ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 05, pp.50454-50457, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

STUDY THE EFFECT OF *ANDROGRAPHIS PANICULATA* FLOWER EXTRACT AS A GASTROPROTECTIVE AND ANTIOXIDANT IN PEPTIC ULCER

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ARTICLE INFO

Article History:
Received 21st February, 2017
Received in revised form
20th March, 2017
Accepted 14th April, 2017
Published online 23rd May, 2017

Key words:

Andrographis paniculata,
CRS induced ulcer,
Acidity,
Ulcer index.

ABSTRACT

Peptic ulcer is a most common ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. This pathological condition is caused by chronic inflammation due to *Helicobacter pylori*, excessive use of NSAIDs like aspirin and smoking. This disorder also results in release of massive amount of toxic free radicals which results in oxidative stress. Ethnobotanically, the flower of Andrographis paniculata has been reported to be used in the treatment of various disorders including stomach and skin diseases. Antiulcer activity of the 50% ethanolic extracts in order to validate ethnobotanical claims regarding the plant, used in the above disorders. Four groups of six albino rats in each group were used. They were pretreated with (0.25% w/v) carboxymethyl cellulose (negative control, 10 ml/kg), 50 mg/kg ranitidine (positive control), Andrographis paniculata flower extract (200 and 400 mg/kg/body weight) and their effect was studied on aspirin induced ulcer, cold-resistant stress-induced ulcers, pylorus ligation and ethanol-induced ulcers. The results of the present study showed that the flower extract of Andrographis paniculata possessed gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by physical and chemical agents with a maximum of 87.15 % therapeutic efficiency (400 mg/kg b.w.) in cold resistant stress-induced ulcers. The present study was also aimed to investigate the effect of this extract on oxidative stress by measuring the level of various oxidative markers. The result of enzyme assay and lipid peroxidation clearly indicates the andrographis paniculata flower extract have significant antioxidant effect on ulcer pathology. Flower extract have decreased LPO ($p< 0.001$) and SOD ($p<0.01$) with concomitant increase in catalyse activity in cold resistant stress-induced ulcers.

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Citation: Anil K.Yadav, Ruqsana Khatoon and Chandana V. Rao. 2017. "Study the effect of *Andrographis paniculata* flower extract as a gastroprotective and antioxidant in peptic ulcer", *International Journal of Current Research*, 9, (05), 50454-50457.

INTRODUCTION

Peptic ulcer is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to aggressive factors. In spite of the vast amount of research on ulcer, the cause of chronic peptic ulceration is still not clear. Although in most of the cases the etiology of the ulcers is unknown, it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defence mechanisms (Piper and Stiel, 1986). To regain the balance, different therapeutic agents have used. Traditional plant remedies have been used for very long time in the treatment of ulcer, but only a few of them have been significantly evaluated. Therefore, the present work aimed to evaluate the effect of purified standard extract of Andrographis paniculata flower on ulcer induced by different method and its efficacy in oxidative stress. Andrographis paniculata a medicinal plant well known as "Kalmegh" and "Green chiratta"and form the principal ingredient of the domestic medicine "Alui" (Bengali) it is referred as a wonder drug in Siddha and Ayurvedic formoulation used for gastrointestinal ailments. Pharmacological and clinical studies suggest that Andrographic paniculata possess anti-inflammatory, anti-pyretics, anti-viral, immunostimulatory, hepatoprotactive, and cardio protective activites. In the present study the gastroprotective and antioxidant activities of ethanolic extract of Andrographic paniculata was studied in Asprin induced ulcer, cold resistant stress induced ulcer, Pylorus ligated induced ulcers and ethanol induced ulcer.

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MATERIALS AND METHODS

Collection of plant material

The flower of the *Andrographis paniculata (Acanthceae)* was collected from Botanical Garden of N.B.R.I (National Botanical Research Institute), Lucknow, India in month of

June 2011. The plant materials were authenticated by Dr. Sayeeda Khatoon, chemotaxonomic at National Botanical Research Institute, Lucknow and voucher specimens were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference.

