

ANTI-ULCER ACTIVITY OF *WITHANIA SOMNIFERA* IN STRESS PLUS PYLORIC LIGATION INDUCED GASTRIC ULCER IN RATS

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Abstract: Anti-ulcer activity of methanolic extract of *Withania somnifera* (Ashwagandha) and its action against stress plus pyloric ligation induced gastric ulcer in rats has been reported. Treatment with *Withania somnifera* extract (100 mg/Kg/day p.o.) for 15 days significantly reduced ulcer index as compared to control group. Extract also significantly reduced volume of gastric secretion, free acidity and total acidity. Significant increase in total carbohydrate (TC) and TC / total protein (TP) ratio of gastric juice was also observed. No significant change in the total protein was noted. A significant increase in antioxidant enzymes viz. catalase, superoxide dismutase (SOD) but decrease in malondialdehyde (MDA) was observed. *Withania somnifera* extract was found to be an effective antiulcerogenic agent, whose activity can well be compared with that of ranitidine hydrochloride. The present study suggests that *Withania somnifera* have not only inhibitory effects on release of gastric hydrochloric acid but it also increase various defensive factors including antioxidant defense to protect gastric mucosal damage.

Key words: Anti-ulcer activity, *Withania somnifera*, Stress plus Pyloric ligation

INTRODUCTION

Peptic (gastric and duodenal) ulcer is a common gastrointestinal disorder. It can be attributed to either *H. pylori* or NSAIDs-induced mucosal damage [1] besides, imbalance between aggressive and defensive factors [2]. Many herbal drugs are being used as ayurvedic or in Indian traditional medicinal system for the management of peptic ulcer. Shatawari (*Asperagus racemosus*) and ashwagandha (*Withania somnifera*) are commonly used in ayurvedic prescriptions. However, experimental evidences in favour of their antiulcerogenic nature is lacking. *Withania somnifera* commonly known as "Ashwagandha" or Indian ginseng or winter cherry is classified as "Rasayana" or rejuvenator in traditional Indian medicinal system. It is one of the most commonly used drug, not only as an antistress and adaptogenic agent, but is also known to increase life span and delay aging [3]. Bioactive constituent

isolated from ashwagandha withaferin has also shown antitumor activity [4]. It is also known to prevent tardive dyskinesia neurological syndrome [5]. Drug is mild sedative and help in reducing excitement and pain. Its antistress activity is mentioned along with tulsi (*Ocimum sanctum*) [6]. Methanolic extract of the root exhibited significant antistress property [7-10]. Ashwagandha is a main constituent of various adaptogenic and antistress tonics. Antioxidant properties and anxiolytic action of ashwagandha extract has been also reported [11]. Bhattacharya et al. [7] for the first time showed antioxidant activity of withanolides. They showed dose dependent increase in SOD, CAT and GPX activity in frontal cortex and striatum after 21 days treatment. Scartezani and Sreroni [12] also showed antioxidant property of ashwagandha root powder. Dhuley [13] and Panda and Kar [14] demonstrated anti-lipid peroxidation properties as well. In a recent study Misra et al. [15] showed therapeutic action of ashwagandha in chromium(VI)

induced thyroid hormones (TSH, T3, T4) disturbances. Present study was undertaken to explore the anti-ulcer and antioxidant activity of *Withania somnifera* root extract using swim stress plus pyloric ligation induced gastric ulcer model in rats.

MATERIALS AND METHODS

Animals: Adult (one and half month old, body weight 225 to 275 g) albino rats (Wistar strain) of the either sex were used in the present study. The animals were acclimatized for ten days in polytharaine cages in laboratory conditions. All the rats were fed with standard laboratory diet and given water *ad libitum*.

Before experimentation rats were deprived of food but allowed free access to water for 12 hours. They were subjected to swim type restraint stress. Care was taken to avoid coprophagy. Pyloric ligation was carried out only for the collection of gastric juice. Drinking water was withheld one hour prior to pyloric ligation. The animals were maintained as per the norms of IAEC and the experiments were cleared by IAEC and the local institutional ethical committee.

Drug preparation: Dried roots of ashwagandha were purified using absorption method by keeping them in contact with brick powder. After purification, the roots were packed in high quality filter paper and methanolic extract was prepared by continuous extraction method with the help of Soxhlet extractor. After vacuo evaporation crude extract was suspended in 0.5 % carboxy methyl cellulose (CMC).

