absorbance at 598 nm. The Calculated the quantity of Alcian blue extracted per gram of glandular tissue.

Tissue homogenate sample preparations for assessment of PGE2, SOD, CAT and MDA

Assessment of PGE2, SOD (Cayman, Cat#. 706002), CAT

Table 1: Effect of *T. crispa*(T. c) on the value of liver biochemical parameters to the female rat.

Groups	Total protein g/l	Albumin g/l	Globulin g/l	Alkaline Phosphatase IU/l	Aspartate Aminotransferase IU/l	Alanine Aminotransferase IU/l
Vehicle control	69.7 ± 2.07	13.3 ± 1.03	56.3 ± 2.7	64.5 ± 7.89	174.3 ± 28.7	38 ± 5.9
T. c 50 mg/kg	69.2 ± 3.9	13 ± 0.9	56.5 ± 3.2	73.5 ± 19.17	179.8 ± 20.6	38.8 ± 4.02
T. c 500 mg/kg	69.3 ± 1.4	14.7 ± 0.5	54.7 ± 1.4	96 ± 14.5*	198.5 ± 21.8	39.7 ± 6.08
T. c 2000 mg/kg	69.7 ± 3.8	14.7 ± 1.9	55 ± 2.2	92.8 ± 19.1*	181 ± 21.8	39.5 ± 6.05

Results are presented at mean ± SD. *Significant p<0.05

Table 2: Effect of *T. crispa*(T. c) on the value of liver biochemical parameters to the male rat.

Groups	Total protein g/l	Albumin g/l	Globulin g/l	Alkaline phosphatase IU/l	Aspartate aminotransferase IU/l	Alanine aminotransferase IU/l
Vehicle control	60 ± 1.8	12 ± 0.89	48.5 ± 1.4	145.92 ± 29.9	171.8 ± 32.95	52.5 ± 1.34
T. c 50 mg/kg	63 ± 4.97	12.7 ± 1.2	50.7 ± 3.8	215.2 ± 70.08*	177.3 ± 15.1	47.08 ± 8.6
T. c 500 mg/kg	63.5 ± 5.3	13.2 ± 1.3	50.7 ± 4.3	257.7 ± 14.4*	197.8 ± 33.6	56 ± 1.5
T. c 2000 mg/kg	63 ± 4.9	13.2 ± 1.3	50.2 ± 4.1	261.8 ± 12.96*	193.08 ± 31.7	56.5 ± 1.8

Results are presented at mean ± SD. *Significant p<0.05

(Cayman Cat#. 707002) and MDA (Cayman Cat#. 10009055) in gastric tissue homogenate. the gastric tissue is weighed, then minced, and homogenized on ice in 5 ml of cold PBS buffer by using of Teflon homogenizer ((Polytron, Heidolph RZR1, Germany). After tissue stomach piece fully homogenize, centrifuge the homogenize mixture at 10.000 x g for 15 min at 4°C. Then the supernatant is collected in sterile tube and kept at (-80°C) until de used.

Preparations of blood sample for assessment of serum gastrin and pepsin

After killing the rat's blood samples are collected in blood tube, allow blood to clot for 30 min at 25°C centrifuge at 2000 x g for15 min at 4°C, then serum is collected and preserve at -80°C will be used.

Measurement of PGE2, gastrin and pepsin by ELISA

Measurement of PGE2 by Sandwich ELISA (Cayman PGE2 assay ELISA kit; Cat# 500141), Gastrin by using Abnova Rat Gastrin ELISA kit (Cat#. KA0319 V.01) and Pepsin by using Cusabio Rat pepsin assay ELISA kit (Cat#. CSB-E 08920r). The assays are performed as per the detailed instruction of the manufacturer. The detection limited of these assays is ≤ 15.6 pg for PGE2, ≤ 78.10 pg for serum Gastrin and ≤ 0.8 ng for serum pepsin.

