Advances in Bioresearch
Adv. Biores., Vol 14 (5) September 2023: 275-282

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.5.275282

Advances in Bioresearch

ORIGINAL ARTICLE

Leaves of Curly Kale Aqueous Extract Ameliorates the Anti-Ulcer Efficacy in Rats with Experimentally Induced Ulcers

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ABSTRACT
Peptic ulcers are abscess that extend innermost encircling the abdomen along with uppermost fraction of small intestine. Leaves of Curly Kale showed antioxidant, cardioprotective, neuroprotective, anti-inflammatory and anti-tumor effect. The goal of the study is to determine whether the Kale aqueous extract has any protective effects contrary to ethanol and swim stress breed stomach ulcers in rats. Amid evaluating Curly Kale, the active constituents reported the latency of alkaloids, glycosides, carbohydrates, flavonoids and tannins. The extract was examined prior to the dosage of 2000 mg/kg. The ethanol [1ml/kg p.o.] and swim stress model for 3hrs induced gastric ulcer in rats exhibited substantial decline in free acidity, total acidity, ulcer index, ulcer score and C-reactive protein compared to control group and standard drug ranitidine [150 mgs/kg p.o.] at dosage [250 and 500 mgs/kg p.o.] using aqueous extract leaves of Curly Kale. Significantly increasing gastric pH and percentage of ulcer protection than compared to positive control group and standard drug ranitidine. The aqueous extract of Curly kale leaves defends gastric mucosal damage resulting in an inhibitory effect on discharge of gastric hydrochloric acid by minimizing possible side effects and found to be an effective anti-ulcerogenic agent.
Key words: Curly Kale, Ranitidine, Ulcer index, Determination of pH, Percentage of ulcer protection, C-reactive protein.

Received 22.05.2023 Revised 11.06.2023 Accepted 24.08.2023

How to cite this article:
Seema BR, Jyothi. Y, Yamini BR, Chethan Kumar N Bhovi, Vivek Aradhya MV, Yashas R Kumar. Leaves of Curly Kale Aqueous Extract Ameliorates the Anti-Ulcer Efficacy in Rats with Experimentally Induced Ulcers. Adv. Biores., Vol 12 (5) September 2023: 275-282.

INTRODUCTION

Peptic ulceration is an extensive condition of gastrointestinal system affecting world population by 10% with distinct aetiology. pH of gastric juice remain constant and mucosal aegis must decline for an abscess to form. Two major factors reducing the mucosal dissent to impairment are NSAIDs and infection by *H. pylori* [1-2]. Tendency of peptic ulcers is descending perhaps as an outcome of the escalation usage of PPIs and declining status of *infection* caused by *H. pylori* [3-4]. The presently accessible medications for ulcers are capable to amend the indication of the disease beyond procrastinating its betterment though antiulcer drugs like pantoprazole effect is 74.8% and ranitidine effect is 44.7% [5] and also involved with other complications like constipation, diarrhoea and hypersensitivity immune response provoked by PPI fever with leukocytes and a transitory raise in CRP [6]. Hence, herbal drugs diminish the degrading factors and are found to be safe and clinically efficacious, having improved patient indulgence, proportionately limited cost and extensively combative. A green leafy vegetable Curly Kale belongs to the folk Brassicaceae. It is prominent in Southern United States merely too in Northern Asia and in Europe. Major constituents reported are glucosinolates, flavonoids and carotenoids [7]. Kale is loaded with powerful antioxidants like quercetin and kaempferol. Other constituents like Vitamin C, alimentary fiber and ash [8-10]. Curly Kale possesses cardioprotective, neuroprotective, anti-inflammatory, anti-hypertensive, anti-hypercholesterolemic, anti-diabetic and anti-tumor effects [11-13]. Kaempferol and quercetin in Curly Kale exhibited to be active in treating ulcers [14-16] by protecting the stomach by hindering neutrophil inflation modulating pro-inflammatory cytokine extent and enhancing NO management to sustain gastric mucosal

glycoprotein levels [17]. The present study was designed and gastric ulcer induced against ethanol and swim stress model in rats to determine whether the leaves of Curly Kale aqueous extract are effective.

MATERIAL AND METHODS

Preparation of plant extract

Kale leaves were collected from Urbana superfoods and farms ventures and washed under tap water, chopped into tiny slice and dried at ambient temperature (28±1ºC) for 2 weeks and blended to fine powder and stored in airtight bottles. The taxonomist at ICAR, Hessarghatta lake post, Bengaluru-560089 identified Curly Kale (*Brassica oleracea var sabellica*) family Brassicaceae.

