

**PHARMACOLOGICAL SCREENING OF HYDROALCOHOLIC EXTRACT FOR
CAMELLIA SINENSIS OF MANAGEMENT OF ULCER****Roshni Mehra, Kanika Dhote*, Vinod K. Dhote, Surendra Jain****Truba Institute of Pharmacy, Bhopal (M.P.)***Corresponding Author's E mail: kanikadhote@gmail.com

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ABSTRACT

Medicinal plants possess an important source of pharmacological effects that acts as new anti-infections, antioxidant and anti-cancer agents. The most important bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids alkaloids, tannins and glycosides which serve as valuable starting material for drug development. Tea (*Camellia sinensis*) is consumed worldwide and is second only to water in its popularity as a beverage. It has ascribed many health benefits viz reduction of cholesterol, protection against cardio-vascular diseases and cancer. By concerning all these studies, we have traced out the presence of phytochemical in *Camellia sinensis* leaves. The phytochemical analysis showed the presence of alkaloids, flavonoids, steroids and tannins by changing the colour of medium when treated with respective reagents.

Keywords: Phytochemical, *Camellia sinensis*, Antiulcer activity, Hydroalcoholic, terpenoids, carotenoids.

INTRODUCTION

Phytochemicals are the bioactive compounds which are present in the medicinal plants¹. The most important bioactive compounds present in *Camellia sinensis* leaves are alkaloid, flavonoids, steroids and terpenoids which serve as valuable starting material for the medicine development²⁻³. *Camellia sinensis* possess an antimicrobial activity because of the bioactive compounds⁴. The plant materials have shown the antimicrobial activities against various pathogenic microorganisms therefore consumption of tea has been associated with reduced risk of major diseases⁵⁻⁶. The beneficial effects of the tea have been attributed to the strong antioxidant activity due to the phenolic compounds⁷. The carotenoids,

flavonoids, benzoic acid, ascorbic acid, tocotrienols, cinnamic acid, folic acid, tocopherols are some antioxidants produced by the plants for their substance⁸.

MATERIAL AND METHODS

Collection of the plant leaves:- The selection of plant was based on traditional use such as antiulcer activity. The leaves of *Camellia sinensis* were collected in the month September from the online markets in Bhopal and identified by department of Botany 'Safia college of Science' Bhopal. (M.P.).

Preparation of Extraction:- Green tea leaves powder was extracted by maceration method using 96% of ethanol. The macerate was then evaporated with a rotary evaporator at a temperature of 40-50 °C to obtain a viscous extract. The yield value was calculated by comparing the obtained extract with the original amount of green tea powder and stated as percentage³⁻⁴.

Phytochemical Test: - Carbohydrates, Proteins and amino acid, Flavonoid glycosides, Lipids and Alkaloid⁷.

Experimental Methods: -

In-vivo test

Aspirin-induced gastric lesions: Aspirin (0.2 g/kg x 3 day) were administered once per day to groups of animals for the number of days specified¹⁴. Animals of control group received 1% carboxy methyl cellulose (CMC) suspension and test group received *Camellia sinensis* suspension orally at two dose levels (200 and 400 mg/kg) for 10 days³. From day 8 the animals received CMC/*Camellia sinensis* two hours prior to the administration of aspirin. Overnight fasted animals were sacrificed by cervical dislocation one hour after the last dose of ulcerogen. The stomach was incised along the greater curvature and examined for ulcers⁷⁻⁸.

Animal group

- Group I- Control- normal saline (normal water)
- Group II- Negative control- Aspirin
- Group III- Aspirin + Standard drug
- Group IV- Aspirin+CS extract (200mg/kg) (Aspirin+ *Camellia sinensis* extract)
- Group V- Aspirin +CS extract (100mg/kg) Aspirin+CS extract (200mg/kg)

Ex vivo:

Macroscopic and microscopic evaluation:

Stomachs were immediately removed after sacrifice, opened along the greater curvature and washed with saline solution (0.9% NaCl). After examination by a blinded pathologist, tissues were photographed and