Extraction of Andrographis paniculata

The fresh flower of Andrographis paniculata where dried and powdered homogenously. The powdered material put in 50% ethanol for 24 hours. Ethanol extract was filtered and concentrated under pressure in rotary evaporator (R110 Buchi, Switzerland) at 60 °C and dried to a constant weight in an oven set at 40 °C. The dried extract gave a yield of 28.13% (w/w) and was stored in an air-tight container at about 4 °C until required. The extracts obtained was further subjected pharmacological investigation to dryness to one third of orginal volume and stored overnight at 35^0C filtrate was lyophilized and the drug material obtain was used for study.

Animals

Swiss albino rats weighing (160-200 gm) and were procured from National Botanical Research Institute (Lucknow). They were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free excess to water. The composition of diet is 10% protein, 4% arachis oil, 1% fibers, 1% calcium, 1000 IU/gm vitamin A and 500 IU/gm vitamin D (Zimmerman M, 1983).

Experimental Procedure

In the experiment, the rats were divided into four groups (n = 6). Group 1 was the control group which received suspension of 1% carboxymethyl cellulose in distilled water (10 ml/kg). Groups 2 and 3 received APE in doses of 200 and 400 mg/kg b.w.. Group 4 received ranitidine in the dose of 50 mg/kg body weight. These were administered orally twice daily at 10:00 and 16:00 h, respectively, for five days for acute ulcer protective studies.

Aspirin (ASP)-induced ulcers

ASP in dose of 200 mg/kg (20 mg/ml) was administered to the animals on the day of the experiment and ulcers were scored after 4 h (Goel *et al*., 1985). The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9% NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index was calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The pooled group ulcer score was then calculated according to the method of Sanyal *et al*. (1982).

Cold-resistant stress (CRS)-induced ulcer

Rats were deprived of food, but not water, for about 18 h before the experiment. On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4–6 ◦C (Gupta *et al*., 1985). Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described above. Extent of lipid peroxidation (LPO) was also estimated under the stress condition using the standard method of (Okhawa *et al*. (1979). The activity of superoxide dismutase (SOD) was determined by monitoring the inhibition of the autoxidation of pyrogallol (Marklund and Marklund, 1974). CAT activity was determined by monitoring the enzyme catalyzed decomposition of hydrogen peroxide by potassium permanganate (Cohen *et al*., 1970).

Pylorus ligated induced ulcers

Drugs were administered for a period of 5 days as described above and the rats were kept for 18 h fasting. Animals were anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period (Shay *et al*., 1945). After 4 h, stomachs were dissected out and cut open along the greater curvature and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach.

Ethanol - induced ulcer

The gastric ulcers were induced in rats by administrating ethanol (1 ml/200 g, 1 h) (Hollander *et al*., 1985) and the animals were sacrificed by cervical dislocation and the stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm2/rat).

Table 1. Effect of ethanolic flower extract of *Andrographis paniculata* on ulcer index in Asprin induced ulcer

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	Protactive ratio (%)
I	Vehicle	PL	19.4±0.07	
II	APE	200	8.3±0.08[b]	63.4±0.14
III	APE	400	7.2±0.09[d]	67.3±0.13
IV	Ranitidine	50	7.3±0.13[b]	60.3±0.13

Values are mean±S.E.M. (n=6)
a $P < 0.001$ compared to respective control group.
b $P < 0.01$ compared to respective stress group.
c $P < 0.05$ compared to respective stress group.
d $P < 0.001$ compared to respective stress group.

Table 2. Effect of ethanolic flower extract of *Andrographis paniculata* on ulcer index in cold resistant stress induced ulcer

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	Protactive ratio (%)
I	CR stress		26.2±0.08	
II	APE	200	14.1±0.04[c]	56.2±0.08
III	APE	400	5.3±0.13[d]	87.2±0.07
IV	Ranitidine	50	6.2±0.11[d]	80.4±0.13

Values are mean±S.E.M. (n=6)
a $P < 0.001$ compared to respective control group.
b $P < 0.01$ compared to respective stress group.
c $P < 0.05$ compared to respective stress group.
d $P < 0.001$ compared to respective stress group.