Preliminary phytochemical studies

Preliminary phytochemical analysis was undertaken as per definitive methods for the comparative assimilations of phytoconstituents [18-19].

Experimental animals

The animals used were Albino Wistar rats weighing 200-250g. The experimental methodology that the animals are using has received IAEC approval are housed in the College's animal house under standard circumstances, Krupanidhi College of Pharmacy, Bangalore. Maintaining temperature (23±2^{0}C) and humidity; receiving 12h light (7.00am-7.00pm). They were kept in wire mesh cages, fed on nutritional pellets, and given drinking water *ad libitum*. The care of the animals complied with National and Institutional Guidelines for Animal welfare protection. IAEC (Institutional animal ethics committee) of CPCSEA provided ethical committee approval (Ref. No.378/PO/ReBi/S/01/CPCSEA).

Acute Toxicity study in rats (LD_{50} determination)

Female wistar rats were chosen for study of acute oral toxicity in accordance with OECD Directive No.425. During 14 days, the animals are monitored for symptoms and mortality by administering different dosage of aqueous extract of Curly Kale at 50, 100, 200, 500, 800, 1000, 1500, and 2000 mgs/kg *p.o.* at the outset of experiment [20].

Gastric lesions induced by Ethanol

Female wistar rats weighing between 200-250 gm were chosen. Each animal was divided into five groups of six: -

Group 1: - Negative control group treated with vehicle
Group 2: - Positive control group treated with ethanol (1ml/gm *p.o.*)
Group 3: - Treated with Low Dose of Curly Kale aqueous extract (250mg/kg *p.o.*)
Group 4: - Treated with High dose of Curly kale aqueous extract (500 mg/kg *p.o.*)
Group 5: - Treated with Standard drug ranitidine (150 mg/kg *p.o.*)

The experiment was carried out for 7 days. Before treatment animals were fastened for twenty-four hours on 8th day received test drugs and standard drug respectively 30 minutes before administering ethanol. All of the animals were euthanized by a carbon-dioxide overdose an hour after receiving ethanol. Untreated group served as control. Stomachs of each animal were removed, sliced with a broader arc, and rinsed with normal saline (0.9% NaCl) and examined under microscope [16].

Gastric lesions induced by Swim stress

Female wistar rats weighing between 200-250 gm were chosen. Each animal was divided into five groups of six: -

Group 1: - Negative control group treated with vehicle
Group 2: - Positive control group stress induced by forced swimming in water for 3hrs
Group 3: - Treated with Low Dose of Curly Kale aqueous extract (250mg/kg *p.o.*)
Group 4: - Treated with High dose of Curly kale aqueous extract (500 mg/kg *p.o.*)
Group 5: - Treated with Standard drug ranitidine (150 mg/kg *p.o.*)

The experiment was carried out for 7 days. Before treatment animals were fastened for twenty-four hours on 8th day received test drugs and standards drugs respectively 20 min prior to forced swimming in water. Untreated group served as control. After three hours of forced swimming in water to the height of 35 cm using glass cylinder of height 45cm and diameter 25cm for 3 hours asserted at 25^{0}C. By over-dose of carbon-dioxide all the animals were euthanized the stomachs are removed, sliced with a broader arc, and rinsed with normal saline (0.9% NaCl) and examined under microscope [16].

Determination of anti-ulcer activity parameters [16,21]

a) Macroscopic evaluation of stomach

The scores of ulcers and severity of lesions are specified as follows: -

Scores: - 0= No destruction, 1= typical mucosa, 2= vascular obstruction, 3= 1 or 2 lesions, 4= serious injuries, 5= highly aggressive lesions, 6= lesions all over mucosa.

b) Ulcer Index Determination

The equation is utilised to calculate the ulcer index for each group: -

$$U.I. = [\text{Ulcerated area/total stomach area}] \times 100$$

The % of ulcer prevention index is figured out as follows:

$$\%\text{Ulcer inhibition} = \frac{[U.I. \text{ in control} - U.I. \text{ in test}]}{[U.I. \text{ in control} \times 100]}$$

c) Measurement of gastric acid secretion and pH

After excising the stomach was shaped more curvaceously using eppendroff tubes stomach acid were recollected and centrifuged at 1000RPM for 10min. The supernatant volume expressed as ml/100g and calculated. Using digital pH meter supernatant of pH was determined [14].

d) Determination of free and total acidity Free and total acidity

In a conical flask by using distilled water, Topfer's reagent, and phenolphthalein as an indicator, titrate 1 ml of gastric juice against 0.01N NaOH expressed as mEq/L/100g [15].

$$\text{Acidity(mEq/L)} = \frac{\text{Volume of NaOH X Normality of NaOH X100}}{0.1/100gm}$$

e) Evaluation of C-reactive protein in ethanol induced group

Under anaesthesia using cardiac puncture blood samples were collected from animals, by separating serum C-reactive protein is determined using CRP kit.