Measurement of TNF-alpha & TGF-β1 by ELISA

After sacrifice the rats blood samples are collected in blood tube, allow blood to clot for 30 min at 25°C centrifuge at 2000 x g for 15 min at 4°C using refrigerated centrifuge Rotofix 32 (Hettich Zentrifugen, Germany), then serum is collected and preserve at -80°C until be used. Measurement of Rat TNF-alpha by ELISA kit (Thermo Scientific, Cat#. ER3TNFA) & TGFB1 by ELISA kit (Abnova, Cat#. KA0279; version: 04) are performed as per the detailed instruction of the manufacturer. The sensitivity limited of these assays is ≤ 15 pg/ml for TNF-alpha and for TGF-β1 is 7.8 pg/ml.

Statistical analysis

The values communicated as mean ± Standard Division (S.D.). The statistical examination of data is through Post Hoc LSD test (comparing the treated groups with control) using a 5% level of significance.

Results

Acute toxicity

During the 14 days of experimental period, in both sexes of rats was no record of death and *T. crispa* stems extract in all doses (50, 500, and 2000) mg/kg nor appeared any change on the skin and hair, in all the rats. In the groups that treated with *T. crispa*, biochemical tests of the kidney and liver was in the normal level except the elevated the level of alkaline phosphatase significant (P<0.05) at the doses (500, 2000) mg/kg in the female and all doses groups in the male when compare with vehicle control group (Table 1 and 2). In the histological examination (Figure 1,2), the results showed that in the rat administration dose 500 mg/kg and 2000 mg/kg, in spite of the normal hepatic structure but there are few inflammatory cells infiltration associated with mild Congestion in portal area, few blood vessels congested indicate to resolution stage from hepatitis. Based on the circumstances of this severe examination the LD50 value for *T. crispa* is more than 2000 mg/kg body weight.

Gastric lesions

The effect of *T. crispa* in the gastroprotective and the enhancement of the mechanism defense of gastric ulcer are investigated in the

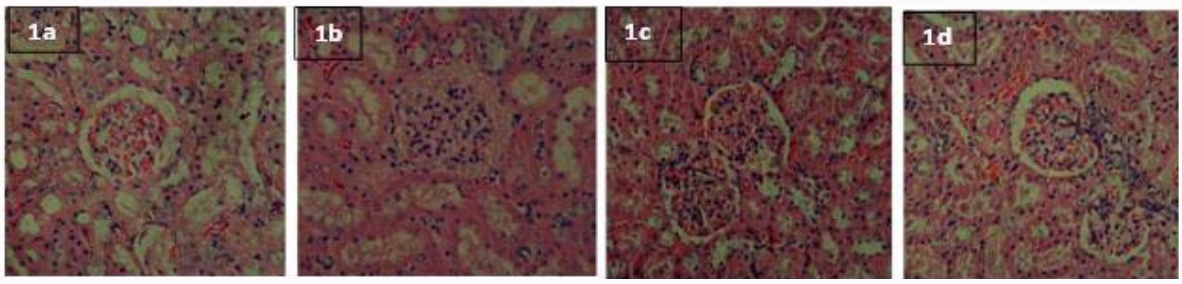

Figure 1: Effect of *T. crispa* on liver and kidney. Rat (1a) treated with 5ml/kg vehicle as control group. Rat (1b) treated with 50 mg/kg; Rat (1c) treated with 50 mg/kg and Rat (1d) treated with 2000mg/kg) of *T. crispa* (hematoxylin and eosin staining 10x).