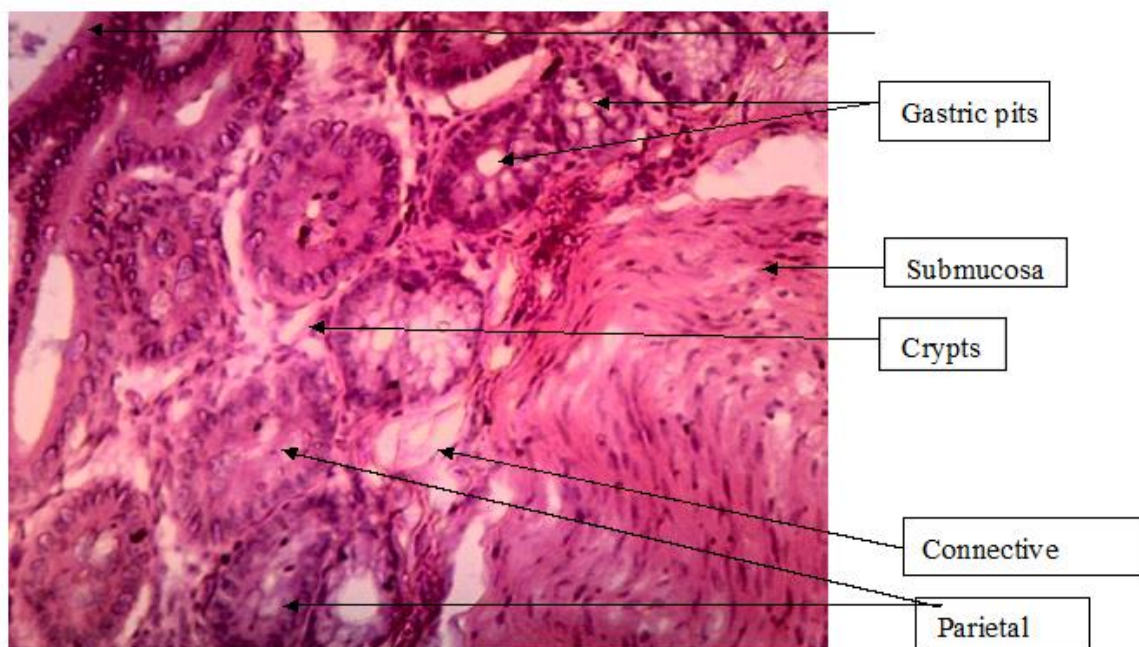
then fixed in buffered formalin to undergo histological preparation ^{3,9-12}. The scoring system from macroscopic and microscopic evaluation used in this work is presented in table 1.

