Research Article

Evaluation of *Canthium parviflorum* on experimentally induced ulcer in rats

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Abstract

Objective: Objective of present research work was to evaluate the effect of *Canthium parviflorum* on experimentally induced ulcer in albino wistar rats. **Materials and methods:** Ethanolic extract of leaves of plant *Canthium parviflorum* (EECP) were investigated for the presence of various phytoconstituents. Antiulcer activity of EECP was carried out by ulcerogenic pylorus ligation, aspirin and cold stress induced rat models. EECP 100, 200 mg/kg and standard drug ranitidine 20 mg/kg body weight dose administered orally 30 min prior to induction of ulcer. Biochemical parameters namely pH, free acidity, volume of gastric juice, mucin, total acidity, pepsin and protein contents were accounted to asses antiulcer activity. Further, Histopathological study of gastric mucosa was carried out. **Results:** Experimental data indicate that EECP reduces the gastric secretion, its free acidity and total acidity level was less, but its pH level was more. It was observed that extract increases the level of protective factor like mucin but reduces the aggressive factor like pepsin dose dependently. **Conclusion:** The present finding suggests that leaves of *Canthium parviflorum* possess anti-ulcer property.

***Keywords*:** *Canthium parviflorum*, antiulcer, antisecretory, ulcer index, ranitidine

Introduction

About 8-10% of world's population is majorly known to suffer with Peptic ulcer. Globally, the prevalence of gastrointestinal disease is almost 40% in the developed countries and 80% in the developing countries countries (Adinortey et al., 2013; Asali et al., 2018). Symptoms mainly are pain at abdomen region, burning sensation, nausea and vomiting. Peptic ulcer is wound like lesion in mucus membrane of gastrointestinal tract (GIT) mainly stomach, intestine or both. Various factors cause ulcer some majorly attributed to stressful modern life style, smoking, alcohol consumption, H-pylori infection and excess usage of drugs namely non-steroidal anti-inflammatory drugs (NSAIDs). Also due to less secretion of protective factors like prostaglandins (PGs), bicarbonate, nitric oxide, antioxidants, mucus and less resistance of mucosal cells (Tripathi, 2011; Narayan et al., 2004; Lanas, 2017). All above mentioned factors leads to high secretion of gastric acid, pepsin, lipid peroxidation and formation of reactive oxygen species (ROS).

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Current treatment for peptic ulcer involves drugs namely antihistamines (H_2 blockers), proton pump inhibitors, anticholinergics, antacids, ulcer protectives and anti-H pylori. Most of these drugs have some drawbacks, H_2 antihistamines bioavailability was less and adverse effects are dizziness, bowel upset and rashes. Proton pump inhibitors have adverse effect like loose stool, abdominal pain, joint pain and dizziness. Antacids not inhibit release of acid but cause acid rebound and bowel upset. Ulcer protectives have side effects like constipation and dry mouth (Tripathi, 2011).

The *Canthium parviflorum* Lam. Belongs to family Rubiaceae, a small shrub and woody plant, grows up to 3 meters in height is widely distributed throughout India and Asia (Magadi, 2001). Plant has been used traditionally as laxative, wound healing, antidysenteric, gastrointestinal disorder and constipation (Harihar NS 2012; Ayyanar et al. 2008; Mohideen et al., 2003). Phytoconstituents like flavonoids, tannins, saponins and terpenoids present in other plants reported for antiulcer activity (Gadekar et al., 2010). Hence, the present study was planned with an objective of evaluating antiulcer activity of ethanolic extract of *Canthium parviflorum* in models namely pylorus ligation, NSAIDs (aspirin) and cold stress induced ulcer on experimental Wister Albino rats.

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Materials and methods

Drugs and chemicals

Ranitidine (Kopran Pharma Ltd., Mumbai, India), Aspirin (SIGMA Chemicals co (St Louis, MO, USA)) other reagents, chemicals and solvents of analytical grade were obtained.

Source of crude drug

Fresh plant leaves of *Canthium parviflorum* were collected from terrestrial area of Raichur, Karnataka, India. Identification and authentication were obtained from Dr. B. S. Angadi Department of Botany, P.C. Jabin Science College, Vidyanagar, Hubballi, Karnataka.

