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Evaluation of the gastroprotective potential of *Chloris paraguaiensis steud*

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ABSTRACT

A peptic ulcer is one of the most common and aggressive disorders in human beings recently. Excessive use of proton pump inhibitors has lead to many other complications including bone weakness and cardiac conditions. In view of this, herbal extracts are found to be the best alternatives so far and thus serve us as sources of new drugs and lead molecules. Due to the presence of phytoconstituents like flavonoids, Glycosides and phenols, it was estimated that the ethanol extracts of *Chloris paraguaiensis steud* exhibit the proposed activity. The gastroprotective efficacy of an ethanolic extract of *Chloris paraguaiensis steud* was evaluated by inducing gastric ulcer using ethanol and pylorus ligation methods at two doses as 250mg/kg and 500mg/kg body weight. The percentage of ulcer protection was 82% for *Chloris paraguaiensis* and 89% for standard, Lansoprazole. The dose at 500mg/kg showed significant and promising activity in comparison with the standard drug. Hence, the gastroprotective effect observed in the present study might be due to phytochemicals such as a flavonoid, glycoside and also it is a dose dependent activity.

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INTRODUCTION

Peptic ulcer is considered as the most aggressive disorder involved related to acid-pepsin, leukotrienes, prostaglandins, mucus secretion release of reactive oxygen species and blood flow along with the other causative agents like alcohol, bacterial infections caused by H.Pylori, stress and excess intake of non-steroidal anti-inflammatory drugs (Nash *et al.*, 1994). To treat this disease, the therapy that is being used includes proton pump inhibitors, antibiotics that can act against H.Pylori infections along with other agents that can reverse the inflammation occurred during ulceration. But, still the disease condition is a recurrent one, and it is not completely curable. In spite of that the excess usage of proton pumps inhibitors the use of antacids, mucosal secretory agents are also used vigorously. Most of them are known to cause side effects such as bone weakness and cardiac disorders which are common for synthetic drugs (Hardman *et al.*, 2001). In this instinct, the extracts obtained from medicinal plants are considered as best available alternatives and used as sources of new molecules for treating gastric ulcers. Many kinds of research had been

performed on ulcers induced in animal models and found herbal extracts having the potential to replace synthetic drugs. Moreover, herbal extracts treating gastric ulcers induced in animal models were shown promising results in many cases assuring their efficacy (Gisbert, 2005).

MATERIALS AND METHODS

Reagents and chemicals

The whole plant of *Chloris paraguaiensis Steud* was collected from in and surroundings of Tirupati and Tirumala hills and was authenticated by Dr.Madhavachetty, Department of Botany, Sri Venkateswara University, Chittoor, Andhra Pradesh, India. The total plant was collected and shade dried to powder coarsely. The powdered plant material was extracted with ethanol by using a Soxhlet apparatus. The extract was used for the determination of preliminary phytochemical analysis and to study the gastroprotective activity (Jones and Raud, 2001).

Lansoprazole was used as the standard drug that was purchased from Sigma Aldrich chemical company, and all the other chemicals and reagents used in the experimental study were of analytical grade.

Phytochemical analysis

The obtained extract is utilised to determine the phytochemical constituents present in the whole plant. Constituents like Alkaloids, Glycosides, Tannins, Phenols, Terpenoids, Saponins were determined by following the standard testing procedures.

Acute toxicity studies

Acute toxicity studies were conducted as per OECD 420 Guidelines by using Wistar albino rats. The pharmacological and acute toxicity studies were approved by the Institutional animal ethical committee bearing the Reg NO: SVCOP/IAEC/007/2016-17.

Gastroprotective activity studies

Wistar albino rats weighing about 200-220g of either sex were selected and fed with a standard diet of pellets and water ad libitum. All the selected animal animals were grouped into 5 groups each bearing 6 animals. The groups were treated as per the experimental protocol (Jugdutt, 2007). The extract was found to be safe at 2000mg/kg body weight. As per LD50 dose calculation 1/8th and 1/4th doses were selected for *invivo* studies, i.e., 250mg/kg and 500mg/kg body weight were used for carrying out the pharmacological activity.

Group, I served as control group received saline orally.

Group II animals served as an ulcerogenic group received ethanol orally.