Table1. Macroscopic and microscopic score evaluation-

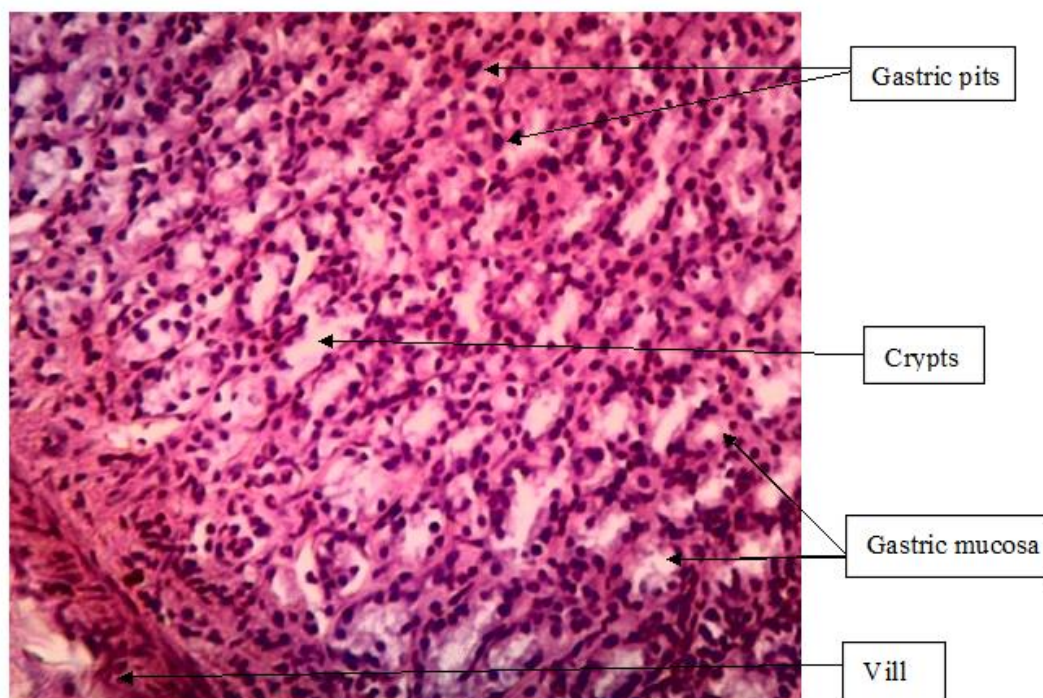
Macroscopic	Score1	Score2	Score3
Hemorrhage			
Size	Punctiform (focal<2.5mm)	Mild (2.5-5.5mm)	Intense bands(>6mm)
Number	0-4	5.5-6.4	>_7
Site	Unilaterals	Bilaterals	
Microscopic	Score1	Score 2	Score 3
Depth of the erosion	Up to 1/3 of total mucosa depth	Up to 1/3 of total Mucosa depth	Total mucosa
Hemorrhage	Focal	Mild	Severe
Inflammation	Light	Mild	Severe
Apoptosis	Light	Mild	Severe

The microscopic evolution score was based in gastric mucosa haemorrhage. The sum of the scores for the different parameters allows calculation of the total macroscopic score, with a maximum possible of 8. The microscopic evaluation score was based in histological analysis. The sum of the scores for the different parameters allows calculation of the total microscopic score, with a maximum possible of 12.

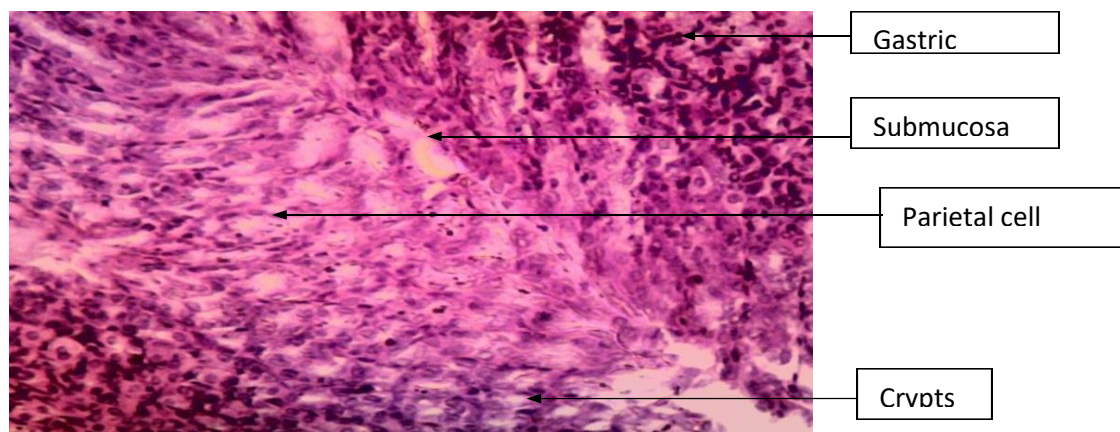
Histological studies: For histopathological examinations, stomachs were embedded in paraffin blocks, sectioned at 5 um intervals, mounted on glass microscope slides and with hematoxylin and eosin. Histological characterization comprised the following parameters: tissue erosion depth, and presence of hemorrhage, inflammation and apoptosis. Representative histology images of each score are presented ^{7, 13-15}.