Statistical analysis

Data are expressed as mean±SEM after statistical analysis using one way analysis of variance using Tukey's test in GraphPad Prism version 9. When compared to the control group, the data show statistically significant differences at $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ and $^{****}p<0.0001$.

RESULT

Preliminary phytochemical studies

Curly Kale aqueous extract leaves were subjected to a quantitative phytochemical screening that identified the presence of alkaloids, glycosides, carbohydrates, flavonoids, and tannins [Table No.1].

Acute oral toxicity study

As a result of acute oral toxicity study Curly Kale aqueous extract showed maximum tolerated dose, the therapeutic low dose selected was 250 mgs/kg *p.o.* and high dosage selected was 500 mgs/kg *p.o.* for this study, and has been free from clinical signs of toxicity and mortality for 14 days.

Estimation of CKAE on Ethanol Induced Gastric Ulceration

The animals which received CKAE decreased the free acidity, total acidity, ulcer index and CRP and mean while shown pH of gastric juice rising in comparison to the positive control group. The parameters evaluated in a rat's model of ethanol-induced ulcer are ulcer index, % of ulcer protection, pH, free acidity, total acidity and CRP shown in [Table No. 2]. The effect of Curly Kale aqueous extract at 250 and 500 mgs/kg *p.o.* dose-dependence, unveiled an % ulcer protection of 51.87±1.973 and 65.66±2.13 respectively, standard drug ranitidine unveiled an % ulcer protection of 76.36±2.43 compared to positive control group. Macroscopic evaluation of stomach using ethanol induced ulcers shown in [Fig no. 1].

Estimation of CKAE on Swim stress Induced Gastric Ulceration

The animals which received CKAE decreased the free acidity, total acidity, ulcer index and CRP and mean while shown pH of gastric juice rising in comparison to the positive control group. The parameters assessed in swim stress induced model in rats are the ulcer index, % of ulcer protection, pH, free acidity, total acidity and CRP are shown in [Table No. 3]. The effect of Curly Kale aqueous extract at 250 and 500 mgs/kg *p.o.* dose-dependence, unveiled an % ulcer protection of 46.27±1.5626 and 68.28±2.350 respectively, standard drug ranitidine unveiled an % ulcer protection of 73.84±1.5626 compared to positive control group. Macroscopic evaluation of stomach using swim stress induced ulcers shown in [Fig no. 2].

DISCUSSION

The goal of the current investigation was to actuate ulcer defensive effect of Curly Kale aqueous extract to prevent ethanol-induced and stress induced stomach ulcer in comparison to positive control group and ranitidine. Hence using animal models of gastric ulcers that provoke the status which humans exhibit [22]. Since drugs available in the market are associated with side-effects comprise proton pump inhibitors, antacids, H2 antagonists and anti-cholinergic used cause adverse reactions like gynaecomastia, haemopoietic changes, anaphylactic reactions, nephrotoxicity and hepatotoxicity. Thus, medicative herbs are preferred due to less side-effects and are preferred substitute for the use of gastric ulcer.

When ethanol is taken orally it invades abdominal mucosa and causes lesions which ultimately damages the stomach tissues [23]. Haemorrhage, edema in sub-mucosa and inflammatory cells intrusion is peculiarity of gastric lesions [24,25]. Ingestion of ethanol to rat's releases O2.- and HO2. free-radicals [26]. Ethanol when administered to animals causes variations in the mediators of inflammation and reported to induce hemorrhagic shock [27]. Moreover, causes necrosis of gastric mucosa which damages micro-vessel of stomach and stagnates blood flow [28]. The anti-oxidant effect of Curly Kale indicates that ulcer developed animals when treated with Curly Kale reduce gastric ulcer [29].

Swim stress model reduce mucosal blood circulation and mucus content and also raise secretion in stomach by releasing histamine associated with physical and psychological stress. Moreover, release free-radicals by destructing gastric mucosa and reduce secretion of bicarbonate and mucin [30,31].