Table 3. Effect of ethanolic flower extract of *Andrographis paniculata* on ulcer index in Pylorus ligation induced ulcer

Group	Treatment	Dose mg/kg)	Ulcer index mm^2/rat	Protactive ratio (%)
I	Vehicle	_	14.4±0.15	_
II	APE	200	9.4±0.1	48.4±0.11
III	APE	400	7.3±0.14	69.3±0.08
IV	Ranitidine	50	6.3±0.1	64.5±0.12

Values are mean±S.E.M. (n=6)
a $P < 0.001$ compared to respective control group.
b $P < 0.01$ compared to respective stress group.
c $P < 0.02$ compared to respective stress group.
d $P < 0.001$ compared to respective stress group

Table 4. Effect of ethanolic flower extract of *Andrographis paniculata* on ulcer index in Ethanol induced ulcer

Group	Treatment	Dose mg/kg	Ulcer index mm^2/rat	Protective ratio (%)	Gastric wall mucus (µg/g wet glandular tissue)
I	Ethanol	1ml/200g	25.4±0.01	_	175.3±0.12
II	APE	200	20.3±0.11	19.2±0.07	178.3±0.12
III	APE	400	16.3±0.14	38.4±0.12	185.3±0.13
IV	Ranitidine	50	12.5±0.12	58.3±0.11	269.3±0.11

Values are mean±S.E.M. (n=6)
a $P < 0.001$ compared to respective control group.
b $P < 0.01$ compared to respective stress group.
c $P < 0.02$ compared to respective stress group.
d $P < 0.001$ compared to respective stress group.

Table 5. Effect of flower extract of Andrographis paniculata on cold-restraint stress-induced gastric ulcers and LPO, SOD and CAT activity in rat gastric mucosa

Group	Treatment	Dose (mg/ kg)	LPO (nmol of MDA formed/h/100 mg protein)	SOD(nmol /gtissue)	CAT(unit/ mg protein)
I	CR stress	_	$53±0.07^b$	$238±8.2^b$	$23.3±1.3^b$
II	APE	200	$29±0.01^a$	$210±4.3^a$	$32.1±4.2^a$
III	APE	400	$13±0.01^b$	$179±5.7^d$	$41.51±2.5^d$
IV	Ranitidine	50	$41±0.02^b$	$135±1.5^d$	$40.31±1.8^d$

Values are mean±S.E.M. (n=6).
a $P < 0.001$ compared to respective control group.
b $P < 0.001$ compared to respective cold-resistant stress group.
c $P < 0.05$ compared to respective cold-resistant stress group.
d $P < 0.01$ compared to respective cold-resistant stress group.

Statistical analysis

The statistical analysis of all the pharmacological analyses was carried out using SPSS 13.0 for Windows. The values are represented as mean±S.E.M. (n=6). Paired *t*-test (Newman-keuls multiple comparison test).was used for reporting the *p* value and significance with respect to the control group and ANOVA for the comparison between more than two groups.

