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THERAPEUTIC EFFECT OF LEAVES OF *AYAPANA TRIPLINERVIS* IN INDOMETHACIN INDUCED MILD, MODERATE AND SEVERE GRADE GASTRIC ULCER

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Keywords:

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ABSTRACT: Peptic ulcer disease (PUD) is increasing gradually in India as well as worldwide. Excess use of non-steroidal anti-inflammatory agents (NSAID) and lifestyle factors cause gastric mucosal damage. This study was designed to search out the gastro-protective efficacy of hydromethanolic extract of *Ayapana triplinervis* on different graded ulcers. Mild to severe grade of gastric ulcer has been developed by oral administration of IND at the doses of 10mg, 20mg and 40mg/kg body weight for 15 days. Gastric ulcer were assessed by elevation of ulcer score, ulcer index, pepsin activity and gastric volume in IND-treated groups in respect to control group. Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) levels were elevated in IND-treated groups in respect to control due to withdrawal of prostaglandin and cyclooxygenase (COX-2) enzyme induced suppression. Significant protection in the levels of these ulcerogenic parameters were observed after the treatment of *Ayapana triplinervis* (200mg/kg) when compared with the standard drug omeprazole (20mg/kg). The important antioxidant enzymes activities were decreased but lipid peroxidation levels elevated in all the ulcerated groups($p<0.05$). Gastric-occult test was positive in 40mg IND-treated group. Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in serum measured for toxicity assessment. All the parameters that altered in ulcerated groups were significantly protected towards the control level after *Ayapana triplinervis* or omeprazole treatment. This significant protective efficacy observed in histopathological evaluation of gastric tissue. *Ayapana triplinervis* significantly protect the mild, moderate and severe grade ulcers by its antioxidant activity and withdrawal of IND-induced suppression of prostaglandin and COX-2 levels.

INTRODUCTION: Gastric ulcer (GU) is a major public health burden in the present century [1] with a high morbidity of about 5–10 %. Annually 200,000 people are hospitalized with the diagnosis of ulcer and the financial cost of the treatment of this disease reaches about 4 billion dollars [2].

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Infection of *Helicobacter pylori*, smoking, alcohol consumption, distress, haemorrhagic shock, sepsis, nutritional deficiencies and long-term use of NSAIDs are major causes of gastric ulceration [3]. Around 25% cases of gastric ulceration are caused by NSAIDs [4].

Indomethacin (IND) is mainly used for the treatment of stroke, pain control, rheumatoid arthritis, osteoarthritis, tendonitis and other inflammatory diseases [5]. Experimentally, IND is widely used for the induction of GU due to its higher ulcerogenic potency compared to other NSAIDs [6, 7].

Gastric mucosal damage including erosion, ulceration, bleeding and perforation are the most significant adverse effects associated with NSAIDs [8]. Many synthetic anti-ulcer drugs have been developed For gastric ulcer treatment. However, these agents are facing major problems due to their partial effectiveness against gastrohelcosis and severe side effects, for instance, dizziness, diarrohea, gynecomastia, hypoacidity, impotence, osteoporotic bone fracture, hypergastrinaemia, skin rash and cardiovascular disease risks [9, 10, 11]. Thus, new drugs with high efficacy and low toxicity are desirable for the prevention and treatment of gastric ulcers. For safety and therapeutic efficacy, the World Health Organization (WHO) recommends medicinal plants [12]. Medicinal plants and their extracts have an important therapeutic role against many diseases without significant side effects [13]. There are many plants with ethno-pharmacological background that have been gastro-protective and used in traditional medicine [14, 15]. The purpose of this study design is to develop different graded gastric ulceration in experimental model and to search out whether our selected plant *Ayapana triplinervis* (AT) has protective efficacy even on severe grade ulcers or not [16, 17]. Rats were treated with three different doses of indomethacin 10mg, 20mg and 40mg/kg body weight/day for 15 days **Table 1** and after sacrifice accordingly to clinical manifestation of gastric environment and different gastric secreting parameters, we have divided these three different doses of indomethacin treated experimental rats in mild, moderate and severe grade ulcerated group. Now the gastro-therapeutic efficacy of hydromethanolic extract of *Ayapana triplinervis* was investigated on mild, moderate and severe grade ulcers.

TABLE 1: DIFFERENT DOSES OF INDOMETHACIN TREATMENT AND ULCER MANIFESTATION

	Mild (IND10)	Moderate (IND20)	Severe (IND40)
Ulcer score	10-15	15-30	<30
Ulcer lesion	Mild ulcer small lesion	Damaged tissue	Deep ulcerating bleeding
Smell	No smell	Bad smell	Foul smell
Discharge	No discharge	Mild discharge	Profuse discharge
Stomach wall	Smooth	Rough	Very Rough

MATERIAL AND METHODS:

Chemicals: Indomethacin purchased from Tokyo Chemical Industry, Mumbai, India. Omeprazole procured from Alfa Aesar (UK). GOT, GPT kits were purchased from Span Diagnostic Limited, Surat, India. Prostaglandin E_2 and COX-2 ELISA Kit purchased from Elabscience biotechnology Co., Ltd, Wuhan, China. TNF-α and IL-6 ELISA kits obtained from Raybiotech Co., Peachtree Corners, United States. Alcian blue obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). All other chemicals were of the highest purity grade commercially accessible.