Studies done erstwhile suggest that the active compounds like flavonoids and tannins curtail ulcer formation. Curly Kale aqueous extract amid examination of phytochemical revealed presence of flavonoids, tannins, glycosides, alkaloids. Since Curly Kale contains flavonoids gastrointestinal system by disintegrating oxidants which are reactive and counteracting raise in PG, bicarbonate and mucus secretion and also reported with a rise in stomach juice's pH and % of ulcer protection [32-35].

The results attained from our study propose that Curly Kale employed in the study diminished the ulcer evolution as estimated found on the ulcer index at both the doses (250 and 500 mg/kg *p.o.*). The resultant shown in Table 1 and Table 2. There was also depletion in parameters like free acidity, total acidity, CRP which is involved in the acid formation. OH- radical collection absolutely measure antioxidant property by reduction in oxidative stress reported by ranitidine [36]. Ultimately Curly Kale extract possessed strong antioxidant effect compared to ranitidine.

Table No. 1: Preliminary phytochemical analysis of Curly Kale

Chemical constituents	Curly Kale
Alkaloids	**+**
Glycosides	**+**
Carbohydrates	**+**
Flavonoids	**+**
Tannins	**+**

Table No. 2: Ethanol-induced ulcer model's assessment of the impact of CKAE on various ulcer parameters in rats

Treatment	Ulcer Index	% of ulcer protection	pH	Acidity mEq/L/100g		CRP (mg/dl)
				Free	Total	
Vehicle control	0	0	2.89±0.060	5.00±0.365	11.5±0.76	5.67±1.140
Ethanol induced	13.73±1.50$^{\#}$	0	1.28±0.083$^{\#}$	8.33±0.33$^{\#}$	25.6±2.20$^{\#,\#}$	23.33±5.030$^{\#}$
Low dose of CKAE (250 mg/kg *b.w. p.o.*)	8.92±1.10**	51.87±1.973	1.53±0.108^{ns}	6.33±0.33*	21.00±1.75^{ns}	18.23±0.052^{ns}
High dose of CKAE (500 mg/kg *b.w. p.o.*)	7.21±0.62***	65.66±2.13	1.89±0.095***	5.66±0.55**	15.33±1.20**	12.32±0.033*
Standard drug Ranitidine (150 mg/kg *b.w. p.o.*)	3.02±0.046****	76.36±2.43	2.22±0.088****	5.50±0.670**	12.6±0.68***	10.40±1.44**

The Mean±SEM all results are graphically depicted and subjected to one-way ANOVA and Tukey's test analysis. By contrasting the ethanol inducer group with the vehicle group, the statistical significance of the findings was evaluated (# P<0.05, ## P<0.01, ### P<0.001, #### P<0.0001). Ranitidine treated group are

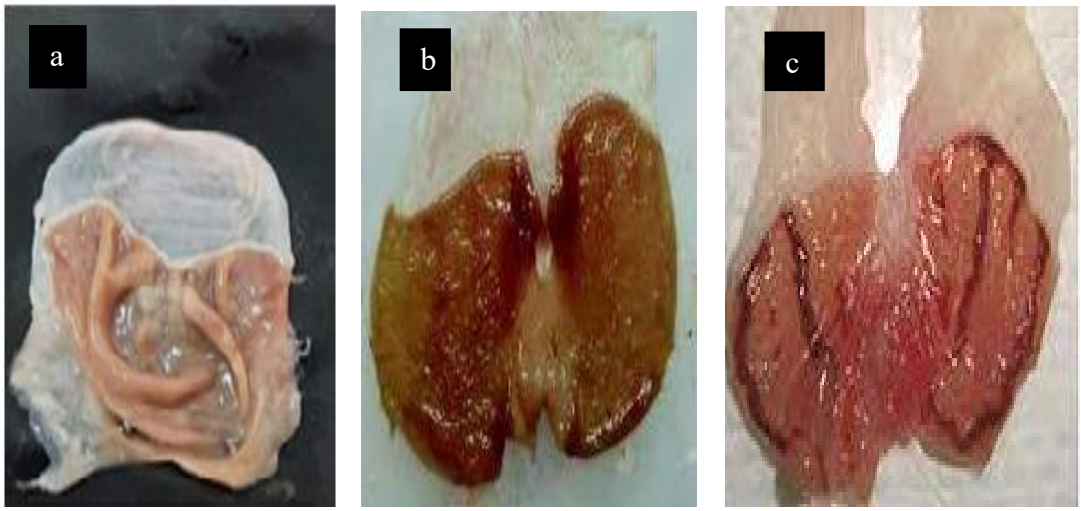
contrasted with ethanol inducer low and high dose groups (P<0.05) ns: no significance, *P <0.05, ** P<0.01, ***P<0.001, ****P<0.0001.