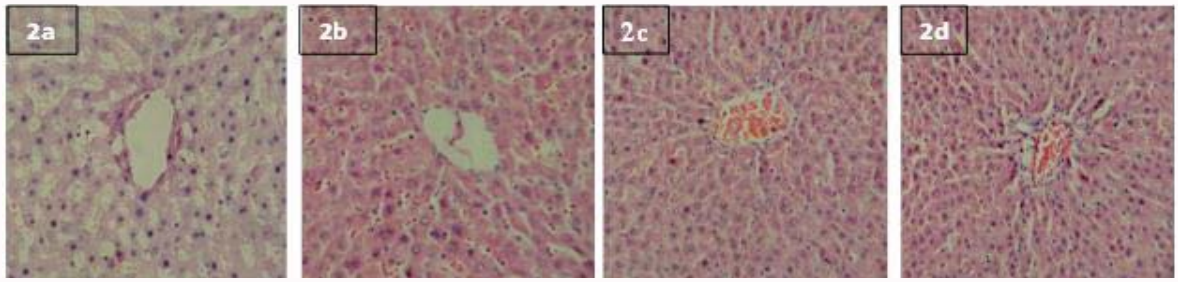

Figure 2: Effect of *T. crispa* on liver and kidney. Rat (2a) treated with 5ml/kg vehicle as control group. Rat (2b) treated with 50 mg/kg; Rat (2c) treated with 50 mg/kg and Rat (2d) treated with 2000 mg/kg) of *T. crispa* (hematoxylin and eosin staining 10x).

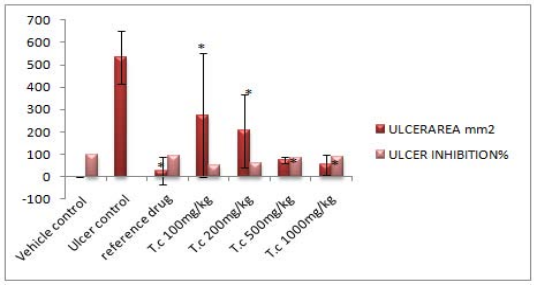

Figure 3: Effect of pretreatment of *T. crispa*[*] (T. c) stems extract on the ulcer area, ulcer inhibition. The data expressed as mean ± SD, * significant p<0.05 compare to the ulcer group.

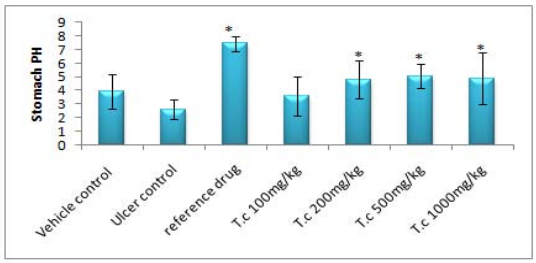

Figure 4: Effect of pretreatment of *T. crispa*[*] (T. c) stems extract on gastric PH. The data expressed as mean ± SD, * significant p<0.05 compare to the ulcer group.

induction of gastric ulcer in the rat by using necrotizing agent as ethanol that results mucosal injury characterized by submucosal edema, increasing secretory products of the cell, disturbances in microcirculation.

Pretreatment with ethanol extract of *T. crispa* stems at dose of 100 mg/kg, 200 mg/kg, 500 mg/kg, 1000 mg/kg or omeprazole at 20 mg/kg inhibited the formation of gastric ulcer lesions in the rats. The intensity of ulcer is detected by ulcer area shows that in the pretreated groups (*T. crispa* or omeprazole) statistically significant (P<0.05) lower than ulcer control group and the ulcer inhibition percentage result is reflected that the pretreatment with all doses of the *T. crispa* stems or omeprazole statistically significant (P<0.05) higher than ulcer control group (Figure 3).

Gastric PH

The gastric PH in experimental groups pretreated with *T. crispa* stems or omeprazole increased significantly compared with the ulcer control group (P<0.05, Figure 4).