Plant Collection: Fresh leaves of *Ayapana triplinervis* were collected from the local area of Medinipur, identified and authenticated by the taxonomist. The voucher number of the specimen is VU/CM/102.

Preparation of Extracts: Fresh leaves of *Ayapana triplinervis* were washed in normal distilled water and shed dried at room temperature and crushed. The crushed leaves were dissolved in hydro-methanol (40:60) in a glass jar in the air tight condition for 7 days with occasional shaking. The filtrate was collected and dried at 40°C under reduced pressure in a rotary evaporator (N1200AS-W, EYELA, Japan). Finally, this was kept in an owing bottle and stored at 4°C to use in experiment [18].

Experimental Animals: Albino rats of Wistar strain having body weight 150±10g, were used for this study approved by the Institutional Animal Ethical Committee with the ethical number No.VU/IAEC-I/CM-1/3-6/19 dated 11/12/19. Rats were collected from Saha Enterprise, an authorized dealer of CPCSEA registered under the Ministry of Environment and Forest. Rats were divided into four cages and kept for 15 days for acclimatization in our animal houses with adequate temperature, humidity along with proper diet and water.

Induction of Gastric Ulcer: Gastric ulceration was induced in the animals by oral administration of indomethacin at the dose of 10mg, 20mg and 40 mg/kg body weight/day. They were deprived of

food before 2 hours of indomethacin treatment but had free access to water [19]. 2 hours after the indomethacin treatment rats were treated with AT (200mg/kg) or omeprazole (20mg/kg).

Animal Grouping and Treatments: Sixty male albino rats were randomized into ten groups equally.

Group I: Control group (CON): Rats of this group received only vehicles.

Group II: Indomethacin treated group (IND 10): Rats were given indomethacin at the dose of 10 mg/kg b.w /day.

Group III: Ayapana treated group (IND10AT): Rats were treated with indomethacin (10mg/ kg/day) followed by *Ayapana triplinervis* at the dose of 200 mg/kg b.w.

Group IV: Omeprazole treated group (IND10OMZ): Rats were treated with indomethacin (10mg/ kg/day) followed by omeprazole at the dose of 20 mg/kg b.w.

Group V: Indomethacin treated group (IND 20): Rats of this group were treated with indomethacin at the dose of 20mg/kg b.w.

Group VI: Ayapana treated group (IND20AT): These indomethacin (20mg/kg/day) treated rats were also administered with *Ayapana triplinervis* at the dose of 200 mg/kg b.w.

Group VII: Omeprazole treated group (IND20OMZ): These indomethacin (20mg/kg/day) treated rats were treated with omeprazole at the dose of 20 mg/kg b.w.

Group VIII: Indomethacin treated group (IND 40): Rats were treated with indomethacin at the dose of 40mg/kg b.w.

Group IX: Ayapana treated group (IND40AT): Rats were also treated with indomethacin at the dose of 40mg/kg b.w. After 2 hours these rats were again treated with *A. triplinervis* at the dose of 200mg/kg b.w.

Group X: Omeprazole treated group (IND40OMZ): These rats were also treated with indomethacin at the dose of 40mg/kg followed by omeprazole treatment at the dose of 20mg/kg b.w.

The experiment was continued for 15 days. On 16th day animals of all the groups were sacrificed and the abdomen was opened with clamping of the pyloric end for the collection of gastric juice. Stomach was collected and stored in -20°C for biochemical study. Pylorus ligation shows the possible changes of other gastric parameters like gastric content, volume, total acidity and pH. One piece of vertically incised stomach portion kept in bouins solution for histological study. Blood was collected and serum was separated for biochemical and immune assay.

Estimation of Gastric pH: After sacrifice, gastric content was collected and centrifuged for 10 min at 1000 rpm (4°C) then the clear supernatant was collected and used for the determination of pH by using pH meter [20].

Gastroccult Test (Gastric Occult Blood Test): The Gastroccult blood test is based on alpha guaiaconic acid with hydrogen peroxide reaction in the presence of heme to produce a highly conjugated blue quinone compound.

It is a qualitative screening method for detecting the existence of occult blood and determining the pH of gastric aspirate or vomitus. Results cannot be considered conclusive evidence of presence or absence of gastrointestinal bleeding [21].

Ulcer Quantification: Rats were Sacrifice and stomachs were opened and gastric ulcer score was measured by standard protocol [22]. In the 10mg dose treated group 10-15 ulcerated areas are observed and we considered this group as mild ulcers. In the 20mg treated group 15-30 ulcerated areas are found. We consider these groups as moderate ulcerated groups and in the 40mg treated group above 30 ulcerated areas are observed and it is the severe ulcerated group **(Table 1).**

Determination of Pepsin Activity: Pepsin activity was determined by using hemoglobin as the substrate. Specific pepsin activity expressed by ug/ml [23].