Extraction of *Canthium parviflorum* leaves

The fresh shade dried leaves was pulverized with help of mortar and pestle into a coarse powder before grinding into fine particles. Briefly, 100 gm of powdered leaves were extracted by hot Soxhlet method using 250 ml of 70% ethanol. The extract was filtered and dried on hot water bath. The dried material was weighed and stored in air tied container and used for further study (World Health Organization 1998).

Preliminary phytochemical investigation of ethanolic extract

Preliminary phytochemical screening was carried out to check the existence of various phytoconstituents like steroids, triterpenoids, alkaloids, flavonoids, polyphenols, carbohydrates, tannins, saponins and glycosides (Harborne, 2009).

Animals

Wistar albino rats of both sex (150 to 200 gm) were procured from KLE College of Pharmacy, Hubballi. All animals acclimatized in laboratory for seven days in standard conditions. Animals kept in polypropylene cage and maintain in an environmentally controlled room (temperature 25 ± 2^0C) with 24 hour dark/light cycle. Animals were get standard feed and sufficient water. Ethical clearance was taken from the institutional animal's ethical committee (06/KLEUSCOPH/16).

Acute oral toxicity studies

The acute oral toxicity study for the ethanolic extract of *Canthium parviflorum* was done using mice as per Organization for Economic Co-operation and Development (OECD, 423). The female mice (18-25 gm) were used for the present investigation. Rats fasted overnight prior to administration of extract. After fasting, they were weighed and kept in separate groups each containing three mice. Doses were prepared using distilled water and tween 80 (1%), each animal EECP was given orally and the dose was 2 gm/kg body weight. After administration food was not given for up to 4 hour. The animals were observed for any changes for the first 30 min, 1, 2, 3, 4 and 24 hour once daily for 14 days. Animals were examined for mortality and behavioral changes.

Preparation of drug solution

Ranitidine was dissolved in cold citrate buffer (pH 4.5), EECP solution were prepared freshly in 1% Tween 80 and used for the study.

Experimental procedure

The animals separated into 3 groups of 24 each, for pylorus ligation, cold stress and aspirin induced gastric ulcers. All 3 groups was further divided into 4 subgroups, each consists 6 rats.

Pylorus ligation induced ulcer

The ulcer was induced as per method of Shay (Dai et al. 1973). Animals were fasted for 24 hour and divided into four group each consisting of 6 animals. Control Group I received only normal saline (p.o.), Group II animals received Ranitidine (20 mg/kg., p.o.)(Bhajoni et al., 2016). Group III and IV received EECP (100 mg/kg) and (200 mg/kg., p.o.) respectively. Following 1 hour of administration, pylorus ligation was done by a midline abdominal incision under the anesthesia (35 mg/kg., b.w.). Food and water were not given after ligation. Rats were sacrificed after 3h by over dose of ether anesthesia, stomach was separated and opened at the grater curvature and ulcer scoring was done (Yusuf et al., 2004). Gastric juice was taken, to estimate acid volume, pH, free and total acidity, ulcer index, mucin, total protein and pepsin (Deshpande et al., 2003). Estimations were done according to the standard procedures.

***Macroscopic evaluation of stomach*:** The stomach opened at greater curvature, gastric content was collected and cleaned using saline water. Tested for blood clots and scored by 10X magnification. The total mucosal, ulcer area and severity was observed (Robert 1979; Melese et al., 2011). Ulcer was scored as follows (Dashputre et al., 2011).

Formula to calculate Ulcer Index (UI):

$UI = 10/x$

x = Total mucosal area / ulcer area

Table 1. Ulcer index

Sl. No.	Evaluation	Ulcer scores
1	Normal color stomach	0
2	Red coloration	0.5
3	Spot ulcer	1
4	Hemorrhagic streak	1.5
5	Deep ulcers	2
6	Perforation	3

Formula to calculate Percentage inhibition (PI) of ulcer formation:

$$\% \text{ Inhibition of ulceration} = \frac{\text{UI (control group)} - \text{UI (test group)}}{\text{UI (control group)}} \times 100$$

Biochemical Estimations

Volume of gastric contents

Gastric juice was taken, centrifuged at 3000 rpm at 25°C for the period of 10 min and measured in milliliters.