Group III animals received ethanol extract of CP at a dose of 250 mg/ kg orally.

Group IV animals received ethanol extract of CP at a dose of 500 mg/ kg orally.

Group V was orally administered 20 mg/kg (IP) Lansoprazole as a standard drug.

Acute gastric ulcer induced by absolute alcohol

Acute gastric ulcer was induced to the experimental animals (group II) by administering the 1ml of absolute ethanol to each animal of the group before 30min of the activity through oral route of administration. Ethanol administered animals were sacrificed by euthanasia technique, and stomach part of the animal is removed and flattened at greater curvature. The ulcer affected area of the stomach is observed and measured under a dissected microscope [10X] with a micrometre. Then the ulcer index is calculated as the sum of all the ulcer lesions in mm2 area.

The percentage of gastroprotection was calculated by using the formula given below;

% Gastro protection = UIC-UIT * 100/UIC

UIC= ulcer index in the control group of animals; UIT= ulcer index in a treated group of animals

Ethanol-induced gastric mucosal lesions in pretreated animals

All the selected animals were divided randomly into five groups 6 rats in each. The Gastric lesions were induced with ethanol (96%) at a dose of 0.2 ml/ animal (Moraisa *et al.*, 2010). Forty-five minutes after treatment with plant extract and Lansoprazole, each animal was given orally 0.2 mL of ethanol (96%). and they were sacrificed 30 min later. After 30 min, the rats were sacrificed, and the stomach was removed. The gastric content was collected, and centrifuge for 5 min at 2000 x RPM and the supernatant was separated. The PH, volume of gastric fluid, and total acidity, free acidity, number of gastric ulcers formed and percentage of ulcer protection in gastric ulcer induced animals were determined.

Ulcer induced by a pylorus ligation method

The selected experimental rats were divided into IV groups each of 6 animals. After the fasting period, the rats were anaesthetized with diethyl ether, and the abdomen was opened through which the pyloric end was ligated with a thread. All the test samples were administered 1hr prior to pyloric ligation. Group-I received 1% CMC (1ml/kg,p.o.) that act as control. Group-II received Lansoprazole (20

Table 1: Effect of *Chloris paraguaiensis* on pH, Volume, free and total Acidity, Ulcer lesion, the percentage of ulcer inhibition (Protection) in control and experimental rats

Groups	pH	Gastric volume	Free acidity	Total acidity	Gastric ulcer (No)	% of ulcer protection
Control	1.27±0.01	3.2±0.004	319.3±0.14	348.0±0.9	93.6±0.88	0.0±0
Std. drug	2.64±0.02***	1.26±0.002	123.4±0.17	185.48±0.16	4.76±0.07	89.08±0.29***
250mg/ml	1.78±0.06*	2.60±0.004	187±0.15	263.35±0.15	2.233±0.03	72.53±0.15*
500mg/ml	2.41±0.04**	2.23±0.018	221.7±0.71	245.30±0.56	3.63±0.056	82.1±0.463**

Data is expressed in terms of mean ± S.E.M. for six rats in each group and analysed by one-way ANOVA followed by Dunnett's test. *P<0.05, **P<0.01, ***P<0.001 as compared to disease control group(n=6)

Table 2: Effect of *Chloris paraguaiensis* on pH, Volume, free and total Acidity, Ulcer lesion, percentage of ulcer inhibition (Protection) in control and experimental rats

Groups	pH	Gastric volume (ml)	% of ulcer inhibition	Ulcer area (mm^2)	Ulcer index	Mucus production (mg)
Control	1.28±0.02	3.5±0.03	—	72.93±0.265	13.4±0.07	20.5±0.1
Stnd. drug	2.697±0.03	1.08±0.04	73.15±0.28*	23.365±0.170	3.68±0.06***	52.65±0.4*
250mg/ml	1.61±0.03*	2.7±0.06*	52.8±0.32	51.43±0.24**	9.715±0.07**	29.06±0.2**
500mg/ml	2.5±0.04**	1.51±0.06	67.7±0.32**	46.05±0.1***	5.225±0.13*	48.71±0.14***