Determination of Mucin Content: The conventional approach was used to determine the mucin content [24]. Optical density of mucin content was determined at 605 nm and standard curve developed with various mucin concentrations.

Determination of Free Acidity and Total Acidity: Gastric free acidity and total acidity were measured by the standard method [23]. Gastric juice was taken followed by addition of few drops of Topfer's reagent and phenolphthalein respectively for free and total acidity. Titrated against NaOH, and the volume of NaOH added was recorded. The formula for the calculation of gastric acidity was given bellow:

Acidity = (Volume of NaOH × actual normality of NaOH × 100)/0.1 mEq/L.

Determination of Prostaglandin E_2 (PGE_2) and Cyclooxygenase-2 (COX-2): Serum PGE_2 levels and COX-2 measured in gastric tissue were measured using ELISA kits [25] according to manufacturer protocols. Value was expressed as pg/ml and ng/ml [26].

Determination of Tumor necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6) in Stomach: TNF-α and IL-6 in stomach tissue homogenate were measured by using standard ELISA kits according to the protocol provided by the manufacturer. The values were expressed as pg/ml [27].

Antioxidant Activity:

Biochemical Estimation Catalase (CAT) and Superoxide Dismutase (SOD), Glutathione peroxidase (GPx) and GSH: The stomach tissue was homogenized in ice cold 0.5 M Tris-HCl buffer (pH 7.0) and centrifuged. Supernatant was collected and catalase activity was measured by standard method at 240 nm [28].

The activity of superoxide dismutase was estimated using the standard method [29]. The stomach tissue was homogenized and centrifuged. 10 mM pyrogallol and 50 mM Tris-HCl (pH 8.4) was mixed with the supernatant and absorbance was taken at 420 nm in a spectrophotometer using Tris-HCl as blank. The mucosal surface of the stomach was collected by scraping, and then weighed and homogenised in 2 mL of 50 mM Tris-HCl buffer containing 20 mM EDTA and 0.2 mM sucrose (pH 7.5). Then the homogenate was immediately precipitated with 0.1 mL of ice-cold 25% trichloroacetic acid, centrifuged at 4000 rpm for 40 min at 4°C. The supernatant was used for the determination of GSH levels. Absorption was measured at 412 nm using a spectrophotometer. The GSH levels in the gastric mucosa were expressed as nmol/mg tissue [30, 31].

Malondialdehyde (MDA) and Conjugated Diene (CD) Level: Phosphate buffer (pH 7.4) was used to homogenize the stomach tissue. Using thiobarbituric acid-trichloro acetic acid (TBA-TCA) mixture the homogenates were boiled for 10 minutes at 100^{o}C and cooled at room temperature. The supernatant was collected after centrifugation at 4000×g for 10 min and OD was measured at 535 nm [32]. Phosphate buffer (pH 7.4) was used for homogenization of stomach tissue. Chloroform: methanol (2:1) mixture was used for the extraction of the lipid layer and centrifuged at 1000×g for 5 min then evaporated. Cyclohexane was used to dissolve the lipid residue and absorbance was measured at 233 in a UV-spectrophotometer [33].

Histological Study: Stomach tissue processing was done according to the standard method and the section was prepared at 5 µm using a Leica RM2245 microtome [34].

Statistical Analysis: Inhibition against ulceration was expressed in percentage. Other results were expressed as Mean ± SEM. One-way analysis of variance (ANOVA) followed by multiple comparisons two tailed ‘t test’ using the SPSS software package for differences between means was used to detect any significant difference ($p<0.05$) between the treatment groups in this study [23].

RESULTS:

Behavioral Changes: Remarkable physical and behavioral changes were observed in indomethacin treated all the ulcerated groups. We have carefully observed the activities, weaknesses and movement of the experimental animals. It has been found that in mild ulcerated groups, weakness, abnormal movement was not observed but activities were reduced remarkably. In moderate and severe ulcerated groups remarkable signs of weakness, abnormal activities were noted and normal activities were reduced. Sign of weakness and abnormal movement and reduced normal activities were most remarkably observed in the severely ulcerated group than the other ulcerated groups **(Table 2)**.

TABLE 2: BEHAVIORAL CHANGES IN MILD, MODERATE AND SEVERE ULCERATED RAT

Observation	Mild (IND 10)	Moderate (IND 20)	Severe (IND 40)
Indomethacin dose(mg/kg bw/day)	10	20	40
Sign of weakness	-	+	+
Number of rats with signs of weakness	-	++	++++++
Abnormal movement	-	+	+
Number of rats with abnormal movement	-	+	+++
Reduced activities	+	+	+
Number of rats with reduced activities	+	++	++++

Key: + signifies present while – signifies absent.

