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EFFECT OF *GYMNEMA SYLVESTRE* LEAVES EXTRACT ON GASTRIC ULCER

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ABSTRACT

Recently the world is turn to green/herbal pharmacy because the allopathic drug having major side effect. The main aim of present study was to evaluate the anti ulcer activity of *Gymnema sylverstre*. For the gastric ulcer there are varieties of drugs available but these drugs having major side effects. Bone structure alteration, iron deficiency anemia, risk of vitamin B_{12} deficiency may cause by Cimetidine, Famotidine, Ranitidine, Sucralfate drugs use in gastric ulcer. Cardiac problems may also cause by proton pump inhibitors. Anti ulcer activity study was studied using different leaves extract like pet-ether, chloroform, alcohol, aqueous of *Gymnema Sylverstre* on different models of gastric ulcer, such as acetic acid induced chronic gastric ulcer, pylorus ligation and stress induced ulcer models. The extracts administered orally with the dose of 200mg/kg. All the extracts increase the healing process of ulcer ($P< 0.05$). But alcoholic extract was more significant when it compare with standard ($P<0.001$). In the case of pylorus ligation and stress induce ulcer models alcoholic extract gave more significant anti ulcer activity ($P< 0.001$). So we concluded that alcoholic extract of *Gymnema sylverstre* possess significant anti ulcer activity.

KEY WORDS: anti-ulcer, *Gymnema sylverstre*, acetic acid induce ulcer model, pylorus ligation, stress induce ulcer model.

INTRODUCTION

Gastric ulcer form by the secretion of gastric acid and against it decreases in the secretion of bicarbonate in gastric mucosa. Because of gastric ulcer sharp burning is occur in epigastrin. In the cephalic phase of acid secretion excess amount of acid get secret and it will damage the epithelial layer of stomach.

Allopathic treatment is base on the observation and etiology of disease is base on manifestation of an abnormal physiology. Disillusioned by the non availability of drug to cure in chronic disease and failure of drugs in current use to give long term relief, that is why people are look after for alternative drug therapies as hope of a permanent cure. Unconfirmed and wrong claim of cure provided by traditional drugs leading to lots of misuse. Therefore it is a major area of interest to work on such claims and claims in literature of traditional drugs for exacts scientific search before they are give to humans.

It is fascinating to know that there is advance synthetic chemistry but we are fail to synthesize new molecule which having high therapeutic value and less adverse action than existing molecule. By searching in an ancient literature, identification of natural drug molecule used in local and tribal medicine pursue investigation into the phytochemical profile are emerging as new trend in drug discovery. Isolation of active molecule, finding of their chemical structure and testing of biochemical activity is also advance fame of work. On a basis of ancient literature and tribal medicine very less work has been done and in India there is rich biodiversity so world researcher are eager to study on a dug use in India with hope that they may be potential source for the discovery of newer medicine.

In the last decade rise in awareness to growing biological concept result an increase interest in herbal formulation throughout world. In the search of traditional medicinal plants always they care the hope of new and safe medicines. The WHO has also taken steps to search a new medicine from the plants. With the use of valid phytopharmaceutical techniques efficacy of a number of herbal formulations has been tested.

Recent trend now shifted towards the polyherbal formulation for the treatment of peptic ulcer, because it has been proved that herbal formulation is more advantageous than the synthetic drugs.[1,2] The polyherbal formulation carries various effects like inhibition of acid secretion, decrease formation of free radical and erosion of mucosa etc. by its individual substance or may be by its synergistic effects.

Gymnema sylvestre

Gymnema sylvestre belongs to family Asclepiadaceace[3] it is also known as Madhunashini, Gudmar. It is native to India. For the treatment of diabetes or madhumeha this plant leaves was used in India for over 2000 years. Gudmar or sugar destroyer name is given depend on its function, chewing the leaves it destroy the ability to discriminate the sweet test. Plant

contains resins, saponins, stigmasterol, gymnemic acid, quercitol and amino acid derivative betaine, choline and trimethylamine.