Data is expressed as mean ± S.E.M. for six rats in each group and analysed by one-way ANOVA followed by Dunnett's test. *P<0.05, **P<0.01, ***P<0.001 as compared to disease control group(n=6)

Table 3: Phytochemical analysis of ethanol extract of *Chloris paraguaiensis*

S. No.	Constituents	Presence/Absence
1	Alkaloids	+
2	Glycosides	+
3	Tannins	+
4	Flavonoids	+
5	Saponins	-
6	Phenols	+
7	Terpenoids	+

+ indicates Presence, - indicates Absense of constituents

mg/kg, p.o.) act as a standard. Group-III received CP ethanolic extract (250mg/kg, p.o). Group IV received CP ethanolic extract (500 mg/kg, p.o). After 4 hours of pyloric ligation, all the animals were sacrificed to observe gastric lesions. The gastric juice was collected and centrifuged at 1000 rpm for 10 minutes. The volume of gastric juice (ml), as well as pH of gastric juice, was noted. The gastric ulcer score was recorded according to the method described by Tan *et al.* (1996); Nguelefack *et al.* (2005); Hirohashi *et al.* (1993). Gastric contents were assayed for total acidity by titration against 0.01N NaOH using phenolphthalein as indicator. The volume of gastric content was measured, and the total acidity and free acidity were estimated.

Biochemical estimations

Determination of gastric juice volume and pH

The volume and pH of centrifuged gastric contents were measured by pipette and digital pH meter respectively. The volume was expressed in the units ml.

Determination of total and free acidity

The total and free acidity of experimental animals were determined by titrating with 0.01N NaOH using methyl orange and phenolphthalein.

Procedure

1 ml of filtered gastric contents were pipetted out into a beaker, and 2 to 3 drops of methyl orange was added followed by titrating with 0.01 N NaOH until all the traces of red colour got disappeared and changed to yellowish orange. The volume of alkali added indicates free acidity. Then add 2 or 3 drops of phenolphthalein and continue titrating until a definite red tinge reappears. Note the total volume of alkali added that indicate total acidity. The results expressed as Meq/l (Varely, 1998).

Statistical analysis

Values were expressed as mean ± SD for six rats in each group, and statistically significant differences between mean values were determined by one- way analysis of variance (ANOVA) followed by the Dunnett's test for multiple comparisons. The results

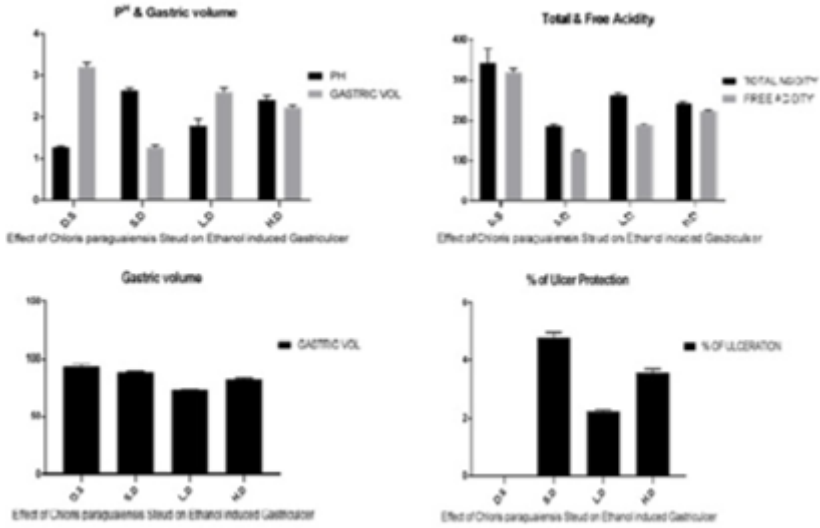

Figure 1: Graphical representation of various parameters of gastroprotective activity of ethanol extract of *Chloris paraguaiensis* in ethanol-induced ulceration