Histological estimation of gastric ulcer

Grossly appearance of the rat pretreated with *T. crispa* extract

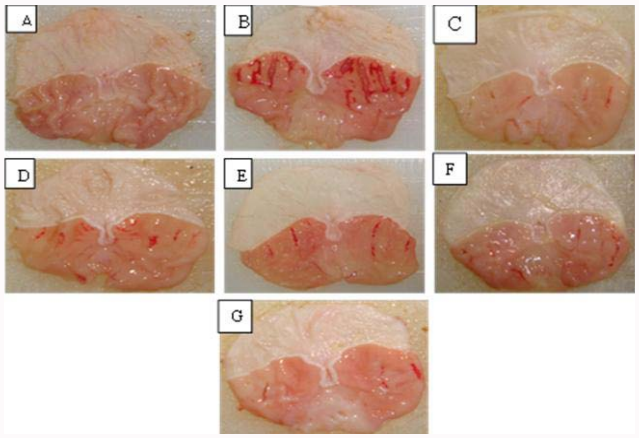

Figure 5: Effect of *T. crispa* on the gross appearance of the gastric mucosa in rats. A) Rats pretreated with 5 ml/kg Teen 20, no injuries to the gastric mucosa are seen. B) Rats pretreated with 5 ml/kg teen 20 + EtOH; severe injuries to the gastric mucosa are seen. EtOH produced extensive visible hemorrhagic necrosis of gastric mucosa. C) Rats pretreated with omeprozole (20 mg/kg); mild injuries are seen in the mucosa layer. D,E) Rats pretreated with *T. crispa* extract (100 mg/kg & 200 mg/kg) consecutively; injuries are seen to the gastric mucosa. F,G) Rats pretreated with *T. crispa* extract (500 mg/kg & 1000 mg/kg) consecutively; mild injuries are seen to the gastric mucosa.

at doses (100 mg/kg, 200 mg/kg) consecutively; injuries are seen in the gastric mucosa, but in the rat pretreated with *T. crispa* extract (500 mg/kg, 1000 mg/kg) consecutively, mild injuries are seen in the gastric mucosa. The extract reduces the formation of gastric lesions induced by ethanol (Figure 5).

The microscopy analysis of the severity of the ulcer lesion presents in (Figure 6) the results show damage throughout the gastric mucosa layer, edema and leukocyte infiltration in the submucosa layer in the ulcer control group. While in the histology section of the stomach rat that pretreated with *T. crispa* extract (100 mg/kg, 200 mg/kg) consecutively; disruption of the surface epithelium mucosa is seen. There is edema and leucocytes infiltration of the submucosal layer. In the rat pretreated with *T. crispa* extract (500 mg/kg, 1000 mg/kg) consecutively; there is mild disruption to the surface epithelium and no edema or leucocytes infiltration of the submucosal layer has better protection from the injury caused by ethanol. This result clearly appears in the reduction of ulcer area, less damage to the gastric mucosa, reduction the edema in the submucosa layer and infiltration leukocyte. This plant shows to extend the gastroprotective effects in a dose-dependent manner.

Mucus barrier of the protective stomach

Mucus barrier content presents in the Figure 7. We find the

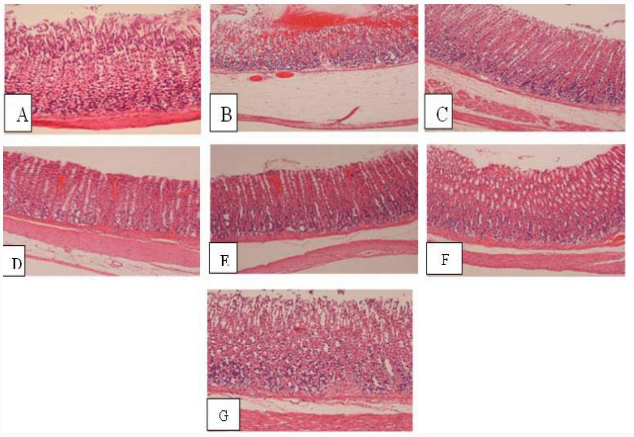

Figure 6: Effect of *T. crispa* on the histological appearance of EtOH induced gastric mucosal damage in rats. (A) Rats pretreated with 5 ml/kg Tween 20. no injuries to the gastric mucosa are seen. B) Rats pretreated with 55 ml/kg tween 20 + EtOH. There is severe disruption to the surface epithelium and necrotic lesions penetrating deeply into mucosa and extensive edema of submucosa layer and leucocytes infiltration is present. C) Rats pretreated with omeprozole (20 mg/kg); mild disruption of the surface epithelium mucosa is seen. D,E) Rats pretreated with *T. crispa* extract (100, 200 mg/kg) consecutively; disruption of the surface epithelium mucosa is seen. There is edema and leucocytes infiltration of the submucosa layer. F) Rats pretreated with *T. crispa* extract (500 mg/kg) consecutively, mild disruption to the surface epithelium and edema, no leucocytes infiltration of the submucosal layer. G) Rats pretreated with *T. crispa* extract (1000 mg/kg) consecutively, mild disruption to the surface epithelium and no edema, nor leucocytes infiltration of the submucosal layer (H&E stain 10x).