Gymnema sylvestre is a diuretic, stomachic, refrigerant, tonic,[4] along with that it is use in heart disorder, leucoderma, inflammation, piles, curing burning sensation, bronchitis, asthma, biliousnessand ulser.[5]

Gymnema sylvestre possessed anti-diabetic activity by enhancing the secretion of insulin from beta cell of pancreas. Along with that it increase the enzyme activity those who increase the glucose uptake and utilization, and inhibits peripheral utilization of glucose by somatotrophin and corticotrophin. Plant extract have also reported to inhibit epinephrine-induced hyperglycemia.

MATERIAL AND METHOD

Pharmacognostic study

Standardization of Plants

Plant materials *Gymnema sylvestre* was collected from forest of Dharampur, Dist- Valsad, Gujarat and authenticated by Dr. S.B. Narkhede, Asso. Prof Department of Pharmacognosy. The specimens of above plant will place in the herbarium of Smt. BNB Swaminrayan Pharmacy College, Salvav (PCOG.H-221).After the authentication plant was dried at room temperature until they become free from moisture.

Plant material was powdered and passed through sieve #40, and subjected to standardization with the different parameter.

Determination of Alcohol soluble extractive,[6, 7] loss of drying,[7] total Ash, Acid-insoluble Ash,[8] (Table no. 1)

Physicochemical values

Table No. 1: Physicochemical values of root extract of *Gymnema sylverstre*

Sr. No.	Parameters studied	Gymnema sylvestre (leaves)
1	Loss on drying	1.60% w/w
2	**Ash value**	
	Total Ash	6.63% w/w
	Acid soluble Ash	4.20% w/w
	Water soluble Ash	5.15% w/w

3	**Extractive values**	
	Alcohol	12.3% w/w
	Water	16.5% w/w
	Chloroform	5.40% w/w
	Pet-Ether	3.2% w/w

Phytochemical Study

Extraction Methodology

Extraction is defined as treatment of plant or animal tissues with solvent were by the medicinally active constituent are dissolved and most of the inert matter remains un-dissolved.

Methodology for maceration process:[9]

A wide mouth bottle or conical flask which can be well stopper is used for the maceration process. A close container is essential to prevent the evaporation of menstrum in this process the drug is placed with the whole menstrum in closed container for seven days the liquid is strained and marc is pressed. The expressed liquid is mixed with strained liquid. It is than filter to make a clear liquid. The final volume is not arrested. The filtrate is evaporated in vacuum to give the final residue.

Gymnema sylvestre subjected for extraction by using chloroform water. The extract obtained by above described method ware stored in refrigerator for further research process.

Methodology for continues hot extraction process

Gymnema sylvestre

The shade dried leaves of *Gymnema sylvestre* was reduced to fine powder (# 40 size mesh) and around 400gm of powder was subjected to successive continues hot extraction (soxhlet) with petroleum ether (40-60^{o}C), chloroform and alcohol. Each time before extracting with the next solvent the powder material dried in hot air oven at 50^{o}C for one hour. After the effective extraction, the solvents were distilled with each solvent weighed. Its percentage is calculated in term of air dried weight of plant material.

The obtained extracts were subjected to chemical investigation.

Table No. 2: Percentage yield of leaves extracts of *Gymnema sylvestre*

Sr.No.	Solvent	Nature of extract	Colour	% yield
1	Pet-ether	Semisolid	Dark yellow	3.30
2	Chloroform	Semisolid	Dark green	3.90
3	Alcohol	Semisolid	Dark green	9.56
4	Aqueous	Semisolid	Dark green	17.7

Preliminary Phytochemical Screening [10, 11]

Table No. 3: Preliminary Phytochemical investigations of extracts of *Gymnema sylvestre*

Sr, No	Phytoconstituent	Pet-ether	Chloroform	Alcohol	Aqueous
1	Test for carbohydrates	-	+	-	-
2	Tannins	-	+	+	-
3	Amino acid	+	-	-	+
4	Vitamin C	-	-	-	-
5	Phytosterol	-	-	-	-
6	Flavonoids	-	-	+	-
7	Triterpenoids	-	-	+	-
8	Saponins	+	-	+	-
9	Alkaloids	-	-	+	-
10	Fats & oils	-	-	-	-

+ Present, - Absent

Pharmacological activity

1. Animal selection
2. Extract used
3. Acute toxicity study
4. Method of screening

1. ANIMAL SELECTION

Female albino wistar rats of adult age 120gm-150gm, taken from the animal house, Smt BNB Swaminarayan Pharmacy College, Salvave, Vapi (CPCSEA Reg No. 1276/a/09/CPCSEA) were utilize in study. The animals were allowed free access to food and water. Rats were housed in a group of six in clean cages at 25^0C. The bedding materials of the cages were change every day.