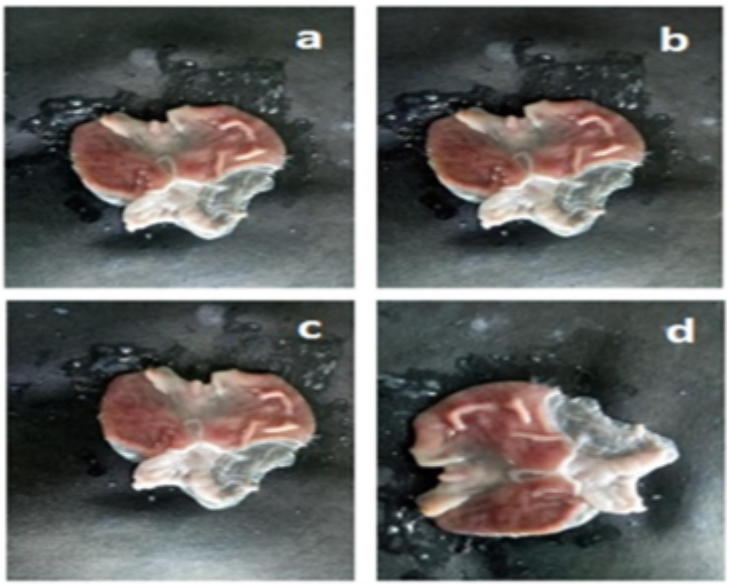

Figure 2: Sectioned stomach of rats treated with ethanol extract of *Chloris paraguaiensis* and standard drug in ethanol-induced ulceration a. Control; b. Standard; c. Extract 250mg/kg; d. Extract 500mg/kg

were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 7 and $p<0.05$; $p<0.01$ and $p<0.001$ were considered to be significant.

RESULTS AND DISCUSSION

Phytochemical analysis

With the help, the standard procedures the determination of phytochemical constituents was performed, and it was evidenced with the presence of flavonoids, alkaloids, glycosides, polyphenols and terpenoids in the extract which is illustrated in Table 1.

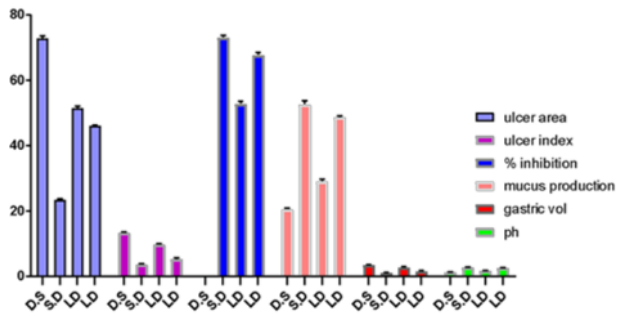

Figure 3: Graphical representation of various parameters of gastroprotective activity of ethanol extract of *Chloris paraguaiensis* in pylorus ligation induced ulceration

Ethanol-induced gastric mucosal lesions in pretreated animals

Oral administration of absolute ethanol (1mg/kg body weight) has produced superficial or deep erosions ulcers. However, pretreatment with ethanol extract of *Chloris paraguaiensis Steud* has reduced the severity of ethanol-induced gastric ulcer as in Figure 1. The gastroprotective effect of 250mg and 500 mg/kg doses of *Chloris paraguaiensis Steud* on the ethanol-induced gastric ulcer was determined and measured for various gastric parameters that are shown in Table 2.

There was significant variation in the result obtained for the gastric parameters of *Chloris paraguaiensis Steud* (500mg/ml) treated group compared to other groups that received the extract (250mg/ml) and with the vehicle-treated animals.

The remarkable reduction in ulcer lesion was observed in treatment with *Chloris paraguaiensis Steud* (500mg/ml). It is obvious to note that increased the volume, total acidity and free acidity and decreased pH of gastric juice were observed in ulcer control rats as compared to normal rats. Animal groups treated with the *Chloris paraguaiensis Steud* (500 mg/kg,) exhibited a competitive reduction of gastric damage against ethanol-induced gastric ulceration. The percentage of ulcer protection was 82.183 % for extract and 72.5% for lower dose treated group with extract [250mg/ml]. Lansoprazole, the positive control included for the study also offered significant protection (89.8%) against ethanol-induced gastric ulcer (Table 2). The *Chloris paraguaiensis* Steud percentage of inhibition was almost near to the range of the standard drug-treated group.