Table No. 3: Swim stress induced ulcer model's assessment of the impact of CKAE on various ulcer parameters in rats

Treatment	Ulcer Index	% of ulcer protection	pH	Acidity mEq/L/100g		CRP (mg/dl)
				Free	Total	
Vehicle control	0	0	3.273 ±0.1365	4.500 ±0.4282	11.17 ±0.6009	5.850 ±0.2663
Stress induced	12.09 ±0.6365$^{\#}$	0	1.467 ±0.1382$^{\#}$	7.167 ±0.4773$^{\#}$	26.00 ±1.789$^{\#}$	22.05 ±0.4496$^{\#}$
Low dose of CKAE (250 mg/kg *b.w. p.o.*)	7.278 ±0.1176*	46.27 ±1.5626	1.92 ±0.2056^{ns}	6.333 ±0.4216^{ns}	21.33 ±0.8819*	16.05 ±0.3944^{ns}
High dose of CKAE (500 mg/kg *b.w. p.o.*)	5.443 ±0.2760**	68.28 ±2.350	2.011 ±0.1112***	5.000 ±0.3651**	19.33 ±0.8028***	13.50 ±0.3850*
Standard drug Ranitidine (150 mg/kg *b.w. p.o.*)	3.120 ±0.1943****	73.84 ±1.598	2.35 ±0.0671***	4.833 ±0.3073**	12.00 ±0.5774****	11.08 ±0.6031**

The Mean±SEM all results are graphically depicted and subjected to one-way ANOVA and Tukey's test analysis. By contrasting the swim stress inducer group with the vehicle group, the statistical significance of the findings was evaluated (# P<0.05, ## P<0.01, ### P<0.001, #### P<0.0001). Ranitidine treated group are contrasted with swim stress inducer low and high dose groups (P<0.05) ns: no significance, *P <0.05, ** P<0.01, ***P<0.001, ****P<0.0001.

Fig No. 1: - Photographs showing macroscopic evaluation of rat's stomach using ethanol induced model subjected to vehicle (a), ethanol 1ml/gm *p.o.* (b), Low dose Curly Kale AE leaves 250 mgs/kg *p.o.* (c), High dose Curly Kale AE leaves 500 mgs/kg *p.o.* (d), Ranitidine 150 mgs/kg *p.o.* (e).