Experimental groups: Rats were divided into five groups containing four animals in each group:

Group 1: Rats were given only vehicle (0.5% CMC) each day upto 15 days.

Group 2: Rats were given stress (Swimming for six hours every day) for 15 days.

Group 3: Rats were given stress (Swimming) plus drug *Withania somnifera* (100 mg/kg/day, bw, p.o.) for 15 days.

Group 4: Rats were given only *Withania somnifera* (100 mg/kg/day, bw, p.o.) for 15 days.

Group 5: Rats were given stress (Swimming) plus Ranitidine (30 mg/kg/day, bw, p.o.) for 15 days.

On 16th day pyloric ligation was performed and rats were sacrificed. Physical parameters and biochemical investigations were then performed.

Water immersion (Swim) restraint ulcer model: The method described by Brodie et al. [16] and Takagi

and Okabe [17] was employed with small modification. Rats were fasted for 12 hours, care being taken to avoid coprophagy. Rats were immersed in water up to xiphoid process for 6 hours, for 15 days. The temperature of water was maintained at $24 \pm 5^\circ\text{C}$. *Withania somnifera* root extract was administered (orally) 30 minutes prior to the swim stress.

Pyloric ligation: Rats were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al. [18], avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anesthetic ether after four hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The inner surface of free stomach was examined for gastric lesions.

Dose schedule: Dose 100 mg/kg of body weight was given to the rats daily (between 8.00 to 10.00 a.m). Dose was administered orally (using feeding tube) everyday for fifteen days.

Physical parameters: Ulcer Index (Score \pm SEM and $\text{mm}^2 \pm \text{SEM}$) was determined by the method of Ganguli and Bhatnagar [19]. Volume of gastric secretion (ml/100 gm) was determined by the method of Parmar et al. [20].

Biochemical investigation: Free acidity (mEq./L) was measured by method of Hawk [21], total acidity (mEq./L) by Hawk [21] and Goyal [22], total carbohydrate ($\mu\text{g}/\text{ml}$) by Nair [23], total protein ($\mu\text{g}/\text{ml}$) by method of Lowry et al. [24] and total carbohydrate(TC)/ total protein(TP) ratio were determined. Catalase was determined by method of Sinha [25], superoxide dismutase (SOD) by method of Winterbourn et al. [26], malondialdehyde (MDA) by the method of Buege and Aust [27] and ascorbic acid was measured by method of Natelson [28].

RESULTS

Rats were given 6 h swim stress daily for fifteen days showed increase in ulcer index and volume of gastric content (Table1), free and total acidity (Table 2) respectively as compared to control. Contrary to this, total carbohydrate TC/TP ratio (Table 3) were significantly decreased. Study showed no significant

Table 1: Effect of *Withania somnifera* on ulcer index (score and mm²) and volume of gastric secretion in stress induced gastric ulcerative rats.

Group	Treatment	Dose mg/kg/day, bw, p.o.	Free Acidity (MEq./Litre \pm SEM)	Total Acidity (mEq./Litre \pm SEM)
I	Control	Vehicle	11.00 \pm 1.16	41.75 \pm 3.09
II	Stress (Swim)	-----	23.50 \pm 3.18 ⁺⁺	65.00 \pm 4.04 ⁺⁺
IV	Stress + <i>W. somnifera</i>	100	8.00 \pm 1.73 ^{**}	38.50 \pm 2.60 ^{**}
VI	Only <i>W. somnifera</i>	100	10.00 \pm 1.13 ^{**}	43.50 \pm 3.07 ^{**}
VII	Stress + Ranitidine	30	17.50 \pm 0.87	46.50 \pm 5.48 ^{***}

All values are represented as Mean \pm SEM (n = 6) P value: + < 0.001; ++ < 0.01; +++ < 0.05 when compared with control untreated animals. * < 0.001; ** < 0.01; *** < 0.05 when compared with stress induced gastric ulcerative model.

Table 3: Effect of *Withania somnifera* on total carbohydrate (TC), total protein (TP) and TC/TP ratio in stress induced gastric ulcerative rats.