Determination of pH

One ml distilled water added to equal quantity of gastric juice aliquot then the digital pH meter was used to measure pH of the solution (Dashputre et al., 2011).

Determination of total acidity (Dashputre et al., 2011)

In a conical flask (50 ml) take 1 ml of gastric juice and equal quantity of distilled water, add phenolphthalein. Mixture titrated using 0.01N NaOH till color pink seen. The quantity of 0.01N NaOH added was recorded. Formula to calculate total acidity:

$$\% \text{ Inhibition of ulceration} = \frac{\text{UI (control group)} - \text{UI (test group)}}{\text{UI (control group)}} \times 100$$

mEq/L = Milliequivalents Per Litre

Estimation of total protein

The dissolved protein in gastric juice estimated by adding 90% alcohol at 9:1 ratio respectively to get precipitate. Further, to 0.1 ml of precipitate added 1ml of 0.1 N NaOH and take 0.05 ml into separate tube, add 4 ml copper reagent, 10 min later of phenol reagents (0.4 ml) added and wait for 10 min to develop color. Absorbance read at 610 nm by spectrophotometer. Protein level was calculated from the standard curve prepared with bovine albumin, it was expressed as µg/ml of gastric juice (Lowry et al., 1951).

Estimation of pepsin

Centrifuged gastric content (0.1 ml) was added to of bovine albumin (1 ml) and kept for 20 min. at 37°C. Gastric juice with 1 ml of 0.01 N HCl used as blank. Reaction stopped by adding 2 ml of 10% trichloroacetic acid. All tubes heated then cooled for 5 min. to get precipitate; it was removed by centrifugation (9000 × gm for 10 min.). To 1 ml supernatant add of 2.5 N NaOH (0.4 ml) and Folin reagent (0.1 ml), then adjust the volume to 10 ml using distilled water. The absorbance read at 700 nm, then pepsin level expressed as µg/ml of gastric juice (Anson, 1938).

Estimation of mucin

Gastric juice collected, glandular portion excised then opened down along the lesser curvature. The stomach was saturated in 0.1% alcian blue for 2 hour. The dye was detached by two successive washing at 15 and 45 min using 0.25M sucrose solution. The dye with mucus was diluted by immersion in of 0.5M magnesium chloride (10 ml) for 2 h. Blue solution obtained was shaken with same quantity of diethyl ether then absorbance of aqueous phase was read at 605 nm. The mucin content calculated with the standard curve, expressed in microgram/gram of wet tissue (Corne, 1974).

Aspirin induced gastric ulcer model

Aspirin is suspended in 1% carboxymethyl cellulose in water and administered at a dose of 200 mg/kg., b.w., orally to induce ulcer (Williamson and Okpako, 1986). All 24 animals were fasted for 24 hour and divided in to four groups (n=6 in each group). In addition to aspirin; Group I animals treated with saline water, Group II treated with ranitidine (20 mg/kg., p.o.), Group III and IV treated with EECP 100 mg/kg and 200 mg/kg respectively daily once for 7 days. The treatment was given 30 min before the aspirin. On 8th day all group animals fasted for 18 hour and sacrificed. Further, the stomach was removed and opened along the grater curvature, gastric contents were collected and used to measure various biochemical parameters (Robert 1979).

Cold restraint stress-induced ulcer model

The experiment was carried out as described in standard procedures (Vincent et al., 1977). Animals were fasted for 24 hour and divided in to four groups, each group having 6 animals. Control Group I animals received saline water as equal volume of drug, Group II received ranitidine (20 mg/kg) as the reference drugs. Group III and IV were received EECP 100 and 200 mg/kg respectively. After administration for 5 days, animals fasted for 12 hour then made unable to move in a stress cage (steel cage), temperature maintained 3-5°C kept for 3 hour. After these animals were sacrificed, stomachs were collected and ulcer index calculated as described in earlier methods (Deshpande et al. 2003).