2. Extract Used

These all extracts were used for this present study.

Chemical used

Ketamine (Prem Pharmaceutical Ltd, Indore, India), xylazine (Indian Immunologicals, Guntur, India), acetic acid (SD Fine Chem Ltd, Mumbai, India), cysteamine HCl (Hi-media, Mumbai, India) and ethanol (Jebsen & Jessen, Germany), Topfer reagent (Loba chemie, Mumbai), phenolphthalein solution (Nice chemicals, Cochin, India), phenol reagent (Folin Ciocalteau, Loba chemie, Mumbai, India), standard bovine serum albumin (S.D.fine chemicals, Mumbai, India) trichloroacetic acid (Loba chemie Mumbai India).

Toxicity study-Acute toxic class method [12]

As per Organization for Economic Co-operation and Development (OECD) and Control and supervision of experiments on animals (CPCSEA) guideline acute oral toxicity study was carried out.

Methods of screening

Evaluation of Anti ulcer activity.

Acetic acid induced ulcer

The method given by Asad *et al.* (2001) [13] was followed. The animals were fasted for 24 h prior to the experiment. Under ether anesthesia, the abdomen was opened by midline incision below the xiphoid process and the stomach was exposed. Glacial acetic acid (0.05 ml) was added to the cylindrical mould of 6 mm diameter placed tightly over the anterior serosal surface of the stomach and this was allowed to remain there for 60 s. The acid solution was removed by rinsing the mould with normal saline twice or thrice to avoid damage to the surrounding tissues. The stomach was placed back carefully and the abdominal wall was closed. The animals were treated either with different extracts of *Gymnema sylverstre* or with standard drug ranitidine 200 mg/kg p.o. for 10 days after induction of ulcer. On the 11th day, rats were sacrificed by ether anesthesia and the stomachs were removed. The stomach was cut opened along the greater curvature. The ulcerated area and total area were measured and the ulcer index was determined using the formula [14].

Ulcer index = 10/X

Where, X = Total mucosal area / Total ulcerated area.

4.7b Pylorus ligation induced ulcers [15, 16]:

Animals were fasted for 36 h before pylorus ligation with water *ad libitum* by placing them individually in cages to avoid coprophagy and cannibalism. Normal saline (1 mL/rat p.o.) was administered twice daily to all the animals during the fasting period[17]. Under ether anesthesia, the abdomen was opened by midline incision below the xiphoid process. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal wall was closed with sutures. The different drug extracts and standard drug was administered immediately after pylorus ligation. The animals were deprived of food and water during the postoperative period and were sacrificed 6 h after pylorus ligation by overdose of ether anesthesia. The stomachs were isolated and the contents of the stomach were collected and centrifuged.

The gastric juice was used for estimation of free acidity and total acidity[18], pepsin content[19] and total proteins[20]. The stomachs were cut opened along the greater curvature. The ulcer index was determined using the formula mentioned above and the gastric mucin content was determined following method described by Prabha *et al*[21].

Cold restraint stress induced ulcers [22,23]

The ulcers were induced by subjecting the animals to cold restraint stress. Drugs or vehicle was administered 30 min prior to subjection of stress. The animals were placed in a restraint cage and the cage was placed at a temperature of 2-3^{0}C for 2.5 h. The animals were sacrificed and the ulcer index was determined.

RESULT

Acetic acid induced chronic ulcer shows effect healing process

All the extracts produced significant reduction in ulcer index when compared to control. But alcohol extracts in dose of 200mg/kg (p.o.) showed more significant reduction in ulcer index ($p<0.001$) which is similar to the effect of standard drug ranitidine at the dose of 200mg/kg. (Table no. 4, Fing No.1)