Ulcer Score, Ulcer Index, Preventive Index and occult Blood Test: The ulcer score and ulcer index were significantly elevated in 10mg, 20mg and 40mg doses of indomethacin treated group in comparison to control group. Ulcer score and ulcer index were significantly reduced towards the control level in all the *Ayapana triplinervis* (AT) and omeprazole (OMZ) treated groups. Ulcer scores in all the indomethacin treated groups significantly differ from each other and it is highest in 40mg/kg indomethacin treated groups. Preventive index was highest in IND10AT and IND10OMZ treated groups i.e., 80.82% and 82.19% respectively. In case of IND20AT and IND20OMZ treated groups, preventive indexes are 64.06% and 64.84%. Preventive indexes in IND40AT and IND40OMZ treated groups are 61.95% and 59.51%.

Occult blood test of gastric content showed positive in 20mg/kg and 40mg/kg indomethacin treated groups **Table 3.**

TABLE 3: EFFECT OF *AYAPANA TRIPLINERVIS* ON ULCER SCORE, ULCER INDEX, PREVENTIVE INDEX AND OCCULT BLOOD TEST

Group	Treatment	Ulcer scores	Ulcer index	Preventive index (%)	Occult Blood (OB)
	CON	0	0	-	-VE
Mild	IND 10	12.16 ± 0.75^{a}	1216.66	-	-VE
	IND10AT	2.33 ± 0.42^{b}	233.33	80.82	-VE
	IND10OMZ	2.16 ± 0.40^{b}	216.66	82.19	-VE
Moderate	IND 20	21.33 ± 1.92^{c}	2133.33	-	+VE
	IND20AT	7.66 ± 0.66^{d}	766.67	64.06	-VE
	IND20OMZ	7.5 ± 0.42^{d}	750	64.84	-VE
Severe	IND 40	34.16 ± 1.13^{e}	3416.67	-	+VE
	IND40AT	13 ± 0.51^{f}	1300	61.95	-VE
	IND40OMZ	13.83 ± 0.65^{f}	1383.33	59.51	-VE

Values are expressed as Mean ± SEM, n= 6. ANOVA followed by two-tail t-test. Different superscripts (a,b,c,d,e and f) indicate the significant difference ($p<0.05$).

Gastric pH, Free Acidity, Total Acidity and Pepsin Activity: Gastric pH were reduced but gastric free acidity, total acidity and pepsin activity were significantly elevated in 10mg, 20mg and 40mg doses of indomethacin treated groups when compared with control group ($p<0.05$). These parameters were significantly recovered towards the control level in all the AT and OMZ treated groups. More significant restoration was noted after *Ayapana triplinervis* and OMZ treatment in mild (IND10AT, IND10OMZ) ulcerated groups than other ulcerated groups **Table 4.**

Mucin Content: Alcian blue is an indicator of the mucus content in gastric mucosa. Regular treatment of indomethacin at different doses causes significant degeneration of gastric mucosal layer, as well as a significant reduction in alcian blue binding capacity of gastric mucosa in comparison to the normal control group. Whereas treatment of AT and OMZ significantly increase the alcian blue binding with the mucosal layer in mild, moderate and severe ulcerated groups. More significant recovery was observed in the mild ulcerated group than moderate and severe ulcerated group **Table 4.**

TABLE 4: EFFECT OF *AYAPANA TRIPLINERVIS* ON GASTRIC SECRETORY PARAMETERS IN DIFFERENT GRADED ULCERATED RATS

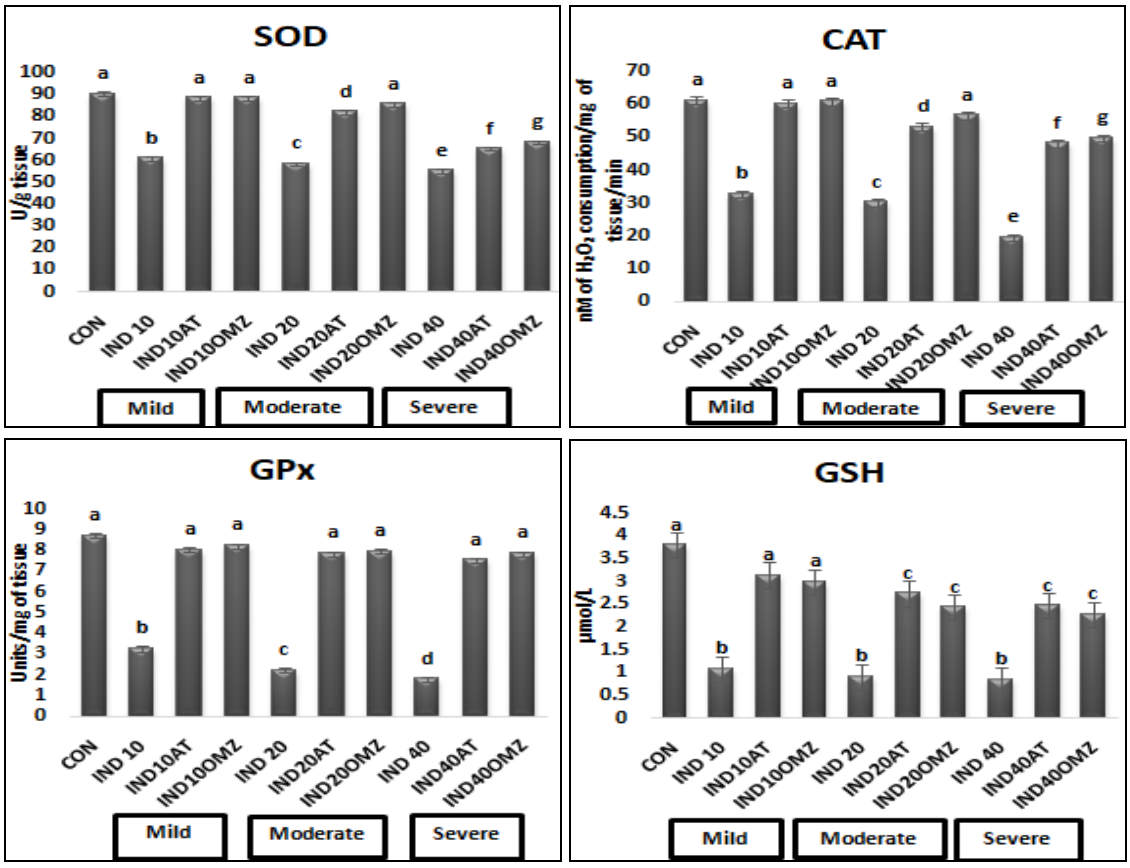
Groups	Parameters	Gastric volume	Gastric pH	Mucin content (µg alcian blue/	Pepsin activity	Free acidity (µEq/L)	Total acidity (µEq/L)