Histopathology study

The animals sacrificed and stomachs were separated, washed using saline and kept in 10% formaldehyde solution for histopathological examination. The staining was carried out by using hematoxylin and Eosin (H&E) and assessed for mucosal damage, oedema, bleeding and necrosis.

Statistical analysis

The experimental data were analyzed statistically by one-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison test. All data were expressed as mean ± S.E.M. (standard error of mean). Every group has six animals (n=6). The significant difference was expressed by *p* values, if *p* value results were <0.05 then it was considered statistically significant. Statistical analysis

of data was computed by using Graph Pad Prism Software version 5.1.

Results

Preliminary phytochemical investigations

The phytochemical investigation reveals the presence of various phytoconstituents like Carbohydrates, Steroids, Triterpenoids, Glycosides, Saponins, Flavonoids, Tannins, Phenolic Compounds and Alkaloids in the ethanolic extract of leaves of the plant *Canthium Parviflorum.*

Pharmacological evaluation of ethanolic extract of *Canthium parviflorum*

Acute Oral Toxicity studies of EEPL & EECP

After administration ethanolic extract of *Canthium parviflorum* at 2000 mg/kg body weight orally to female mice, showed no changes in behavior, side effects and there was no mortality. Hence the extract was considered as safe. Therefore, $1/10^{th}$ (200mg/kg) and $1/20^{th}$ (100 mg/kg) of maximum tolerable dose were considered for the study.

Effect of EECP on ulcer induced by Pylorus ligation in rats

In the pyloric ligation induced ulcer model, control group animals showed significant increase in ulcer index, oral administration of EECP (100 and 200mg/kg) showed significant reduction in ulcer index, gastric volume, free acidity, total acidity, total protein and pepsin, but significant increase in mucin content and pH of the gastric juice was observed as compared to the control group. The mean ulcer index of control group was 7.85 this indicating the ulcerogenic effect of pylorus ligation. The ulcer index of extract 100 and 200 mg/kg were 3.35 and 1.58 respectively indicate that EECP showed protective effect. The percentage protection of EECP 100, 200mg/kg and ranitidine was 57.32, 79.87 and 85.60 respectively (Table 3).

Effect of EECP on aspirin induced ulcer in rats

Aspirin administered to induce ulcer in animals. The mean ulcer index was 3.57 in control group animals indicating its ulcerogenic effect. Pretreatment by oral administration of EECP (100 and 200mg/kg) and ranitidine showed significant reduction in ulcer index, that is 1.57, 1.17 and 1.07 respectively as compare to control group. Extract showed dose dependent reduction of ulcer index. The percentage protection of EECP 100, 200mg/kg and ranitidine was 56.02, 67.22 and 70.02 respectively. The results indicate that the high dose of EECP i.e., 200 mg/kg has more protective effect in aspirin induced ulcer (Table 4).

Effect of EECP on cold stress induced ulcer in rats

The ulcer in animals was induced by cold stress that is hypothermic stress induced ulcer model. The mean ulcer index was 3.48 in control group animals indicating its ulcerogenic effect. Pretreatment by oral administration of EECP (100 and 200mg/kg) and ranitidine showed significant reduction in ulcer index, that is 1.65, 1.37 and 1.15 respectively as compare to control group. Extract showed dose dependent reduction of ulcer index. The percentage protection of EECP 100, 200mg/kg and ranitidine was 51.85, 59.46 and 65.57 respectively. The results indicate that the high dose of EECP i.e., 200 mg/kg has more protective effect and results were comparable to ranitidine (Table 5).

Table 2. Phytochemical screening of ethanolic extract of *Canthium Parviflorum* leaves

Chemical constituents	*Canthium Parviflorum* extract
Carbohydrates	+
Proteins	-
Amino Acids	-
Steroids	+
Triterpenoids	+
Glycosides	+
Saponins	+
Flavonoids	+
Tannins	+
Phenolic Compounds	+
Alkaloids	+

Note: '+' = Present; '-' = Absent.

