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ORIGINAL ARTICLE

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Evaluation of Antiulcer Activity of *Crateva Magna* (Lour.) Dc in Wistar Albino Rats

Abirami J. *, and Shobana G.

[1] *PG & Research Department of Biochemistry, Srimad Andavan Arts & Science college(Autonomous), Tiruchirappalli - 620 005, Tamil Nadu, India.*

*E-Mail:abiramij600@gmail.com

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The Antiulcer activity of aqueous leaf extract of *Crateva magna* (Lour) DC. was investigated on Ethanol induced ulcer model and Aspirin induced model in wistar rats. In both models the common parameters determined was ulcer index, ulcer score, gastric pH, free and total acidity, Antioxidant parameters such as glutathione peroxidase, superoxide dismutase, lipid peroxides. The aqueous leaf extract of *Crateva magna* (Lour) DC. dosage 150, and 300 mg/kg produced significant inhibition of gastric lesion induced by Ethanol induced ulcer and Aspirin induced ulcer. The aqueous leaf extract of *Crateva magna* (Lour) DC. dosage 150, and 300 mg/kg showed significant reduction in free acidity, total acidity, and ulcer index as compared to disease control. The study indicates that aqueous leaf extract of *Crateva magna* (Lour) DC. have potential antiulcer activity in both models. These results indicated that *Crateva magna* (Lour) DC. displayed an antiulcerogenic effect due to its cytoprotective activity. Besides antioxidant potential of plant extract scavenged the free radicals produced by Ethanol induction, Aspirin induced ulcer and healed the ulcers.

Key words: Ulcer score, Super Oxide Dismutase, Crateva magna (Lour) DC., Herbal Medicine

Ulcer is defined as an erosion in the lining of the stomach or duodenum and is caused by the disruptions of the gastric mucosal defense and repair systems. Ulcer of the stomach is called gastric ulcer and in the duodenum, it is called duodenal ulcer and together it is peptic ulcer (Gregory *et al*., 2013). Herbal Medicines are emerging as an alternative medicine and there is an increased demand for these medicines due to their lower cost, affordability, fewer side effects, and perceived effectiveness. Medicinal plants are important source of new chemical molecules with potential therapeutic effects (Wahida *et al*., 2007).

Allopathic treatments are prone to produce side effects such as vomiting, Nausea, and depression. Besides these treatments also incur heavy economic burden, hence there is a need for an alternative, cost effective safe Herbal Medicine. Hence in the present study an attempt was made to develop a human friendly antiulcer herbal drug.

MATERIALS AND METHODS

Selected plant for the study:

***Crateva magna* (Lour) DC.**

Kingdom: Plantae
Order: Brassicales
Family: Capparaceae
Genus: *Crateva*
Species: *C. magna*
Binomial name: *Crateva magna* (Lour.) DC

Parts used: Leaves

The plant *Crateva magna* belonging to family *Capparaceae* is a well known plant in herbal world for its wide range of use in medicinal purposes. It is used as an anti spasmodic, hypotensive, anti inflammatory, hypoglycemic, anti protozoal, Anthelmintic, analgesic purposes. It is also used to increase appetite and for the treatment of various diseases e.g.rheumatism and nephrotoxicity, arthritis, urinary disorders (Shirwaikar *et al*., 2004). The root, bark and leaf is used to treat fever, urticaria, snake bite poisoning, disease of vatam, urinary calculi, ulcers, skin eruptions. (Yoganarassimhan, 1992).

Identification and authentication of selected plant:

The selected plant *Crateva magna* (Lour.) DC. Family *Capparaceae* was collected from areas in and around Tiruchirappalli, identified using flora of presidency of Madras and authenticated by the Botanist of St. Joseph's College, Tiruchirappalli and a voucher specimen was deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamilnadu, India.

Assessment of antiulcer potentials of selected plant by *in-vivo* study

i) Ethanol induced model (Anandan *et al.*, 1998)

Experimental design:

Group Number	No. of Animals	Treatment	Dose	Route of Administration	Duration	Parameters
Group I	6	Vehicle only	-	oral	8	Volume of Gastric juice,pH, Total acidity, Free acidity, ulcer index ulcer score Total protein
Group II	6	Ethanol + Vehicle	1ml/kg	oral	8	
Group III	6	Ethanol + Sucralfate	400 mg/kg	oral	8	
Group IV	6	Ethanol + *Crateva magna*	150 mg/kg	oral	8	
Group V	6	Ethanol + *Crateva magna*	300 mg/kg	oral	8	

ii) Aspirin induced model (Zeeyauddin *et al.,* 2011)