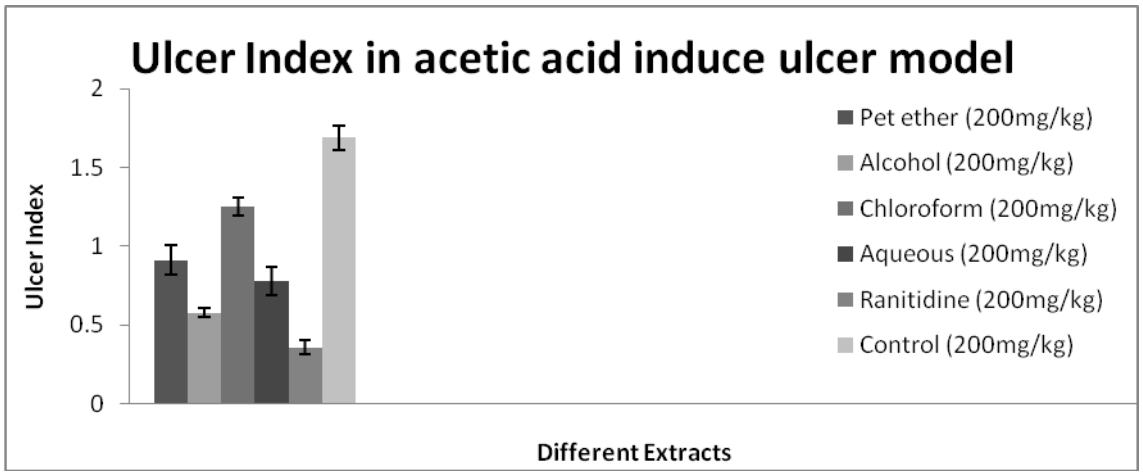

Fig. No.- 1 Ulcer index in acetic acid induce ulcer model

Table No.- 4: Ulcer index of various extracts in acetic acid in duce ulcer model.

Sr No.	Drug extracts	Ulcer Index
1	Pet. Ether 200mg/kg (p.o.)	0.91±0.0937****
2	Alcohol 200mg/kg (p.o.)	0.58±0.0252****
3	Chloroform 200mg/kg (p.o.)	1.25±0.0552**
4	Aqueous 200mg/kg (p.o.)	0.78±0.0865****
5	Ranitidine 200mg/kg (p.o.)	0.36±0.0449****
6	Control 200mg/kg (p.o.)	1.69±0.0784

All values are mean ± SEM, n=6, **p<0.01, ***p<0.001, ****p<0.0001 compared to control group.

Table No 5: Effect of alcoholic extract of *Gymnema sylverstre* on free acidity, total acidity, ulcer index, mucin content, pepsin content and total protein.

Treatment (µg/gm)	Free acidity (mEq/litre) (µmol/6hr)	Total acidity (mEq/litre) (mg/ml)	Ulcer Index	Mucin content	Pepsin content	Total proteins
Control (200mg/kg p.o.)	27.83 ± 1.621	77.00 ± 1.732	1.77 ± 0.083	1.08 ± 0.088	0.46 ± 0.023	10.76± 0.446
Extract (200mg/kg p.o.)	15.66 ± 1.085***	51.50 ± 3.403**	0.7066 ± 0.076***	1.77 ± 0.067***	0.31 ± 0.021***	13.57 ± 0.420*
Ranitidine (200mg/kg p.o.)	10.66 ± 0.881***	38.66 ± 2.404***	0.39 ± 0.041**	2.06 ± 0.051***	0.23 ± 0.016***	15.57 ± 0.420**

All values are mean ± SEM, n = 5-6, *p<0.05, **p<0.01, ***p<0.001 when compared to control group.

Table No. 6: Effect of alcoholic extract of *Gymnema sylverstre* on ulcer index in stress induced gastric ulcers.

Treatment	Stress induced ulcer
Control (200mg/kg p.o.)	1.74 ± 0.0724
Extract (200mg/kg p.o.)	0.8083 ± 0.0780****
Ranitidine (200mg/kg p.o.)	1.13 ± 0.0516****

All values are mean ± SEM, n=6, ****p<0.0001 compared to control group.

Extract effect on Pylorus ligation induce gastric ulcer

Alcoholic leave extract having significant decrease in free acidity (P<0.001), total acidity (P<0.01), and significant increase in mucin content (P<0.001). Extract also produced significant decrease in ulcer index and pepsin content when it compared with control so we can say that alcoholic extract having anti secretary as well as anti ulcer activity. (Table no. 5).