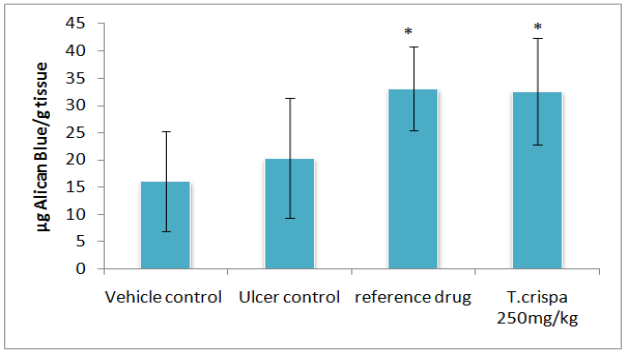

Figure 7: Effect of *T. crispa* extract on mucus content in gastric mucosa of rats. All values expressed as mean ± SD. The mean difference is *significant at the P<0.05 level.

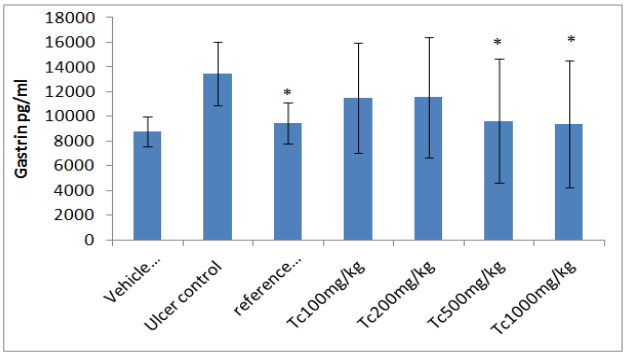

Figure 8: Effect of *T. crispa* (P.m.) on the level of serum Gastrin in ethanol induces gastric ulcer; (6 rats/group). The data was expressed as mean ± SD. * The differences mean between groups significant at P<0.05

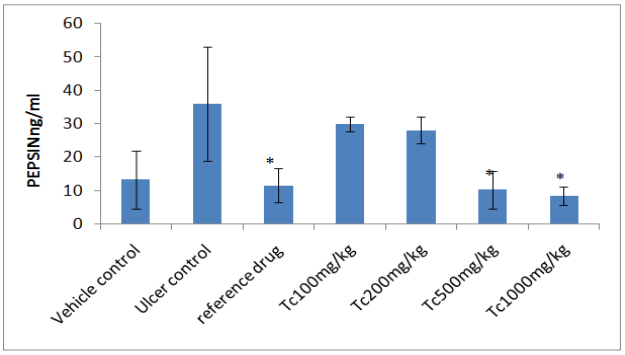

Figure 9: Effect of *T. crispa* (P.m.) on the level of serum Pepsin in ethanol induces gastric ulcer; (6 rats/group). The data was expressed as mean ± SD. * The differences mean between groups significant at P<0.05

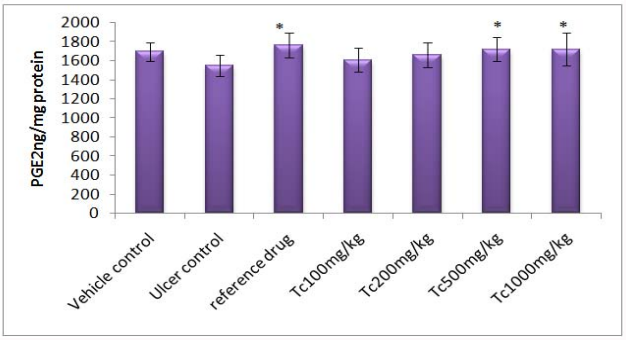

Figure 10: Effect of *T. crispa* (P.m.) on the level of PGE2 in ethanol induces gastric ulcer; (6 rats/group). The data was expressed as mean ± SD. * The differences mean between groups significant at P<0.05

statistically significant (P<0.05) high mucus contents in the pretreated groups with the *T. crispa* extract when compare to reference drug group ulcer control group rats.