Aspirin induced ulcers in rats Animals were divided into four groups, with each group containing six animals. The first group served as a control and was administered vehicle only, second group served as a positive control and were treated with standard drug ranitidine (20mg/kg) third and fourth group served as test groups and were administered at the dose level 150 and 300mg/kg. Plant extract administered for 7 days. After seven days aspirin were administered 30 minutes before 300mg/kg per orally. After 6 hours rats will be sacrificed by anesthesia. Stomachs were dissected out for determination of gastric lesions, washed in tap water and examined ulcers with the help of microscope (10x). Gastric juice collected into centrifuge tubes and centrifuged at 1000 rpm for 10 minutes. The gastric juice volume was noted. Gastric juice of PH was recorded by PH meter. Ulcer score for each animal called as ulcer index. Free and gastric acidity were analyzed.

Parameters Analysed:

1. Determination of ulcer index in gastric tissue (Piyusha *et*

al., 2011).

2. Determination of ulcer score in gastric tissue (Gangale *et al.*, 2010). Ulcer index was scored and percentage protection calculated using the methods of Hemamalini et al., (2012).

3. Determination of gastric juice pH. The volume of gastric juice was measured using a measuring cylinder and the pH was measured by digital pH meter.

4. Determination of free and total acidity in gastric juice (Hemamalini *et al.,* 2012)

5. Estimation of protein (Lowry, *et al.*, 1951). 0.1ml of serum was diluted to 5ml with distilled water. 0.1ml diluted serum was made up to 4ml with distilled water. 4.5ml of alkaline copper reagent was added and incubated at room temperature for 20 minutes. 0.5 ml of Folin's phenol was added and incubated at room temperature for 10 minutes. The colour developed was read at 620 nm using blank. Aliquotes of standard were also treated as above. The protein content was expressed as g/dl.

6. Assay of lipid peroxides (Ohkawa *et al,* 1979). 0.1 ml of tissue homogenate was mixed with 4 ml of 0.85N H2 S04. 0.5 ml of phosphotungstic acid was added and stirred well. The content was centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the sediment was mixed with 2.0ml ofN/12 H2S04 and 0.3 ml of 10% phosphotungstic acid. The mixture was centrifuged for 10 min. The sediment was suspended in 4.0 ml of distilled water and 1 m1 of TBA reagent. The tube was kept in a boiling water bath for 1 hr after cooling, 5 ml of butanol was added and the colour extract in the butanol phase was read at 532 nm using control.

7. Assay of superoxide dismutase (Misra and Fridovich 1972) 0.1ml of tissue homogenate was added to tube containing 0.75 ml ethanol and 0.15ml ice cold chloroform and centrifuged. To 0.5 ml of EDTA solution and 1 ml of buffer were added and mixed well .The reaction was initiated by the addition of 0.5ml of epinephrine and the increase in absorbance was measured at 480 nm.

8. Estimation of reduced glutathione (Sedlak *et al,* 1963). To 0.5ml of homogenate, 20% TCA was added and precipitated. The contents were mixed well for complete precipitation of protein and centrifuged. To an aliquots of clear supernatant, 2.0ml of DTNB Reagent and 0.2 M phosphate buffer were added to make a final volume of 4.0ml. The absorbance was read at 412 nm against a blank containing TCA instead of sample. A series of standards treated in a similar way were also run to determine glutathione content. The amount of glutathione was expressed as nano moles of GSH oxidised/ mg protein.

9. Assay of glutathione peroxidase (GPX) (Rotruckjt *et al.*, 1973).

10. Determination of ulcer score in gastric tissue (Jeetendrakumar gupta *et al* 2010). Scoring for ulcer Normal Stomach = 0 Red Colouration = 0.5 Spot ulcers = 1 Haemorrhagic streaks = 1.5 Ulcers > 3mm < 5mm = 2 Ulcers > 5mm = 3 Ulcers index = UA+US+UP 10 Where: UA = Average number of ulcers per animal US = Ulcer severity score UP = Percentage of animals with ulcers UP = Total ulcers in a group x 100 Total number of Animals 1 Percentage inhibition of ulcer was calculated using the method of Hemamalini et al., (2012) expressed as: Percentage inhibition = UIC – UIT x 100 UIC 1 Where: UIC = Ulcers index of control group UIT = Ulcer index of test group

11. LC MSMS ANALYSIS (Figueirinha *et al.*, 2008). Recently the herbal manufacturing industry has focused on improving its quality assurance and quality control mechanisms to guard against the frequent episodes of substandard quality and possible adulterations. Use of high-performance liquid chromatograms, thin-layer chromatography, atomic absorption spectroscopy, gas chromatography and where necessary more sophisticated techniques such as NMR and LC/MS has now become common in complementary medicines manufacturing industries to ensure the quality of plant materials and final product (Rosen bloom et al., 2011). The emphasis on good manufacturing practice has steadily increased over years. This spectrum indicates may be the presence of bioactive compounds such as alkaloids, flavonoids, glycosides, terpenoids, tannins

RESULTS AND DISCUSSION

The results of the ethanol induced ulcer model study are presented in tables 1, 2 and 3.