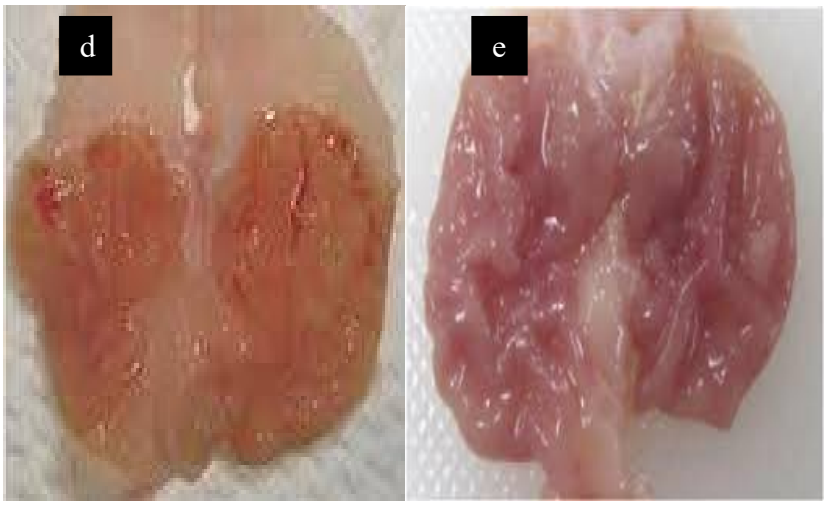
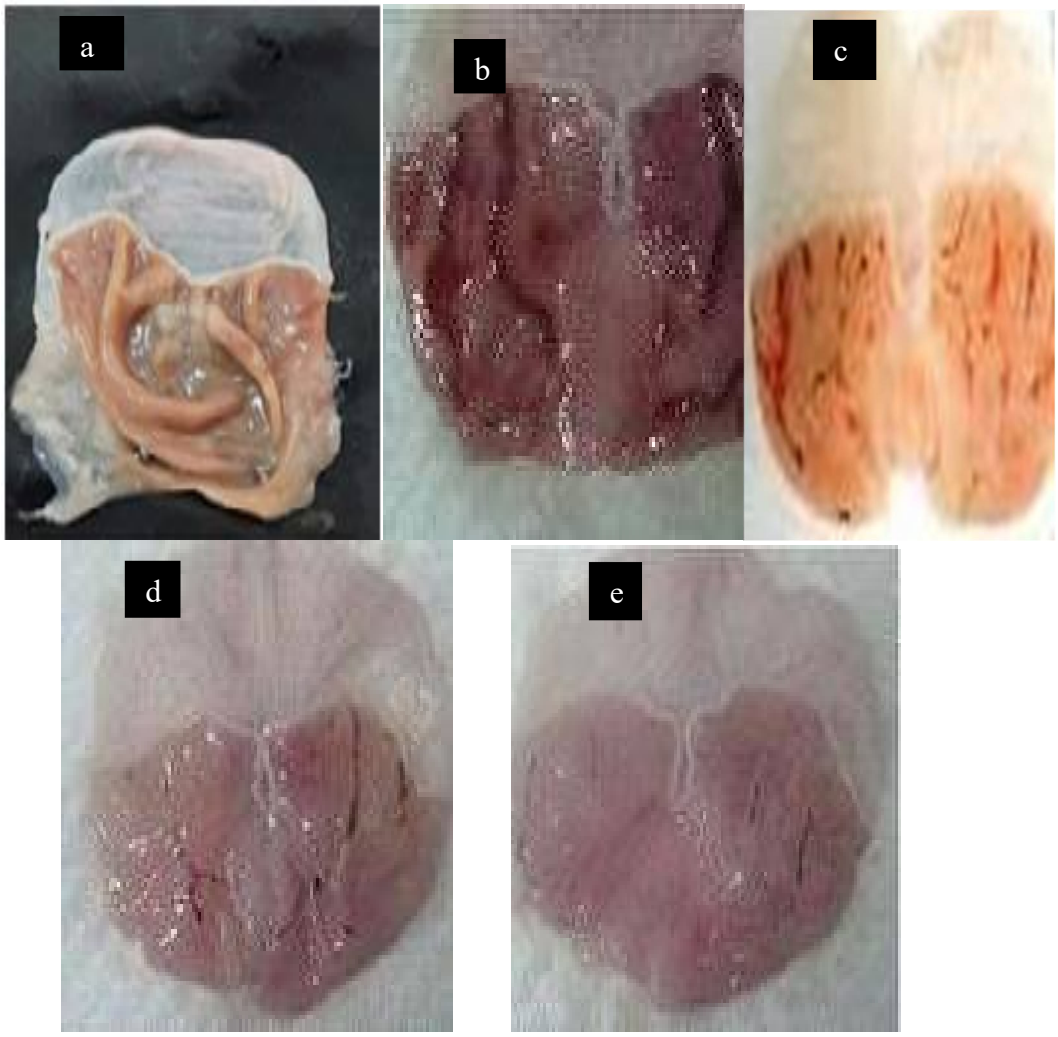


Fig No. 2: - Photographs showing macroscopic evaluation of rat's stomach using swim stress induced model subjected to vehicle (a), ethanol 1ml/gm *p.o.* (b), Low dose AE of Curly Kale leaves 250 mgs/kg *p.o.* (c), High dose AE of Curly Kale leaves 500 mgs/kg *p.o.* (d), Ranitidine 150 mgs/kg *b,w p.o.* (e).



CONCLUSION

Curly Kale aqueous extract through its antioxidant effect attenuated both 250 mg/kg *p.o.* and 500 mg/kg *p.o.* of the ethanol and swim-stress induced gastric ulcers, ranitidine is a promising anti-ulcer medication. The underlying mechanisms of action should be further explored, nevertheless, by additional research.

ACKNOWLEDGEMENT
We are thankful to management of Krupanidhi College of Pharmacy, Bengaluru, Karnataka for the providing laboratory facility, digital library and all facilities for carrying out the work.

ETHICS COMMITTEE APPROVAL
Institutional Animal Ethics Committee approved the above protocol with approval no. (Ref. No.378/PO/ReBi/S/01/CPCSEA).

COMPETING OF INTEREST
We declare that we have no conflict of interest.

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