Evaluation of gastrin, pepsin and PGE2

In the pretreated groups with *T. crispa* and omeprazole the result shows significant decrease (P<0.05) in the Gastrin (Figure 8) and Pepsin (Figure 9) level when compare with ulcer control group. In addition, in the pretreated dose (500 mg/kg and 1000 mg/kg) of *T. crispa* the gastrin level shows significant decrease more than reference drug group. Besides the elevation of PGE2 level in the pretreated groups with *T. crispa* and omeprazole significantly (P<0.05) when compare with ulcer control group (Figure 10).

Estimation of SOD, CAT and MDA level

The effect of *T. crispa* on the antioxidant enzymes and the role in the gastroprotective was investigated in the pretreated groups. The results are showed the effect of the plant on the level of SOD and CAT significantly (p<0.05) by increasing in the level of SOD and CAT in all pretreated groups when compare with ulcer control group. Moreover, decreasing the level of MDA significantly (p<0.05) in all pretreated groups when compare with ulcer control group (Table 3).

Estimation of TGFB1 and TNF-α

The effect of *T. crispa* on inflammatory cytokine the role in the gastroprotective was investigated in the pretreated groups. The results are showed increasing level of TGFB1 significant (p<0.05) at (500 mg/kg and 1000 mg/kg). In contrast, decreasing the level of TNF-α significantly (p<0.05) in all pretreated groups when compare with

Table 3: Effect of *T. crispa*(P.m.) on the level of SOD, CAT, and MDA in ethanol induces gastric ulcer.

Groups	SOD U/mg protein	CAT nmol/mg protein	MDA umol/mg protein
Vehicle control	190.7 ± 112.1	1088.5 ± 586.9	6.16 ± 2.95
Ulcer control	110.4 ± 71.6	540.1 ± 133.3	21.08 ± 6.97
reference drug	314.9 ± 102.6*	3140.1 ± 579.3*	6.95 ± 3.1*
T. c100 mg/kg	215.1 ± 40.3	1839.2 ± 715.8*	12.04 ± 5.34*
T. c200 mg/kg	224.5 ± 114.3	2824.4 ± 387.95*	10.03 ± 5.3*
T. c500 mg/kg	252.98 ± 148.8*	3381.94 ± 102.75*	8.8 ± 4.4*
T. c1000 mg/kg	292.7 ± 160.6*	3601.2 ± 515.3*	6.6 ± 3.96*

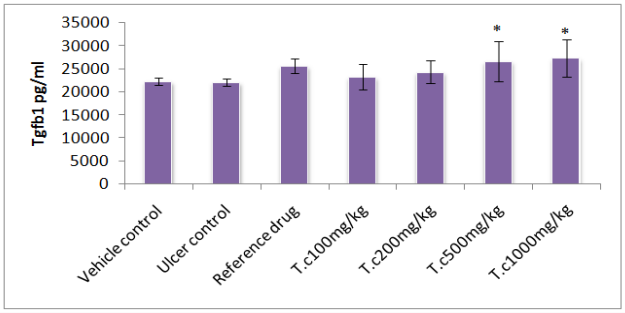

Figure 11: Effect of *T. crispa*(P.m.) on the level of Tgfb1 in ethanol induces gastric ulcer; (6 rats/group). The data was expressed as mean ± SD.
* The differences mean between groups significant at P<0.05

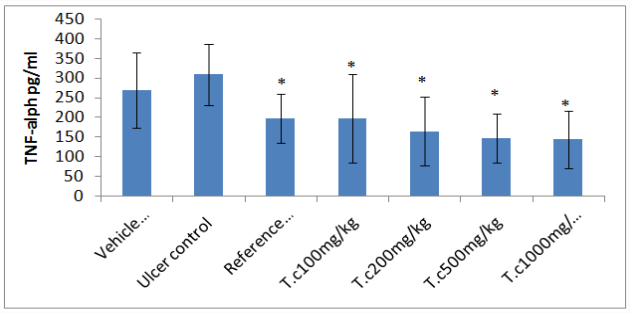

Figure 12: Effect of *T. crispa*(P.m.) on the level of TNF-alpha in ethanol induces gastric ulcer; (6 rats/group). The data was expressed as mean ± SD.
* The differences mean between groups significant at P<0.05

ulcer control group (Figure 11,12).