Ethanol cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa. (Jude *et al*., 2009)

In normal animals, there were no ulcers while administration of ethanol produced severe hemorrhagic gastric lesions. In ethanol induced ulcer (Group II) a significant rise in the volume of Gastric juice, Gastric pH, ulcer score, ulcer index, (Table 1) Total acidity and free acidity (Table 2) were noticed. This is because of

Ethanol induced corrosive effect. It rapidly penetrates into the gastric mucosa, causing cell and plasma membrane damage, leading to increased membrane permeability to sodium and water. It also produced massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium.

On pretreatment with *Crateva magna* (Lour) DC at the dose levels of 150 mg/kg bw, and 300 mg/kg bw and standard drug Sucralfate 400 mg/kgbw significantly reduced the ulcer index, ulcer score, Gastric volume (Table 1) Total acidity, Free acidity, (Table 2) as compared to the positive control group. The pretreatment with plant extract exhibited significant antioxidant activity, with increased levels of GSH and SOD, in response to oxidative stress due to ethanol treatment. SOD converts superoxide to hydrogen peroxide (H_2O_2), which is transformed into water by catalase in the lysosomes or by glutathione peroxidase in the mitochondria.

The results of the aspirin induced ulcer model study are presented in tables 4 and 5.

Aspirin induced ulcer is mediated through tissue damaging free radicals , which are produced from the conversion of hydroperoxyl to hydroxy fatty acids which leads to cell destruction. The hydroxyl fatty acids are generated is generation from the degeneration of mast cells and generalized lipid peroxidation accompanying cell damage.

The pretreatment with *Crateva magna* (Lour) DC at the dose levels of 150 mg/kg bw, and 300 mg/kg bw suggested that the increased levels of SOD, GSH and decreased levels of LPO.

Aspirin is a potent cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogenous prostaglandin biosynthesis and disrupts the mucosal barrier as well as mucosal blood flow. Aspirin increases acid secretion and produce microvasculature damage by generation of free radicals. It is well known that inhibition of prostaglandin synthesis which is essential for mucosal integrity and regeneration will trigger the mucosal lining damage.

It may be stated that aspirin could increase gastric lipid peroxidation therefore generate reactive oxygen metabolites. This could damage gastric cells. This was reflected by decreased amount of DNA in gastric mucosa which, in turn, was responsible for decreased synthesis of gastric muco substances. Induction of aspirin produces severe gastric hemorrhagic erosions and markedly decreases the gastric output because of back diffusion of HCl through the broken barrier, acute inflammation and inhibition of mucosal blood flow.

On pretreatment with *Crateva magna* (Lour) DC at the dose levels of 150 mg/kg bw, and 300 mg/kg bw and standard drug Sucralfate 400 mg/kg bw significantly reduced the ulcer index, ulcer score, Gastric volume Total acidity, (Table 3) as compared to the positive control group.

LC-MS MS Analysis:

LC-MSMS analysis was carried out to identify the major chemical constituents present in the bioactive extract.

Table 1. Effect of *Crateva Magna* (Lour) DC on ulcer score, Gastric volume, ulcer index and Gastric pH in Ethanol induced ulcer in wistar albino rats.

S.No	Groups	Ulcer score	Gastric volume(ml)	Ulcer index	Gastric PH
1.	Group I	0.00 ± 0.00	2.62 ± 0.15	0.00± 0.00	2.67± 0.17
2.	Group II	1.00 ± 0.29	4.17 ± 0.25	6.25 ± 0.46	1.50± 0.13
3.	Group III	0.50 ± 0.13	3.00 ± 0.32	3.77± 0.55	1.75± 0.17
4.	Group IV	0.25± 0.11*	2.78 ± 0.22*	1.67 ± 0.26*	2.50± 0.18*
5.	Group V	0.17 ± 0.11*	2.67 ± 0.25*	1.25 ± 0.00*	2.17± 0.17*

The values are expressed as Mean ±S.E.M n=5, P < (0.01) statistically significant Group IV and V when compared to Group II disease control.