Group	Treatment	Dose mg/kg/day, bw, p.o.	Total Carbohydrate (TC) (μ g/ml \pm SEM)	Total Protein (TP) (μ g/ml \pm SEM)	TC/TP Ratio \pm SEM
I	Control	Vehicle	593.25 \pm 11.12	410.25 \pm 35.46	1.49 \pm 0.16
II	Stress (Swim)	-----	259.00 \pm 7.51 ⁺	475.00 \pm 20.21	0.545 \pm 0.04 ⁺⁺
IV	Stress + <i>W. somnifera</i>	100	424.00 \pm 45.03 ^{***}	390.00 \pm 63.51	1.12 \pm 0.07 [*]
VI	Only <i>W. somnifera</i>	100	492.25 \pm 14.33 [*]	385.00 \pm 11.16 ^{**}	1.28 \pm 0.04 [*]
VII	Stress + Ranitidine	30	399.00 \pm 14.43 [*]	525.00 \pm 25.98	0.76 \pm 0.12 ^{**}

All values are represented as Mean \pm SEM (n = 6) P value: + < 0.001; ++ < 0.01; +++ < 0.05 when compared with control untreated animals. * < 0.001; ** < 0.01; *** < 0.05 when compared with stress induced gastric ulcerative model.

Table 2: Effect *Withania somnifera* on free and total acidity in stress induced gastric ulcerative rats.

Group	Treatment	Dose mg/kg/day, bw, p.o.	Free Acidity (MEq./Litre \pm SEM)	Total Acidity (mEq./Litre \pm SEM)
I	Control	Vehicle	11.00 \pm 1.16	41.75 \pm 3.09
II	Stress (Swim)	-----	23.50 \pm 3.18 ⁺⁺	65.00 \pm 4.04 ⁺⁺
IV	Stress + <i>W. somnifera</i>	100	8.00 \pm 1.73 ^{**}	38.50 \pm 2.60 ^{**}
VI	Only <i>W. somnifera</i>	100	10.00 \pm 1.13 ^{**}	43.50 \pm 3.07 ^{**}
VII	Stress + Ranitidine	30	17.50 \pm 0.87	46.50 \pm 5.48 ^{***}

All values are represented as Mean \pm SEM (n = 6) P value: + < 0.001; ++ < 0.01; +++ < 0.05 when compared with control untreated animals. * < 0.001; ** < 0.01; *** < 0.05 when compared with stress induced gastric ulcerative model.

change in total protein (Table 3). Treatment of *Withania somnifera* extract significantly reduced ulcer index, volume of gastric content (Table 1), free and total acidity (Table 2). A significant increase in total carbohydrate and TC/TP ratio (Table 3) was noticed but no change was seen in total protein. Similarly, treatment with Ranitidine hydrochloride also significantly decrease ulcer index, gastric content (Table 1), total acidity (Table 2) but there was no significant change in free acidity (Table 2). There was a significant increase in total carbohydrate and TC/TP ratio (Table 3) but change in total protein was insignificant.

Antioxidant enzymes (CAT and SOD) as well as MDA and ascorbic acid were measured. In stress animals CAT and SOD were significantly decreased

Table 4: Effect of *Withania somnifera* on catalase and superoxidedismutase (SOD) in stress induced gastric ulcerative rats.