Discussion

This study showed that the ethanolic extract of *T. crispa* has gastroprotective activity as manifested by its significant inhibition in the formation of ulcers induced by ethanol agent. The injury of ethanol-induced ulcers is dominant in the glandular part of stomach. Administration of absolute ethanol orally in rats is destructive for the stomach, affecting on the gastric mucosa topical by disabling its barrier and exciting in few minutes microvascular changes after its application. A strong and rapid vasoconstriction in combination with vigorous and rapid arteriolar dilation caused induction of damage in mucosal capillaries [15,16]. In the previous study was reported that ethanol stimulates the formation of Leukotriene C4 (LTC4) [17], mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa [18]. The necrotic lesion of the gastric mucosa caused by ethanol happens through multiple factors. It causes disruption of the mucus-bicarbonate barrier when reach to the mucosa and cause cell rupture in the wall of blood vessels. These effects caused probably by biological actions, as formation of free radicals, lipid peroxidation, intracellular oxidative stress, effect on the permeability and depolarization of the mitochondrial membrane before cell death [19].

Gastric ulcer defines as the damage resultant disturbance in the gastric mucosal defense that means ulcer caused by imbalance between offensive and protective factors of the gastric mucosa. Pepsin and gastric acid composed of the aggressive factors whose proteolytic effect is buffered by mucosal glycoprotein, mucin secretion, prostaglandins and cell proliferation [20]. *T. crispa* stems extract decrease ulcer area and increase ulcer inhibition percentage by increasing the production of mucin from mucous cells to form the first line defense to protect the mucosal epithelial layer from ulcerogenic action of ethanol. Mucus is an important factor to protect the gastric mucosa. Mucus is a transparent gel composed of water and glycoproteins that coats the gastrointestinal mucosa and preserves the gastric mucosa against excited agents, such as ethanol and HCl [21]. Moreover, the anti-secretory activity of the *T. crispa* stems extract may be important to protect the gastric mucosa. The gastroprotective effect is proven by histological examination. This protection is result from the inhibition of the gastric acids secretion and rising stomach pH by extract. The obtained data revealed that there was rising the stomach pH when compared with the ulcer control group so that the mechanism of mucosa protection is due to anti-secretory activity of *T. crispa* stems and the effect to enhance production of mucin.

The other findings in this study are pretreatment with *T. crispa* stems extract induced a remarkable decrease in the level of serum gastrin and pepsin, while cause increasing in the PGE2 level when compared with the ulcer control group, thus indicating the action of *T. crispa* stems in more than one antiulcer mechanism. The gastric secretion decreased after *T. crispa* stems extract pretreatment. This may be a negative feedback mechanism that is, increase in mucus and PGE2, caused reducing in the gastrin and pepsin release in rats treated with *T. crispa* stems extract.

It is well- known that the protective mechanism depends on the mucus and bicarbonate secretion, to a large range, on PGE2 secretion, the activity of the Cyclooxygenase (COX) enzyme system play an important role in this function [22]. Our results prove the pre-treatment of rats with *T. crispa* stems extract induced increasing of PGE2 levels compared to rats in the ulcer control group. Therefore, these results give a clear indication for involvement of PGE2 in the gastroprotective mechanism of *T. crispa* stems extract. In the stomach, prostaglandins cause elevation the mucus and bicarbonate secretions, keeping the normal response of the gastric environment to intrinsic factors, and inhibition inflammatory mediator released from mast cells and free radical production [23]. Mention the increasing in the blood flow in the ulcer region suggested that it may be caused

by an increase in PGE2 concentration of in ulcerative regions in comparison to other parts of the gastric mucosa, since PGE2 causes vasodilatation. The supply of blood reveals the active reepithelization, which needs an abundant supply of oxygen and glucose [24]. So that, increasing in the production of PGE2 gained in the treatment with *T. crispa* stems extract that clearly indicated exciting of cyto-protective factors participate acceleration of the healing of gastric ulcer.