RESULTS AND DISCUSSION

It has been established that the causative agent for peptic ulcer is use of excessive NSAID's like aspirin, H.coli bacteria and smoking, alcoholism etc. These factors after prolonged duration results into ulcer in gastrointestinal track. This also aggravate oxidative stress in body which may also results into other complication like diabetes, cardiovascular diseases. As we know that there are many synthetic therapeutic agents are available which efficiently controls it, but they further precipitates side effects and creates complication. So there is need arises for the use of herbal medicines which are devoid of side effects. In our present work ulcer have been introduced in albino rat by means of various agent like aspirin, cold, pylorus ligation and ethanol. In case aspirin induced ulcer it has been found that by using dose of 400 mg /b.w. of APE have improved the condition statistically significantly as compared to ranitidine, as shown in Table 1. Also we know that stress plays an important role in etiopathology of gastroduodenal ulceration. Stress-induced ulcers are probably mediated by histamine release with enhancement in acid secretion and a reduction in mucous production. Increase in gastric motility, vagal overactivity (Cho *et al*., 1976) mast cell degranulation (Cho and Ogle, 1979) decreased gastric mucosal blood flow (Hase and Moss, 1973) and decreased prostaglandin syntheses (Rao *et al*., 1999) are involved in genesis of stress-induced ulcers. In cold stress induced ulcer, the protective ratio of APE at dose of 400 mg/b.w. was also found much more significant as compared to ranitidine, as shown in Table 2 which confirmed the histamine antagonistic, anticholinergic and antisecretory effects of extract. Also in case of pylorus ligation induced ulcer the use of extract have showed significantly promising therapeutic action. (Table 3). Alcoholism along with social abuse also proved as a prime reason for the ulceration. Ethanol elevates the pepsin secretion which leads to degradation of endogenous protein in the mucous layer of gastric wall and causes the inflammation. Ethanol also elevates the hypersecreation of acid which further elevates the severity of inflammation. From the results as shown in table 4, it is evident that the APE reduced the ulcer index significantly. This effect achieved due to blockage of H^+K^+ATPase, the enzyme involved in gastric acid secretion. The pepsin reducing effect of APE was found to be significant in ethanol-induced ulcer model. Thus, from these results it is revealed that APE contains flavonoids such as quercitin, formononetin and biochin, which inhibits H^+K^+ATPase activity**.**

It has been established that oxidative stress lies at the root of a number of pathological processes and diseases such as cancer, 126 atherosclerosis, rheumatic arthritis, hematological and neurodegenerative disorders are not exempt with more making the list among which is peptic ulcer. The experimental data revealed that the cold-resistant stress aggravated the ulcer severity and induced oxidative stress as compared to normal rats. Lipid peroxidation is a free radical-induced process leading to oxidative deterioration of polyunsaturated fatty acids under physiological condition, low concentration of lipid peroxides are found in tissue. Elevated level of peroxides is a characteristic feature of chronic ulcer. Superoxide dismutase (SOD) protects tissue against reactive oxygen species (ROS) by catalyzing the removal of superoxide radical (O2.−) which damages the membrane and biological structure. Catalase has been shown to be responsible for detoxification of scavenging enzymes that remove the toxic free radicals in vivo. Reduced activities of SOD and catalase resulted in number of deleterious effects due to accumulation of superoxide radicles (O2.−) and hydrogen peroxide. This effect was significantly reversed by prior administration of flower extract of Andrographis paniculata providing a close relationship between free radical scavenging activity and gastroprotective

effect as Shown in Table 5. The results of the present study showed for the first time that the flower extract possessed gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by physical and chemical agents. These findings have justified, the inclusion of this plant in the management of gastric disorders in ethnomedicine. Also it is now clear from above study that oxidative stress act as a root cause of spread of peptic ulcer, so there is need arises for the study of those plants which posses anti ulcer activity along with the antioxidant potential. These studies have created a new ray of hope for the use of flower of Andrographis *paniculata* in treatment of ulcer.

Conclusion

Recently, the use of herbal medicines for the treatment of peptic ulcer is increasing and most patients consider herbal medicines are more safe as they are of natural origin. Since these herbal medicines have a tremendous potential to combat the oxidative free radical generated during disease with least potential to cause any adverse effects as compared to allopathic drugs. To date the literature describing these herbal drugs with their oxidativepotential is limited and most are in preclinical studies. Hence, more research is required to explore their beneficial therapeutics effect. Andrographis *paniculata found to* possess an efficient antiulcer and antioxidant property which have now increased their demand in market. In present study Andrographis *paniculata* have reduced the ulcer index and also reversed oxidative stress induced in disease. Therefore, the present study concluded that Andrographis *paniculata' flower* is useful in ameliorating the oxidative stress induced in peptic ulcer. However, more mechanism based research work is required to seek out the effect of this herbal medicine on other enzymes which involves in the pathophysiology of peptic ulcer. In future more will be focused on evaluating the efficacy of this amazing drug against bacterial growth and chronic peptic ulcer.

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