		(ml)		g wet stomach)	(µg/ml)		
Mild	CON	0.93±0.01[a]	4.53±0.10[a]	245.66±1.64[a]	99.66±1.38[a]	25.65±0.92[a]	37.53±0.75[a]
	IND 10	1.463±0.10[b]	4.14±0.05[b]	100.33±0.76[b]	225.66±1.33[b]	35.22±0.91[b]	66.75±0.56[b]
	IND10AT	0.96± 0.01[a]	4.373±0.07[a]	243.05±0.94[a]	100.61±0.70[a]	26.98±0.76[a]	39.43±0.39[a]
	IND10OMZ	0.95±0.004[a]	4.52±0.10[a]	242.37±0.84[a]	101.27±0.46[a]	26.72±0.91[a]	39.38±0.68[a]
Moderate	IND 20	1.73±0.07[c]	3.25±0.07[c]	74.16±1.16[c]	262.33±1.56[c]	39.77±0.89[c]	68.61±0.56[c]
	IND20AT	1.05±0.05[d]	3.59±0.16[d]	223.5±0.76[d]	138.08±0.75[d]	33.15±0.83[d]	53.23±0.81[d]
	IND20OMZ	0.98±0.003[a]	4.33±0.09[a]	219.87±1.03[a]	137.08±0.75[a]	27.85±0.64[a]	40.06±0.45[a]
Severe	IND 40	2.33±0.08[e]	2.45±0.06[e]	51.33±0.49[e]	282.66±0.95[e]	48.60±0.65[e]	73.53±0.77[e]
	IND40AT	1.34± 0.13[f]	3.37±0.08[f]	198.5±2.36[f]	169.33±0.49[f]	37.94±0.75[f]	59.87±0.75[f]
	IND40OMZ	1.32± 0.01[g]	3.49±0.12[g]	199.83±0.87[g]	167.30±0.89[g]	40.05±0.77[g]	63.20±0.69[g]

Values are expressed as Mean ± SEM, n= 6. ANOVA followed by two-tail t-test. Different superscripts (a,b,c,d,e,f and g) indicate the significant difference ($p<0.05$).

Activities of CAT, SOD, GPx and GSH in Stomach Tissue: Indomethacin treatment significantly reduced the activities of CAT, SOD, GPx and GSH in stomach tissue. These enzyme activities were significantly recovered in all the hydromethanolic extract of leaf of AT and OMZ treated groups in comparison to the control groups. More significant protection of these parameters were noted to the control level after the treatment of AT in the mild ulcerated group **Fig. 2** than other ulcerated groups.

MDA and CD Levels in Stomach Tissue: Gastric MDA and CD levels were significantly elevated in all the indomethacin treated ulcerated groups when compared with the control group. These parameters were significantly recovered towards the control level in all the AT and OMZ treated groups in comparison to control groups. More significant recovery was noted to the control level after the treatment of AT and OMZ in mild ulcerated groups **Fig. 2.**