Table 3. Effect of EECP on gastric acid secretion and ulcer index in pylorus ligation induced ulcer

Sl. No.	Treatment	Ulcer index	% protection	Volume of gastric juice (mL)	pH	Free acidity (mEq/lt)	Total acidity (mEq/lt)
1	Control	7.85±0.47	—	5.27±0.60	2.80±0.09	30.33± 1.54	55.67±3.03
2	Ranitidine (20 mg/kg)	1.13±0.21***	85.60%	2.45±0.28***	4.42±0.18***	11.33±1.05***	24.17±1.58***
3	EECP (100 mg/kg)	3.35±0.59***	57.32%	3.62±0.32*	3.40±0.13**	19.67±2.62**	31.83±3.27***
4	EECP (200 mg/kg)	1.58±0.42***	79.87%	2.93±0.33**	3.63±0.07***	12.50±1.59***	25.00±1.44***

Table 4. Effect of EECP on mucin, total protein and pepsin levels in pylorus ligation induced ulcer

Sl. No.	Treatment	Mucin (mcg/g)	Total protein (µg/ml)	Pepsin (µg/ml)
1	Control	255.17±7.52	348.17±1.92	11.57±034
2	Ranitidine (20 mg/kg)	320.17±6.58***	315.00±3.54***	5.22±0.15***
3	EECP (100 mg/kg)	289.3±3.56**	331.00±4.51**	6.55±0.09***
4	EECP (200 mg/kg)	302.7±4.06***	335.00±3.41**	6.16±0.23***

Table 5. Effect of EECP on ulcer index and percentage protection in aspirin and cold stress induced ulcer

Sl. No.	Treatment	Ulcer index		Percentage protection	
		Aspirin	Cold stress	Aspirin	Cold stress
1	Control	3.57 ± 0.15	3.48 ± 0.15	—	—
2	Ranitidine (20 mg/kg)	1.07 ± 0.20***	1.15 ± 0.20***	70.02%	65.57%
3	EECP (100 mg/kg)	1.57 ± 0.28***	1.65 ± 0.28***	56.02%	51.85%
4	EECP (200 mg/kg)	1.17 ± 0.25***	1.37 ± 0.25***	67.22%	59.46%

Effect of EECP on histopathology of stomach in pylorus ligation induced ulcer

Inflammation, redness, hemorrhage and ulcers were seen in stomach mucosal layer of control group animals. Ranitidine administered rats gastric mucosa was normal. Pretreatment with EECP (100 and 200mg/kg) significantly reduces disruption of mucosa and development of ulcer dose dependently. Black arrows represent severe disruption of surface epithelium and proliferate of gastric mucosa and red arrows represent the hemorrhagic of mucosa (Figure 1).

Discussion

The research was planned to evaluate the antiulcer activity of ethanolic extract of leaves of plant *Canthium parviflorum* on pylorus ligation, cold stress and aspirin induced gastric ulcer models. The phytoconstituents present in ethanolic extract were carbohydrates, steroids, triterpenoids, glycosides, saponins, flavonoids, tannins, phenolic compounds and alkaloids (Table 2) similar as that of previous study reported (Jeedi et al. 2016). The acute oral toxicity study showed that extract has no side effects, mortality and safe in animals at 2000 mg/kg. Median lethal (LD50) dose of the ethanol extract is more than 2000 mg/kg. The study results support the research carried previously. In the mucosal layer of stomach prostaglandin level elevated by flavonoids and protects against aggressive factors. Flavonoids inhibiting histamine mediated acid secretion, by suppressing formation of histamine in mast cells by inhibiting enzyme histidine decarboxylase. Mucosal layer protected by suppression of Helicobacter pylori, H+/K+-ATPase also from reactive oxygen species (Borrelli et al., 2000). Saponins, tannins, terpenoids and alkaloid protect mucosal layer by different mechanism are useful to treat ulcer (Klein-Júnior et al., 2012). In this study the evaluation of antisecretory and anti-ulcer activity of EECP was done by pyloric ligation, aspirin and cold stress induced ulcer models in experimental animals. The main cause for development of ulcer in stomach is due to reduced protective and increased aggressive factor like mucin and acid respectively.