Reactive Oxygen Species (ROS) like hydroxyl radical and superoxide radical anion considered one of the major causative factors for mucosal lesions through oxidative stress. ROS plays a main role in tissue injury through the pathogenesis of various disorders of the digestive tract [25].

Lipid Peroxidation (LPO), an important indicator of hydroxyl radical induced oxidative damage of membrane known to play a critical role in the pathogenesis of gastric ulceration. Ethanol caused increase in free radical generation and decrease in endogenous GSH production [26]. Endogenous antioxidant enzymes like Superoxide Dismutase (SOD) and Catalase (CAT), which scavenge these ROS and therefore prevent LPO and tissue damage. However, in pathological conditions like gastric ulceration, ROS may be produced in excess and the delicate balance between ROS and endogenous antioxidants or antioxidant enzymes [7]. In such situations, *T. crispa* can augment the activity of ROS scavenging enzymes, prevent LPO, by act as scavenging the ROS, decrease the offensive effect of ROS, and decrease the LPO in pretreated groups. Therefore, inhibition of LPO and increase in activity of free radical scavenging antioxidant enzymes (SOD and CAT) could also be one of the mechanisms involved in the beneficial effect of *T. crispa* in preventing gastric ulceration.

One of the important finding in this study was decreasing the level of pro-inflammatory factors TNF-α significantly in the pretreated groups with *T. crispa* stems extract and increase the level of anti-inflammatory cytokine TGFB1. These results give an indication to the Immunomodulatory effect of the *T. crispa*, may be by effecting in the inflammatory mediator. The gastrointestinal mucosa integrity depends on the balance between the defensive and offensive factors. Many of the components of mucosal defense have proven to be influenced by inflammatory mediators as prostaglandins, leukotrienes and thromboxanes [6]. Prostaglandins can also play a role as anti-inflammatory effects, through inhibition of leukocyte recruitment, which can contribute to the beneficial effects for these materials in situations, inflamed the gastrointestinal mucosa [27]. TNF-α was pointed out to be a key mediator of the intestinal injury exited by endotoxin One of the mechanisms in which TNF-α can develop inflammatory responses and tissue injury is *via* its possibility to regulate expression of receptors for other inflammatory mediators, including leukotriene B4 and Platelet Activating Factor [28].

Prostaglandins are powerful inhibitors of TNF-α release from both the macrophage [29] and the mast cell [6], and this may the cause in the cases of pretreatment with *T. crispa* caused elevation of the PGE2 level, it is in turn caused the inhibition of the production of the TNF-α. Another Immunomodulatory effect of *T. crispa* is exiting of production of TGFB1 an important cytokine in the accelerating gastric ulcer healing. The healing of Gastric ulcer is a complex process, controlled by many factors, hormones and cytokines. TGFB1 is one of the multifunctional peptide growth factors, has a positive regulate healing of gastric ulcer by induces cell migration, angiogenesis and improves extracellular matrix production. TGF-b exerts its action by binding to its trans-membrane serine/threonine kinase receptors, which in turn triggers activation of various intracellular signaling pathways [30]. In the previous study reported the immune-reactive of TGFB1 protein focus to the epithelial cells, in turn of important in the proliferation of gastric glands [31].

Conclusion

In conclusion, our results proof that *T. crispa* pretreatment has protective effects in ethanol-induced gastric ulcers in rats. Moreover, these results provide evidence that these protective effects of *T. crispa* by stimulation of some inflammatory mediators as PGE2, gastrin, TGFB1 and TNF-α. Moreover, important antioxidant enzymes such as SOD and CAT which are scavengers of ROS and therefore prevent gastric injury induced by them.

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