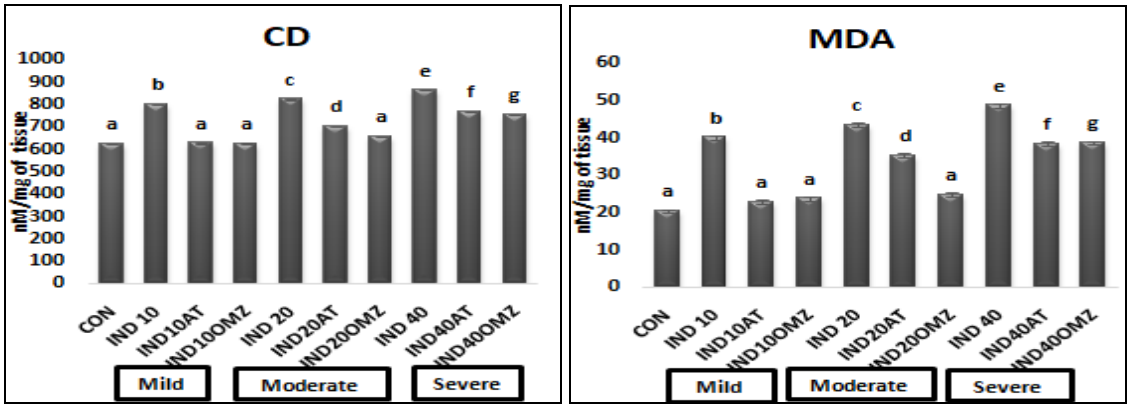


FIG. 2: *AYAPANA TRIPLINERVIS* ON ANTIOXIDANT ACTIVITIES AND LIPID PEROXIDATION LEVELS IN DIFFERENT GRADED ULCERATED RATS. Values expressed as Mean ± SEM, n= 6. ANOVA followed by two-tail t-test. Different superscripts (a, b, c, d, e, f and g) indicate the significant difference ($p<0.05$).

Serum Prostaglandin E_2 (PGE_2) and COX-2 Level: Serum PGE_2 and COX-2 levels were significantly decreased in all the indomethacin induced mild, moderate and severe ulcerated groups in respect to the control group. These two parameters were significantly recovered towards the control level in hydro-methanol extract of leaf of AT and OMZ treated mild, moderate and severe ulcerated groups. In case of PGE_2 at IND10AT (96.84%), IND20AT (91.24%) and IND40AT (78.45%); and in COX-2 the recovery rate were noted IND10AT (97.24%), IND20AT (91.36%) and IND40AT (90.59%) **Fig. 3.** More significant recovery were observed to the control level in the mild ulcerated groups.

Tissue Tumor Necrosis Factor (TNF-α) and Interleukin 6 (IL-6): Gastric tissue TNF-α and IL-6 levels were elevated significantly in all the indomethacin treated groups in respect to the control group. These parameters were significantly recovered in AT and OMZ treated mild, moderate and severe ulcerated groups **Fig. 3.**

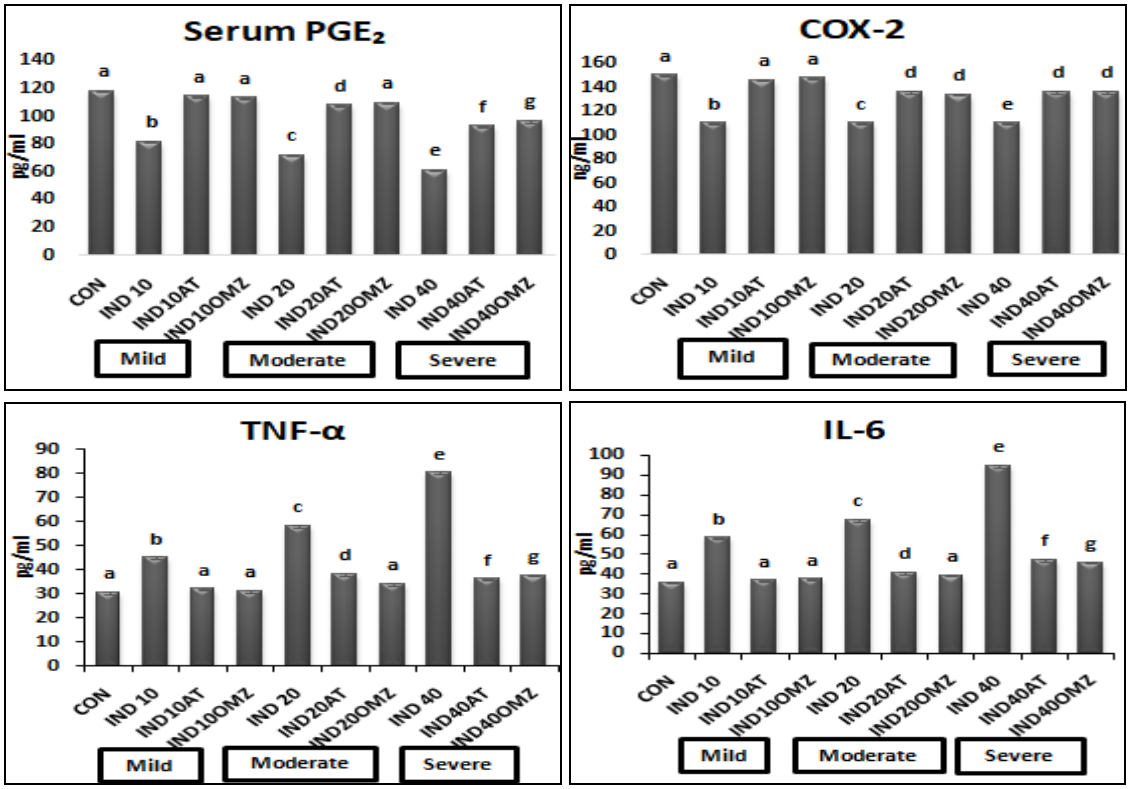


FIG. 3: EFFECT OF *AYAPANA TRIPLINERVIS* ON TNF-α, IL-6, PGE_2 AND COX-2 IN INDOMETHACIN TREATED DIFFERENT ULCERATED GROUPS. Values expressed as Mean ± SEM, n= 6. ANOVA followed by two-tail t-test. Different superscripts (a, b, c, d, e, f and g) the significantly differ from each other ($p<0.05$).