Table 2. Effect of *Crateva Magna* (Lour) DC on free acidity and total acidity in Ethanol induced ulcer in wistar albino rats.

S.No	Groups	Free acidity(meq/l)	Total acidity(meq/l)
1.	Group I	26.00± 0.89	33.33± 0.84
2.	Group II	61.33± 0.84	69.33± 1.69
3.	Group III	36.67 ± 1.23	58.67± 0.84
4.	Group IV	25.33± 0.84*	33.33± 0.84*
5.	Group V	22.00± 0.89*	30.00± 0.89*

The values are expressed as Mean ±S.E.M n=5, P < (0.01) statistically significant Group IV and V when compared to Group II disease control.

Table 3. Effect of *Crateva Magna* (Lour) DC on LPO in Ethanol induced ulcer Induced wistar albino rats.

S.No	Groups	LPO(nm of MDA/g tissue)	SOD(U/mg)	REDUCED GLUTATHIONE(mg/g tissue)
1.	Group I	1.20 ± 0.20*	3.40± 0.10	2.23± 0.24
2.	Group II	3.00 ± 0.60	1.90± 0.02	2.83± 0.12
3.	Group III	1.46 ± 0.29	2.40± 0.13	1.83± 0.09
4.	Group IV	2.40 ± 0.00*	3.30± 0.10	1.22± 0.10
5.	Group V	1.80 ± 0.60*	4.12± 0.13	1.38± 0.12

The values are expressed as Mean ±S.E.M n=5, P < (0.01) statistically significant Group IV and V when compared to Group II disease control.

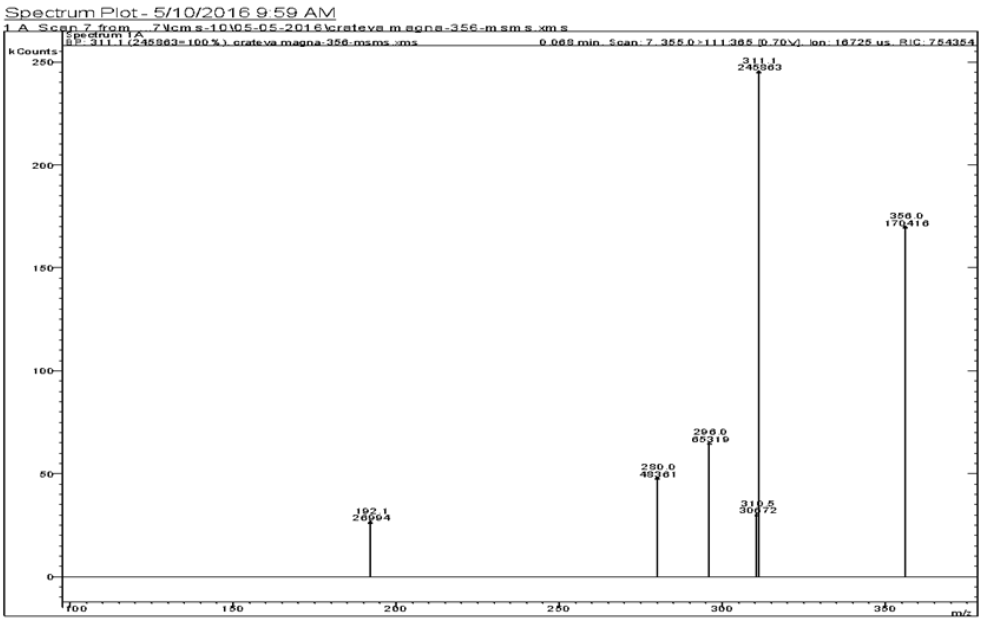
Table 4. Effect of *Crateva magna* (Lour) DC on Gastric PH, in aspirin induced ulcer in wistar albino rats

S.No	Groups	Gastric PH	Ulcer index	Total acidity
1.	Group I	2.08± 0.15	0.00 ± 0.00	30.00 ± 0.84
2.	Group II	1.75± 0.11	6.26± 0.56	69.33 ± 1.69
3.	Group III	1.83± 0.17	3.33± 0.53	58.66 ± 0.84
4.	Group IV	2.00± 0.22*	2.71± 0.50*	33.33 ± 0.84*
5.	Group V	2.25± 0.31*	2.29± 0.21*	30.00 ± 0.84*

The values are expressed as Mean ±S.E.M n=5, P < (0.01) statistically significant Group IV and V when compared to Group II disease control.