Group	Treatment	Dose mg/kg/day bw, p.o.	Catalase (μ moles of H ₂ O ₂ utilized/ min./mg of protein \pm SEM)				Superoxidedismutase (SOD) (Percentage inhibition of NBT reduction \pm SEM)			
			Stomach	Liver	Lungs	Kidney	Stomach	Liver	Lungs	Kidney
I	Control	Vehicle	29.50 \pm 5.78	57.13 \pm 2.73	24.13 \pm 1.20	38.88 \pm 5.94	67.05 \pm 2.11	85.22 \pm 0.85	77.75 \pm 2.03	84.74 \pm 0.76
II	Stress (Swim)	-----	2.50 \pm 0.87 ⁺⁺	21.00 \pm 2.87 ⁺	4.00 \pm 1.44 ⁺	21.50 \pm 2.02 ⁺⁺⁺	35.00 \pm 8.66 ⁺⁺⁺	76.20 \pm 1.33 ⁺⁺	68.12 \pm 3.18 ⁺⁺⁺	75.52 \pm 2.68 ⁺⁺⁺
IV	Stress + <i>W. somnifera</i>	100	7.00 \pm 1.15 ^{***}	39.25 \pm 1.88 ^{**}	16.00 \pm 2.31 ^{**}	45.50 \pm 4.33 ^{**}	72.52 \pm 3.27 ^{**}	82.40 \pm 1.42 ^{***}	79.58 \pm 1.46 ^{***}	83.95 \pm 0.75 ^{***}
VI	Only <i>W. somnifera</i>	100	12.25 \pm 0.72 [*]	36.25 \pm 2.74 ^{**}	12.75 \pm 2.46 ^{***}	36.50 \pm 2.02 ^{**}	65.65 \pm 2.03 ^{***}	86.19 \pm 0.28 [*]	84.40 \pm 0.28 ^{**}	85.31 \pm 1.48 ^{***}
VII	Stress + Ranitidine	30	10.00 \pm 1.73 ^{**}	53.25 \pm 3.33 [*]	13.00 \pm 0.58 ^{**}	34.75 \pm 4.19 ^{***}	70.35 \pm 2.62 ^{**}	82.08 \pm 1.76 ^{***}	80.38 \pm 0.64 ^{**}	81.71 \pm 0.97

All values are represented as Mean \pm SEM (n = 6) P value: + < 0.001; ++ < 0.01; +++ < 0.05 when compared with control untreated animals. * < 0.001; ** < 0.01; *** < 0.05 when compared with stress induced gastric ulcerative model.

Table 5: Effect of *Withania somnifera* on malondialdehyde (MDA) and ascorbic acid in stress induced gastric ulcerative rats.

Group	Treatment	Dose mg/kg/ day, bw, p.o.	Malondialdehyde (MDA) (n moles/ml \pm SEM)				Ascorbic Acid (mg/100 ml \pm SEM)			
			Stomach	Liver	Lungs	Kidney	Stomach	Liver	Lungs	Kidney
I	Control	Vehicle	5.27 \pm 1.04	6.05 \pm 0.99	5.26 \pm 0.96	7.52 \pm 1.29	1.39 \pm 0.09	1.29 \pm 0.16	1.21 \pm 0.16	1.21 \pm 0.06
II	Stress (Swim)	-----	9.68 \pm 0.36 ⁺⁺	11.96 \pm 0.83 ⁺⁺	14.17 \pm 0.76 ⁺⁺	15.34 \pm 1.26 ⁺⁺	0.33 \pm 0.03 ⁺	0.34 \pm 0.04 ⁺	0.26 \pm 0.04 ⁺⁺	0.29 \pm 0.03 ⁺
IV	Stress+ <i>W.somnifera</i>	100	4.82 \pm 0.54*	8.76 \pm 0.64** *	6.79 \pm 0.81*	9.17 \pm 0.38**	0.98 \pm 0.21***	0.80 \pm 0.12** *	0.92 \pm 0.19** *	1.18 \pm 0.10*
VI	Only <i>W.somnifera</i>	100	2.73 \pm 0.21*	6.95 \pm 0.47**	4.80 \pm 0.87*	5.28 \pm 0.21*	1.11 \pm 0.10*	1.17 \pm 0.08*	1.32 \pm 0.10*	1.40 \pm 0.08*
VII	Stress + Ranitidine	30	6.58 \pm 0.59**	8.24 \pm 0.62** *	7.16 \pm 0.38*	10.17 \pm 1.26** *	0.72 \pm 0.09**	0.78 \pm 0.16** *	1.12 \pm 0.12*	0.69 \pm 0.08**

All values are represented as Mean \pm SEM (n = 6) P value: + < 0.001; ++ < 0.01; +++ < 0.05 When compared with control untreated animals. * < 0.001; ** < 0.01; *** < 0.05 When compared with stress induced gastric ulcerative model.

(Table 4). After drug treatment a significant increase in CAT and SOD levels was observed (Table 4). MDA showed significant increase in stress group (Table 5), while ascorbic acid showed significant decrease (Table 5).

Withania somnifera treatment to stressed animals decreased the MDA level (Table 5) but ascorbic acid level was significantly increased as compared to control and only drug treated animals. Administration of ranitidine also showed similar results (Table 5).