More significant recovery of these parameters was observed in the mild ulcerated group in respect to the moderate and severe ulcerated group.

In TNF-α, IND10AT (70.95%), IND20AT (65.74%) and IND40AT (44.87%); and in case of IL-6, IND10AT (62.83%), IND20AT (60.38%) and IND40AT (49.85%) respectively in compared to different ulcerated group such as mild (IND 10), moderate (IND 20) and severe (IND 40).

Activities of SGPT and SGOT: There was significant elevation in SGPT and SGOT activities observed in all indomethacin treated groups when compared with control groups. A significant restoration was noted towards control level in hydromethanolic extract of leaf of AT and OMZ treated all the ulcerated groups **Fig. 4.** More significant recovery was noted in mild ulcerated groups in respect to moderate, severe ulcerated groups.

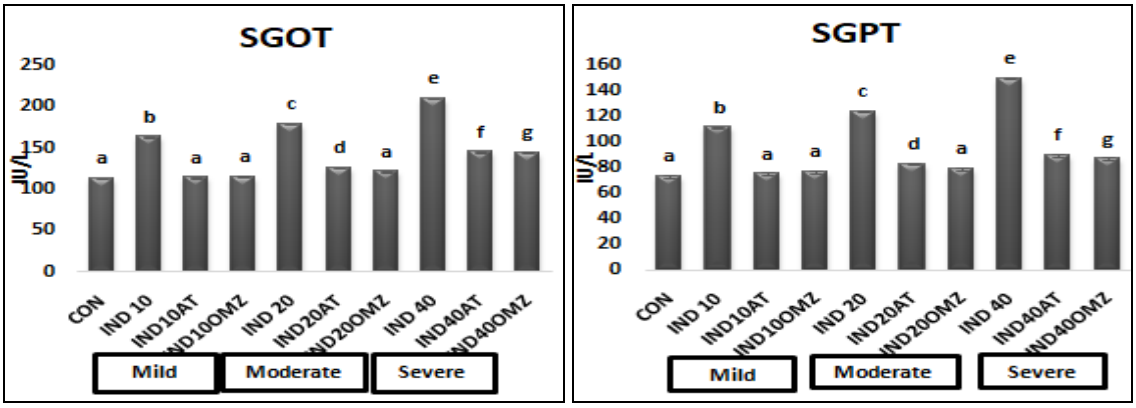


FIG. 4: EFFECT OF *AYAPANA TRIPLINERVIS* ON SERUM GOT AND GPT ACTIVITIES IN INDOMETHACIN TREATED ULCERATED RATS. Values expressed as Mean ± SEM, n= 6. ANOVA followed by two-tail t-test. Different superscripts (a, b, c, d, e, f and g) indicate the significant difference from indomethacin treated groups ($p<0.05$).

Histological Studies: Histomorphological study of gastric tissue carried out by haematoxylin and eosin (H & E) staining. Indomethacin treated groups resulted in marked changes in gastric tissue morphology in respect to control. AT treated rats showed significant recovery of the mucosal layer, thereby indicating gastroprotective effect of *Ayapana triplinervis* against indomethacin induced gastric ulcerative damage in rats **Fig. 5.**