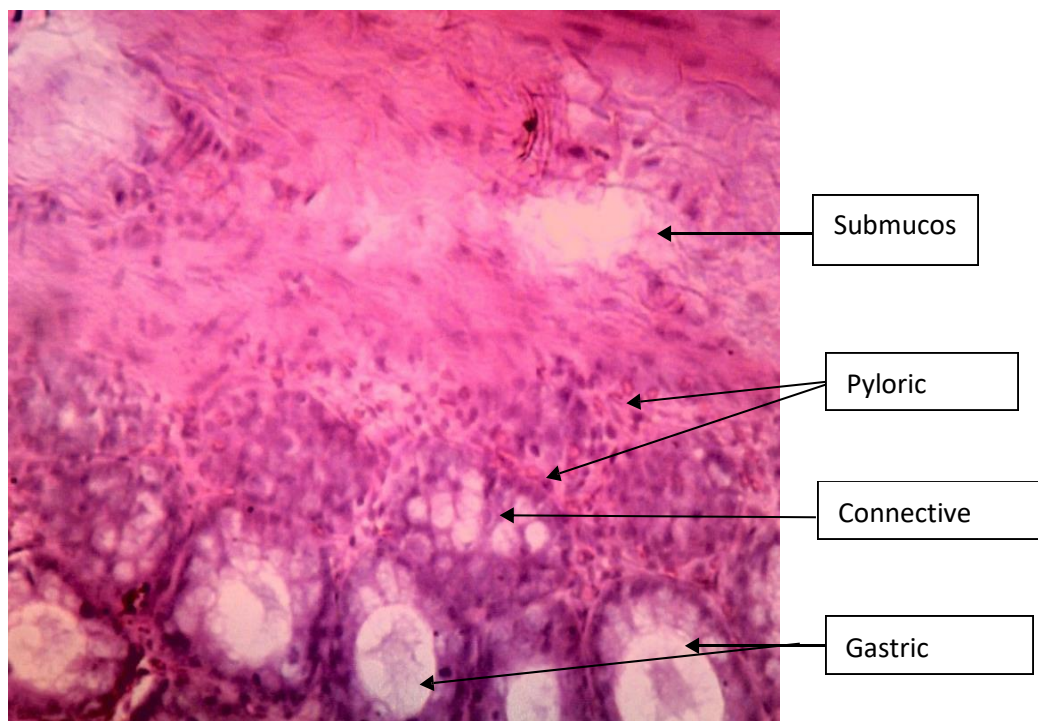
(A) Control- normal saline (normalwater)



(.B) Negative control- Aspirin



(C) Aspirin+CS extract (100mg/kg) (Aspirin+ Camellia sinensis extract)



(D) Aspirin+CS extract (200mg/kg) (Aspirin+ Camellia sinensis extract)

RESULT AND DISCUSSION:Preliminary Quatitative analysis of different extracts of *Camellia sinensis* leaf**Table2. Preliminary Quatitative analysis**

S.No.	Phytoconstituents	Indetification Test	Ee-OH Ext	Aqueous Ext.
1.	Alkaloids	Dandruff's Test	+	+
		Myer Test	+	+
		Wangner's Test	+	+
		Hager's Test	+	+
2.	Corbohydrates	Molish Test	+	+
		Benedict Test	+	+
		Fehling Test	+	+
3.	Flavonoids	Shinoda Test	+	+
		Lead Acetate Test	+	+
		Ammionio Test		
		Vanillin HCl Test	+	+
			+	+
4.	Glycosides	Legal Test	—	—
		Borntagor Bruchard	+	—
		Test	+	+
		Keller Killani Test	+	+
5.	Proteins	Ninhydrin test	—	+
		Biuret test	+	+
		Millons test	+	+
6.	Amino Acids	Ninhgyrin test	—	+
		Biuret test	+	+

Ex Vivo Evaluation:

The gastric ulcer induction assays revealed macroscopic or microscopic mucosal tissue damage in both ethanol Aspirin models. The animals receiving 15mg/kg A ethanol developed a consistent pattern of macroscopic damage, as evidenced by the presence of hemorrhagic ulceration. Stomachs from negative control animals were scored as zero.

Macroscopic and microscopic scores of ethanol Aspirin induced gastric ulcers in control animals (induced non-treated animals; dark grey bars) animals treated with Aspirin (light grey bars) and animals treated with *Camellia sinensis* leaf extract (white bars) .