Table 5. Effect of *Crateva Magna* (Lour) DC on LPO in aspirin induced ulcer in wistar albino rats.

S.No	Groups	LPO(nm of MDA/g tissue)	SOD(U/mg)	REDUCED GLUTATHIONE(mg/g tissue)
1.	Group I	1.20 ± 0.20*	3.40± 0.10	2.23± 0.24
2.	Group II	6.30 ± 0.30	0.57± 0.50	1.94± 0.12
3.	Group III	1.46 ± 0.29	2.40± 0.30	1.83± 0.09
4.	Group IV	4.80 ± 0.00*	1.53± 0.10	1.71± 0.19
5.	Group V	3.90 ± 0.30*	1.60± 0.14	1.31± 0.32



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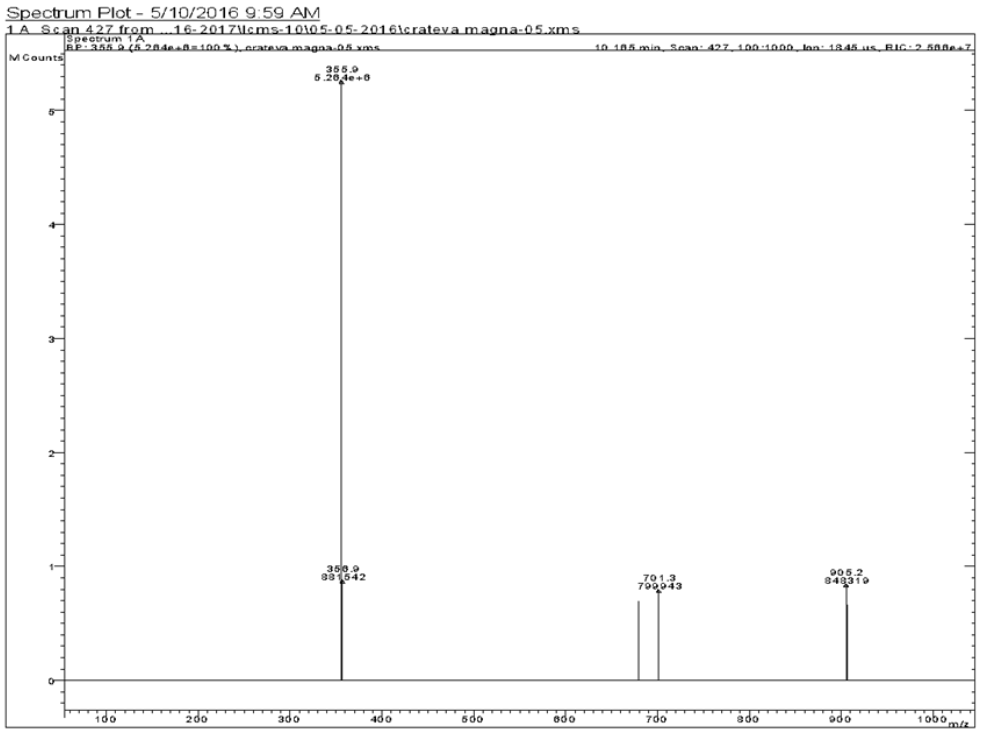

B

Figure 1. A & B. LC-MS MS analysis of the bioactive extract.

CONCLUSION

In the present study two different ulcer models were used to understand the possible therapeutic targets and multiple etiological factors of peptic ulcer disease. It is observed that different mechanisms are involved in the formation of Gastric mucosal lesions in these experimental models studied and the selected plant acted effectively in controlling these various types of ulcers. The models selected are Ethanol induced Ulcer, and Aspirin induced Ulcer.

The data of the results obtained in all these ulcer models revealed that *Crateva magna* (Lour) DC at 150 mg/ kg b.w, and 300mg/kg b.w could significantly protect the gastric mucosa. The emphasis on good

manufacturing practice has steadily increased over years. The LC MS spectrum indicates may be the presence of bioactive compounds such as alkaloids, flavonoids, glycosides, terpenoids, tannins.

The results of the present study further suggested that *Crateva magna* (Lour) DC possessed gastro protective activity due to its significant inhibitory/reducing action on ulcer parameters such as ulcer score, ulcer index, free acidity and total acidity. Besides its efficacy to increase GSH, SOD levels and reduce lipid peroxidation in a dose dependent manner improved the antioxidant status and contributed in ulcer healing.

CONFLICTS OF INTEREST

The author declare that he has no potential conflicts of interest.

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