DISCUSSION

It is evident from the present investigation that the methanolic root extract of ashwagandha possesses antiulcer activity as in stress+pyloric ligation induced gastric ulcer rat model. The treatment of ashwagandha root extract for 15 days (daily single dose) has significantly reduced gastric lesions/haemorrhagic spots and reduced the volume of gastric content, free acidity and total acidity. Decreased ulcer index and gastric content are implicated with the protective effects of drug. Control group animals treated with drug only showed no significant difference in gastric content when data were compared to controls.

Stress plays an important role in etiology of gastric ulceration [29]. Apart from psychological factors, stress induced ulcer is also caused by number of factors such as surgery, head injury, shock, sepsis, neurological disorders, parasitic infections etc. The

etiopathology of gastric ulcer is not known in most cases [2,30], but it is generally accepted that it results from an imbalance between aggressive factors (gastric HCl, pepsin secretion, *H. pylori* infection, alcohol, NSAIDs (Non steroidal anti inflammatory drugs), malignancy etc.) and defensive factors (break down of gastric mucosal barrier, endogenous prostaglandin's, secretin, somatostatin, epidermal growth factors, blood flow etc). These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. Aspirin, phenylbutazone and some non-steroidal anti-inflammatory drugs are also known to cause duodenal and gastric ulceration [31]. Prostaglandin E₂ and I₂ are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant – like phospholipid secretion in the gastric epithelial cells is also stimulated by the prostaglandin [32]. In addition, Brodie [16,33] also showed development of gastric ulcers in pyloric ligation model. Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach by the accumulating acid [18].

Mucin is viscous glycoprotein with physicochemical properties producing relatively resistant acid barrier [34]. It makes up the major part of the mucus, an important pre-epithelial factor that acts as a first line of defense against ulcerogens [35]. The mucus gel

layer is a complex secretion containing inorganic materials, secretory IgA, lactoferrin, high molecular weight glycoprotein (which is composed of mainly galactose, galactosamine, glucosamine, fructose carbohydrate) and proteins containing serine threonine, proline and alanine amino acids [36]. There are approximately 600 carbohydrate side chains per molecule of glycoprotein. The total carbohydrate and total protein (TC/TP) ratio is indicative of the mucus-secreting index [37,38]. Decrease in mucosal secretion is thus considered important in gastric ulceration [2].

In present study both stress + pyloric ligation group showed significant decrease in total carbohydrate and TC/TP ratio, free acidity and total acidity. While treatment with drug extract showed significant increase in TC/TP ratio of the gastric juice. However, they did not have any effect on total protein of the gastric juice. In our study administration of 30 mg/kg/day, bw, p.o. ranitidine (a allopathic drug commonly prescribed to ulcer patients) for 15 days to stress + pyloric ligation groups rats also produced significant decrease in ulcer index, volume of gastric content, free acidity, total acidity and significant increase in the total carbohydrate, total protein and TC/TP ratio. This protection may be due to antisecretory effect of both ashwagandha extract and ranitidine on gastric acid secretion.

The treatments with ashwagandha extract and ranitidine also affect the antioxidant defense as evidenced by significantly increased level of antioxidant enzymes (catalase and superoxide dismutase) in the stomach and liver after pre-treatment with *Withania somnifera* root extract (100mg/kg/day, bw,p.o) as well as ranitidine. The catalase and superoxide dismutase levels were significantly decreased in rats treated with stress followed by pyloric ligation as compared to controls. Interestingly, significant decrease in lipid peroxidation, indicated by decrease in malondialdehyde as compared to stress treated group was also observed after drug (extract) treatment. Malondialdehyde is the end product of the lipid peroxidation [27]. Similarly, ascorbic acid is the water-soluble molecule, found both intra and extra cellularly in most biological systems. It is a scavenger of reactive oxygen metabolites and a predominant plasma antioxidant. Ascorbic acid also regenerates active vitamin E, which is a known free radical scavenger and increases the cholesterol excretion [39] In stress + plus pyloric ligation group rats ascorbic acid content was significantly decreased, while pre-treatment with the

drug extract as well as ranitidine treatment, increased the ascorbic acid level. Overall data clearly indicate that anti ulcerogenic effects of *Withania somnifera* extract are approximately similar to commonly prescribed allopathic drug ranitidine. However, since ranitidine has various side effects, the *Withania somnifera* root extract could be a safe and effective alternative for the treatment of gastric ulceration.

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