Figure 1 shows the gastric protective pattern of the extracts at two doses in comparison with the standard drug in ethanol-induced gastric ulcers. In the figure a shows the widely disrupted mucosal cells of gastric region whereas b indicated the lesser or almost normal mucosal cells when compared to disease control, c indicates the lesser number of ulcer scores when compared to disease control and has higher ulcer range when compared to drug control and high dose treated animals with sample extract, d indicates the presence of lesser number of ulcer score when compared to standard drug-treated (Lansoprazole).

Pylorus ligated method [PL]

Gastric secretion was followed by a highly significant increase in mucus production from 20.5±0.1mg in the PL control to 29.06±0.2mg and 48.710.14mg for the dose of 250 and 500 mg/kg (P<0.001). Lansoprazole (20 mg/kg) similarly produced a complete inhibition of lesion formation which was accompanied by a highly significant reduction in gastric acid levels (Table 3). The PL control rats showed significantly higher gastric juice volume as compared (3.5±0.03ml) to normal rats. Rats pretreated with Chloris paraguaiensis (250 and 500 mg/ kg) showed a significantly lowered volume of gastric juice (2.74±0.06 and 1.51±0.06ml) (P<0.01 and P<0.001) as compared to the PL control group.

The pH of gastric fluid was significantly lower (P<0.001) in animals pretreated with Chloris paraguaiensis (250 and 500 mg/kg) (1.61±0.03 and 2.56±0.046) as compared to PL control rats (1.28±0.02). Chloris paraguaiensis shows a gastroprotective effect on the PL-induced gastric damage in rats. The gastroprotective effect of 250 and 500 mg/kg doses of Chloris paraguaiensis on total and free acidity are shown in Table 3 . Chloris paraguaiensis -treated groups showed discernable changes in the above parameters as compared to the PL control animals. *Chloris paraguaiensis* had significant (P<0.001) gastroprotective effect at a dose of 250 and 500 mg/kg, since it decreased the PH, Gastric volume, Ulcer area (mm2), Mucus production(mg) as compared to PL control rat.

Figure 2 explains the gastric protective activity of the extracts in comparison to the standard and control in pylorus lighted rats. In figure a shows the widely disrupted mucosal cells of gastric region whereas the Fig b indicated the lesser or almost normal mucosal cells when compared to disease control, Fig c indicates the lesser number of ulcer scores when compared to disease control and has higher ulcer range when compared to drug control and high dose treated animals with sample extract, Fig d indicates the presence of a lesser number of ulcer score when compared to standard drug-treated (Lansoprazole).

The anti-ulcer effect of Chloris paraguaiensis was examined against gastric lesions-induced by pylorus ligation and ethanol. The presence of phenols, terpenoids and flavonoids shows the chances of the protective effect of a natural extract against the tissue damage (Sahin and Gumuslu, 2007). Chloris paraguaiensis prevented the mucosal lesions-induced by ethanol and meliorated pylorus ligation-induced gastric damage. Pylorus ligation produces the mucosal damage by altering the level of cytoprotective PGs and cytokines as well as interfering with the gastric mucosal resistance (Bayir *et al.*, 2006). The secretion and accumulation of gastric acid are two important factors responsible for the produc-