Pylorus ligation study results, control group animals with high ulcer index are mainly due to high gastric juice, pH, pepsin, free and total acidity, but significant reduction in the level of mucin (Table 3 and 4). In control group animal's stomach acid gets accumulated leads to severe disruption, digestion of epithelial layer and hemorrhage, perforations in mucosa due to high acid and less mucin (Raju et al., 2009) (Figure 1 (A)). In ranitidine administered group animals gastric mucosa is normal because it inhibits acid secretion and pepsin (Deshpande et al., 2003). Increased mucin level also contributes in normalization of gastric mucosa (Figure 1 (B)). In extract administered group animal's dose dependent improvement were observed, this is due to less acid, pepsin secretion, raised pH and improvement in mucin content. This clearly indicates that gastric parietal and chief cells are suppressed, mucosal layer protected by increasing mucin level by the extract dose dependently. The extract possesses anti-ulcerogenic effect due to its antisecretory and cytoprotective property (Table 3 and 4; Figure 1 (C, D)).

Aspirin a NSAID which is a prostaglandin synthatase inhibitor causes ulcer by preventing prostaglandin synthesis,

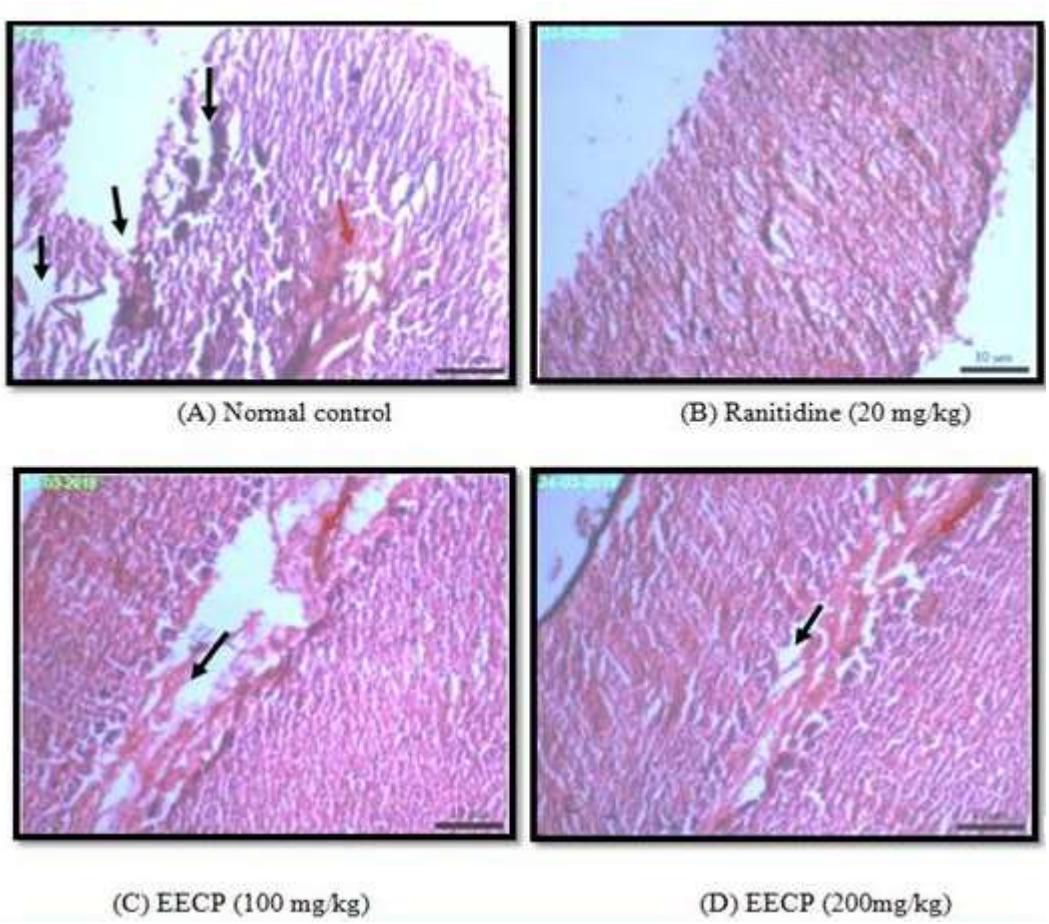

Figure 1. Histopathology of rat stomach mucosal layer in pylorus ligation induced ulcer model. **(A)** Normal control rats: severe damage, ulcer and inflammation to mucosal layer. **(B)** Ranitidine (20mg/kg) treated: gastric mucosa was appearing normal no significant changes **(C)** EECP (100mg/kg): less damage, ulcer and inflammation to mucosal layer compare to normal control. **(D)** EECP (200mg/kg): gastric mucosa was appearing normal, similar as compare to standard drug treated group. Black arrow represents severe disruption of surface epithelium and proliferates of gastric mucosa and red arrow represent the hemorrhagic of mucosa.

increasing acid, pepsin and free radical formation also by reducing mucin and bicarbonate levels in stomach. This may leads to altered permeability and damage of mucosal layer of the stomach (Salim, 1990). Significant increase in gastric acid, pepsin but decrease in the mucin level was observed in control group animals. This is responsible for damage to mucosal layer of stomach and hemorrhage. Extract administered animal's stomach histology and gastric contents were near to normal, protective effect was dose dependent. This may be due to free radicals scavenging, stimulation of mucus neck cells and suppression of parietal cells (Table 5).