Effect of stress induced ulcer

In stress induced model alcohol extracts showed more significant reduce ulcer index (P<0.0001). (Table no. 6)

DISCUSSION

Because of acid peptic secretion in gastric mucosa get degenerate and necrosis is forming which causes peptic/gastric ulcer. Generally it can take place at any area of the alimentary canal but normally it can take place in duodenum and stomach due to exposure of HCL and pepsin. Many kind of medicine get used in the gastric ulcer but they having limitations and they having major side effects. The anti ulcer or anti secretary drugs are given in table no 7.

Table no. 7: Different kind of drugs use in gastric ulcers.

Sr No.	Class	Examples
Reduction of gastric acid secretion		
1	H2 receptor blockers	Cimetidine, Ranitidine, Famotidine
2	Proton pump inhibitors	Omeprazole, Pantoprazole, Lansoprazole, RAbeprazole
3	Anticholinergics	Pirenzepine
4	Prostaglandins analogs	Mesoprostol
Neutralizing of gastric acid		
1	Systemic antacid	Sodium bicarbonate, Sodium citrate
2	Non systemic antacid	Alluminium Hydroxide, Magnesium hydroxide, Magaldrate, Calcium carbonate
Ulcer protective		Sucralfate
Anti- H. Pylori drugs		Amoxicillin, Clarithromycin, Tinidazole

But all these drug having some adverse effect and limitations like Cimetidine, Famotidine, Ranitidine, Sucralfate, Prostaglandins, Omeprazole, Anticholinergics but many case studies shows that they having many major side effects likes; PPIs having higher risk of fracture[24], alter the iron absorption and causes iron deficiency anaemia[25, 26], risk of vitamine B12 deficiency[27,28].

When acid suppressive therapy is given that time higher chance of community acquired pneumonia is present. Both H2 receptor antagonist and PPIs have major effect on cardiac system. It's prolong atrioventricular conducting time, sinus arrest, sinus bradycardia,[29] and higher risk of community acquired pneumonia[30,31,32]. New drugs Famotidine and Nizatidine shown decrease stroke volume [33, 34,] and also shows negative chronotropic effect[33].

To overcome such problems this study is helpful by evaluating anti-ulcer activity of medicinal plant.

The phytochemical constituent like saponoin, [42][35] terpenoids, flavonoids contents[43][36] showed anti-inflammatory, analgesic and antipyretic activities. The *Gymnema sylverstre* contain all these constituents. So, it may shows anti ulcer activity but for understanding its mechanism further studies were carried out to determine its effect on gastric acid secretion and cytoprotection.

The Pylorus ligated model was used to evaluate gastric anti-secretory effect. Ulcer gets produced by accumulation of gastric acid and pepsin in stomach which is a result of ligated pyloric end.[44][15] Alcoholic extract of *Gymnema sylverstre* decrease secretion on gastric aggressive factors, such as free acidity, total acidity and pepsin and increased secretion of the gastric cytoprotective factor mucin. The further investigation is required to know exact mechanism by which *Gymnema sylverstre* shows anti ulcer activity.

Acetic acid induces gastric ulcer model helps to study the healing effect of gastric ulcer. When we introducing Glacial acetic acid on serosal surface of the stomach, it gets penetrating deeply and produce gastric ulcer which is quite similar to human peptic ulcer. Also it has same healing process. The ulcer index was the parameter for this model. This parameter gives an idea about ulcer area and morphology of ulcer area. The oral administration of different extracts of *Gymnema sylverstre* showed anti ulcer activity but out of all extracts alcoholic extract give more beneficial anti ulcer activity. This alcoholic extract was used for the further two anti ulcer models.

Nowadays stress is major factor to cause many kind of disease out of that gastric ulceration is one off it. Stomach will secrete more acid when we apply stress on animal but ulcer production having complex pathophysiology. Histamine is a major component which leads higher acid secretion and a reduce mucus production[37, 38]. Alcoholic extract of *Gymnema*

sylverstre reduce ulcer development as compare to standard and other extracts. Further investigation is required for mechanism of anti ulcer activity of Gymnema sylverstre's extract.

CONCLUSION

From above experiment we concluded that all extract of *Gymnema sylverstre* having significant anti ulcer activity in chronic and acute gastric ulcer model but out of that alcoholic extract was more potent.

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