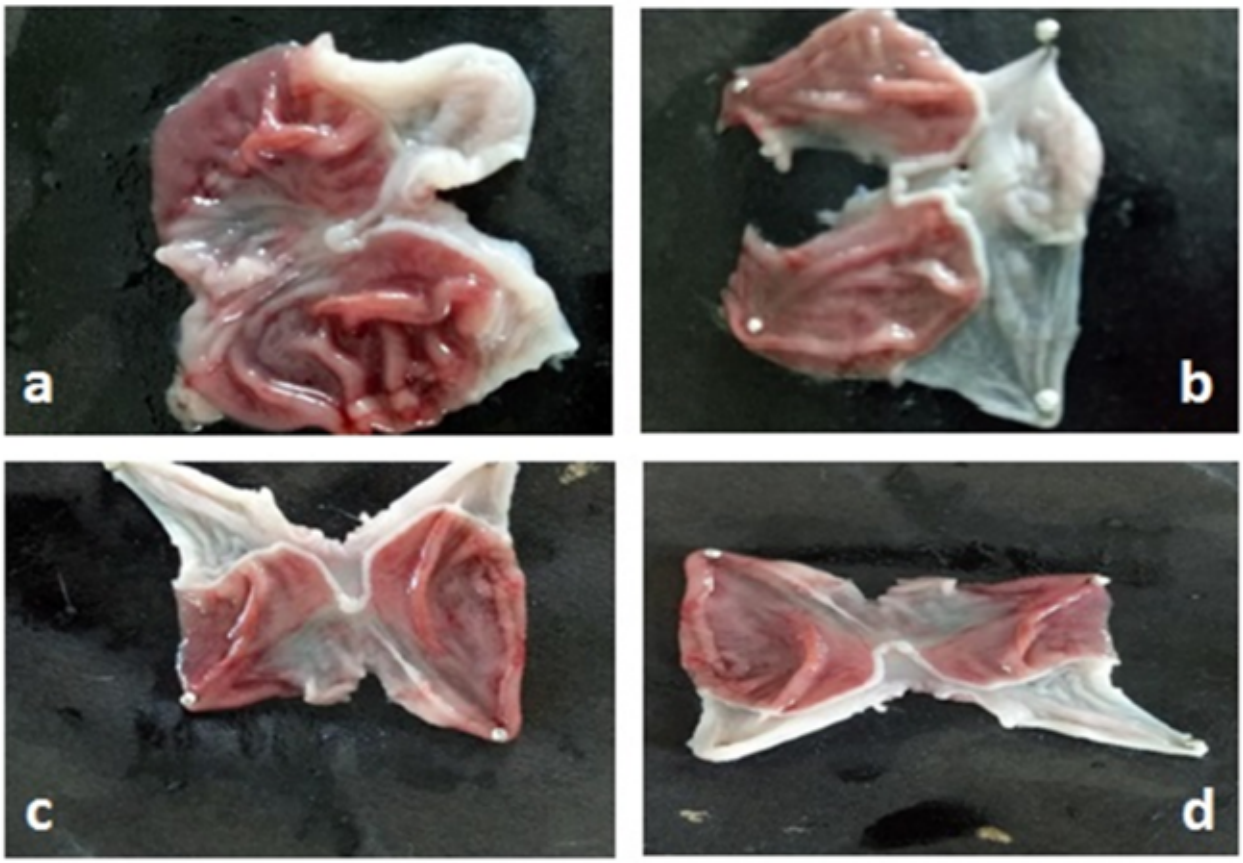

Figure 4: Sectioned stomach of rats treated with ethanol extract of *Chloris paraguaiensis* and standard drug in pylorus ligation induced ulceration a. Control; b. Standard; c. Extract 250mg/kg; d. Extract 500mg/kg

tion of gastric ulcer by pylorus ligation. At the acidic pH 2 pepsinogen is converted to pepsin, whereas its inactivation occurs at pH 6 (Tariq *et al.*, 2007). In the presence of pepsin, acid gets accumulated and causes the gastric ulcer in the pylorus ligated rat and it is successfully treated using ethanol extracts and the activity observed is similar and competent to the standard drug.

CONCLUSION

A gastroprotective potential of ethanol extract of *Chloris paraguaiensis* against gastric ulcer induced by ethanol and pylorus ligation methods were investigated in the present study. Gastric ulcer induced experimental animals successfully indicated the presence of increased gastric volume, acidity and depleted pH. The observed gastroprotection of *Chloris paraguaiensis* is probably attested to a major extent by a gastric mucosal secretion mechanism. The ethanol extract at different doses was able to restore the altered gastric conditions that were induced by ethanol and pylorus ligation methods almost towards normal levels that were also reported by drug control. These combined effects are likely to be accompanied by an upsurge in gastric microcirculation. The percentage of ulcer protection was 82% for *Chloris paraguaiensis* and 89% for standard (Lansoprazole). The positive control selected for the study also offered significant protection against ethanol-induced gastric ulcer. The ethanol extract of *Chloris paraguaiensis* showed a dose-dependent activity wherein the higher dose was competent with the standard drug. Explaining the mechanism, the gastro-protective potential of plant extract observed in the present study was considered likely due to the presence of phytoconstituents such as a flavonoid, a glycoside.

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