Ulcer also developed due to cold stress, it activate autonomic nervous system makes constriction of gastric arteries causes hypoxia, this leads to generation of free radicals (Bandyopadhyay et al., 2002). Accumulation of hydrogen peroxide inhibits prostaglandin synthesis. The results suggest that in the cold stress model, EECP administration significantly suppresses the ulcer formation as compared to control. Structure of mucosal layer was normal, no hemorrhage and ulcer index was very less in extract administered animals indicates its protective effect against stress induced ulcer (Table 5). Flavonoids like antioxidants present in the extract protected mucosal layer against reactive oxygen species and facilitate synthesis of prostaglandins.

Conclusion

In this study ethanolic extract of *Canthium parviflorum* leaves showed antiulcer activity, against pylorus ligation, aspirin and cold stress induced ulcer in experimental Wistar albino rats. Antiulcer activity is mainly due to antisecretory action, stimulation of mucus neck cells and antioxidant property. The activity due to phytoconstituents present in the extract.

Conflicts of interest

There are no conflicts of interest.

References

Adinortey MB, Ansah C, Galyuon I, Nyarko A. 2013. In Vivo Models Used for Evaluation of Potential

Antigastroduodenal Ulcer Agents. Ulcers 13(1): p.1–12.

Anson ML. 1938. The estimation of pepsin, trypsin, papain and catepsin with haemoglobin. Journal of General Physiology 22(1):78–9.

Ayyanar M, Sankarasivaraman K, Ignacimuthu S. 2008. Traditional Herbal Medicines Used for the Treatment of Diabetes among Two Major Tribal Groups in South Tamil Nadu, India. Ethnobotanical Leaflets 12(1): 276–280.

Bandyopadhyay D, Biswas K, Bhattacharyya M, Reiter RJ, Banerjee RK. 2002. Involvement of reactive oxygen species in gastric ulceration: Protection by melatonin. Indian Journal of Experimental Biology 40(6): 693–705.

Bhajoni PS, Meshram GG, Lahkar M. 2016. Evaluation of the Antiulcer Activity of the Leaves of Azadirachta indica: An Experimental Study. Integrative Medicine International 16(3): 10–16.

Borrelli F, Izzo AA. 2000. The plant kingdom as a source of anti-ulcer remedies. Phytotherapy Research 14(8):581–91.

Corne SJ, W.R. 1974. A method for the quantitative estimation of gastric barrier mucus. J Physiol. 242(2): p.116–7.

Dai S, Ogle CW. 1973. A simple method for the production of peptic ulceration in the rat. Life Sciences 12(11): 505–512.