Gastric mucosal damage was attenuated by the administration of Aspirin (15mg/kg) and camellia sinensis leaf extract (10mg/kg) in both models tested macroscopically and microscopically compared to non-treated animals (control groups). Gastric mucosal damage was attenuated by the administration of Aspirin (15mg/kg) and camellia sinensis leaf extract (20mg/kg) in both models tested macroscopically and microscopically compared to non-treated animals (control groups)

Both macroscopic and microscopic parameters are important tools in understanding the cellular processes in models (Aspirin ethanol). At the macroscopic level, both models present total scores calculated from similar partial evaluation scores (size, number, and site of hemorrhagic lesions). However, microscopic observations showed that aspirin resulted in more extensive hemorrhage in greater inflammation.

Camellia sinensis leaf extract is a proton pump inhibitor which inhibits the production and secretion of acid into the gastric lumen, and its potential harmful action is normally neutralized by gastric mucosal protection mechanisms. In the aspirin-induced ulceration model, this protective mucosal barrier is probably damaged and the exposed mucosa is vulnerable to the harmful action of the acid, preventing the healing and regeneration of the damaged tissues and enlarging the already formed lesions.

The action of tea leaf extract is dependent on its absorption by the body and subsequent action at the level of the proton pump in the gastric mucosa. This process certainly does not occur in time for *Camellia sinensis* leaf extract to exert an anti-ulcer action in the aspirin induction model, in which ulceration is achieved in only 1 hour. The ulcerative process is thus sometimes preponderant, leading to highly variable results.

The anti-ulcer action of *Camellia sinensis* leaf extract is independent of acid production and secretion. *Camellia sinensis* leaf extract induces mucosal protection through gastrin receptor-dependent up-regulation of cyclooxygenase-2 in rats. In this work, *Camellia sinensis* leaf extract granules were dissolved in 10ml distilled water, a common vehicle used to suspend and solubilize this drug. In this work, *Camellia sinensis* leaf extract granules were dissolved in 20ml distilled water, a common vehicle

used to suspend and solubilize this drug. The administration of aqueous water can to some extent exert a neutralizing action, increasing the effect of *Camellia sinensis* leaf extract on the mucosa.

The administration of *Camellia sinensis* leaf extract did not prevent the macroscopic effects induced by aspirin. In our study the animals were fasted for 24 hours before each test and the fasting period may have caused some gastric lesions, allowing the proteins exposed to bind to the *Camellia sinensis* leaf extract. Other mucosal areas may not be damaged by the fasting period and these areas may stay vulnerable to the action of aspirin. When *Camellia sinensis* leaf extract is administered, a sucrose-protein-sulfate complex can form that is resistant to peptidic hydrolysis and indifferent to the action of HCL, bile acids and salts. However, it is not known whether this complex resistant to the absolute aspirin volume administered and part of the protective system produced by *Camellia sinensis* leaf extract may be destroyed.

CONCLUSION

Camellia sinensis leaf extract is rich in catechins, caffeine and L-theanine – the main bioactive compounds behind tea's health benefits. *Camellia sinensis* leaf extract may improve heart health by lowering blood pressure, cholesterol, triglycerides and preventing plaque buildup in the blood vessels. Catechins in the extract may help reduce the risk of multiple types of cancer, L-theanine and EGCG in *Camellia sinensis* leaf extract may boost your immune system and help your body fight infections and viruses. *Camellia sinensis* effect mostly, which promotes relaxation and reduces anxiety. Caffeine and EGCG in the extract may promote weight loss, especially when people consume them together. However, some studies have found that consuming

Camellia sinensis leaf extract did not significantly affect weight loss. *Camellia sinensis* leaf extract may also protect against inflammatory, bone, and liver diseases. The extract is considered safe and people usually tolerate it well. However, consuming high amounts may lead to jitteriness, headaches, skin rashes, nausea, diarrhea and even liver toxicity in rare cases. Studies revealed that administration of aqueous water can to some extent exert a neutralizing action, increasing the effect of *Camellia sinensis* leaf extract on the mucosa.

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