Dashputre NL, Naikwade NS. 2011. Evaluation of Anti-Ulcer Activity of Methanolic Extract of Abutilon indicum Linn Leaves in Experimental Rats. International Journal of Pharmaceutical Sciences and Drug Research 3(2): 97–100.

Deshpande SS, Shah GB, Parmar NS. 2003. Antiulcer activity of Tephrosia purpurea in rats. Indian Journal of Pharmacognosy 35(3): 168–172.

Gadekar R, Singour PK, Chaurasiya PK, Pawar RS, Patil UK. 2010. A potential of some medicinal plants as an antiulcer agents. Pharmacognosy Reviews 4(8): 136–146.

Harborne J. 2009. Phytochemical Methods, A guide to modern techniques of plant analysis 2nd ed. Springer Netherlands.

Harihar NS. 2012. Wild medicinal plants of kappat hills, Gadag District Karnataka. Life sciences leaflets 5(1): 37–42.

Jeedi NM, Koti BC. 2016. Antidiabetic and Antihyperlipidemic Activity of Canthium parviflorum Extracts in Streptozotocin-Induced Diabetic Rats. RGUHS Journal of Pharmaceutical Sciences 8(4): 708–715.

Klein-Júnior LC, Santin JR, Niero R, De Andrade SF, Cechinel-Filho V. 2012. The therapeutic lead potential of metabolites obtained from natural sources for the treatment of peptic ulcer. Phytochemistry Reviews 11(4): 567–616.

Lanas ACF. 2017. Peptic ulcer disease. Lancet 390(10094): 613–624.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. The Journal of biological chemistry 193(1): 265–75.

Asali MA. 2018. Risk factors leading to peptic ulcer disease: systematic review in literature. International Journal of Community Medicine and Public Health 5(10): 4617–4624.

Magadi RG. 2001. Botanical and Vernacular Names of South Indian Plants. Bangalore: Divya Chandra Prakashan, Bangalore.

Melese E, Asres K, Asad M, Engidawork E. 2011. Evaluation of the antipeptic ulcer activity of the leaf extract of Plantago lanceolata L. in rodents. Phytotherapy Research 25(8): 1174–80.

Mohideen S, Ilavarasan R, Hemalatha S, Anitha N, Sasikala E. 2003. Wound healing and diuretic activities of Canthium parviflorum lam. Natural Product Sciences 9(2): 102–104.

Narayan S, Devi RS, Jainu M, Sabitha KE. 2004. Protective effect of polyhedral drug, ambrex in ethanol-induced gastric mucosal lesion in experimental rats. Indian Journal of Pharmacology 36(1): 34–37.

OECD. 2011. OECD Guidelines for the testing of Chemicals. OECD Publishing.

Raju D, Ilango K, Chitra V, Ashish K. 2009. Evaluation of anti-ulcer activity of methanolic extract of terminalia chebula fruits in experimental rats. Journal of Pharmaceutical Sciences and Research 1(3): 101–107.

Robert A. 1979. Cytoprotection by prostaglandins. Gastroenterology 77(4): 761–767.

Salim AS. 1990. Removing oxygen-derived free radicals stimulates healing of ethanol-induced erosive gastritis in the rat. Digestion 47(1): 24–28.

Tripathi KD. 2011. Essential of medical pharmacology 7th ed. New Delhi: Japee brothers medical publishers (p) Ltd.

Vincent GP, Glavin GB, Rutkowski JL. 1977. Body orientation, food deprivation and potentiation of restraint induced gastric lesions. Gastroenterol Clin Biol 1(6): 539–43.

Williamson E, Okpako D. 1986. Pharmacological Methods in Phytotherapy Research Chinchester.

World Health Organization. 1998. Quality control methods for medicinal plant materials Illustrate. World Health Organization, Geneva.

Yusuf S, Agunu A, Diana M. 2004. The effect of Aloe vera A. Berger (Liliaceae) on gastric acid secretion and acute gastric mucosal injury in rats. Journal of Ethnopharmacology 93(1): 33–37.