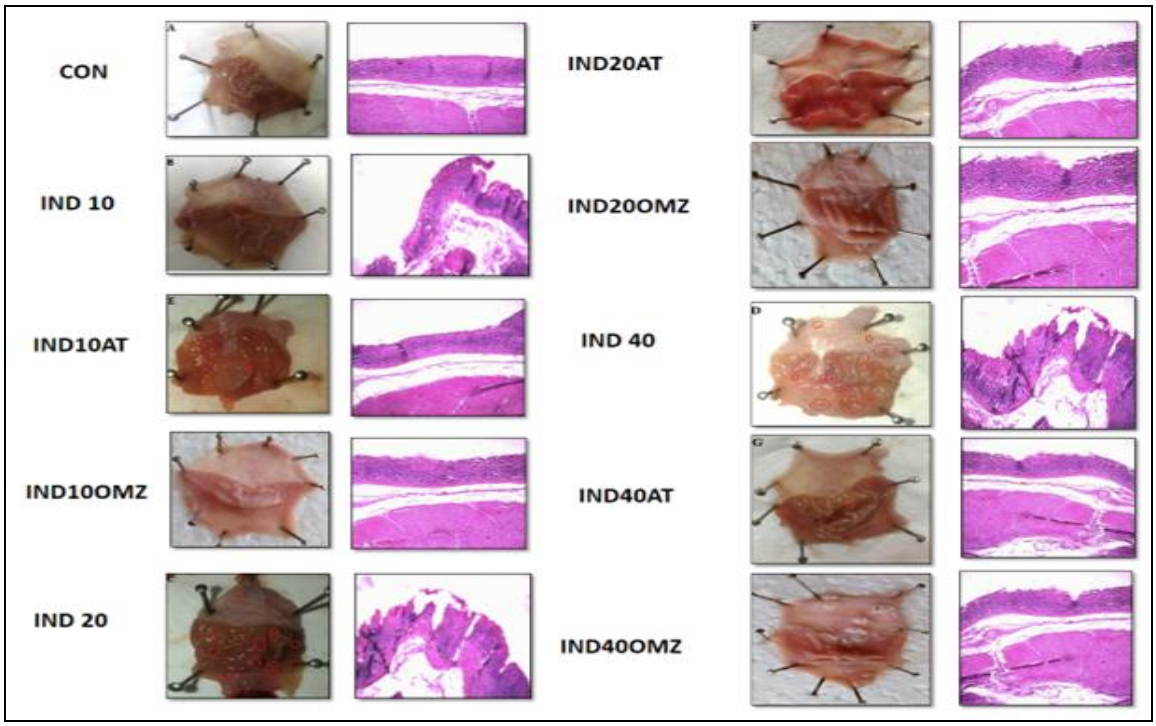


FIG. 5: MAGNIFICATION VIEW OF GASTRIC MUCOSAL SURFACE

CON indicate the normal stomach histoarchitecture of control group. IND 10 showed a very few number of black spots or ulcerated areas. IND 20 showed a significant elevation of black spot or ulcerated areas. IND 40 showed the increasing number of black spot or ulcerated areas. Extract treated groups (IND20AT and IND40AT) shows significant recovery and a decreasing number of the ulcerated area. The IND10AT and omeprazole groups show significant recovery and very less number of ulcerated areas.

DISCUSSION: Stomach ulcers are open sores formed due to the damage of the lining of the stomach. Indomethacin is a potent ulcerogenic agent [35] used for the induction of gastric ulcers in an experimental model [23]. A significant increase in ulcer index [22] and gastric volume [22] was noted in all the indomethacin treated mild, moderate and severe ulcerated groups may be due to either generation of free radicals [36], or inhibition of prostaglandin synthesis [37]due to inhibition of COX [34]. Ulcer index, ulcer score and gastric volume are significantly different in mild, moderate and severe ulcerated groups. Depletion of prostaglandin level stimulates gastric acid secretion and impairs gastric protection that causes mucosal lesion and gastric ulceration [38]. Gastric pH increased in indomethacin treated groups which indicate the decreased hydrogen ion concentration in gastric juice that causes pathogenicity of ulcer [39]. Pepsin activity also increased in indomethacin induced mild, moderate and severe ulcerated groups [22]. Gastric free acidity, total acidity was elevated by indomethacin induced depletion of serum prostaglandin level [34, 38]. In all ulcerated groups, increased pepsin activity with decrease in mucin secretion indicated altered hydrophobicity and reduced protective ability of the mucosal membrane against hemorrhagic erosions, thus resulting in tissue damage. In AT treated group significant restoration was observed due to recovery of prostaglandin levels as well as COX levels.

Gastric mucus forms a continuous adherent layer and gives important gastric defense against various aggressive factors such as pepsin, gastric acid, swallowed material and alcohol [40]. Gastric mucus is also important for gastric ulcer healing [41]. A decrease is gastric mucosa was observed due to exposure of acid and other aggravating factors, further slowing ulcer healing. Furthermore, mucus functions as an antioxidant, which reduces the damaging effects of reactive oxygen species and lubricates gastric mucosal surface [42]. Finally gastric mucus is also essential for tissue regeneration during gastric ulcer healing; it provides a suitable pH environment for gastric epithelial cell restitution and enhances the binding of epithelial growth factor and other growth factors to their receptors [43]. These would promote healing by increasing cell proliferation, granulation tissue formation and re-epithelialization [44]. In moderate and severe ulcerated groups more gastric mucosal lesion was observed due to more acid formation and pepsin activity than the mild ulcerated group. After the AT treatment significant complete recovery in the mucin content and pepsin activity was observed in mild ulcerated groups but partial recovery was observed in severe ulcerated groups. Partial recovery is also observed in OMZ treated groups.

Indomethacin reduces CAT, SOD, GPx and GSH activities and these enzymes reduce intracellular levels of free radicals. SOD acts as the first-line defense system against reactive oxygen species [45]. SOD catalyzes the superoxide anione into H_2O_2 which then converts into water by catalase. Elevation of CD and MDA levels in gastric tissue in indomethacin treated rats indicates the involvement of oxidative injury [46, 47]. Hydro-methanol extract of leaves of AT have the potent antioxidant activity that scavenged the free radicals and protects the rats from indomethacin induced oxidative damage [20].

The important inflammatory markers like TNF-α and IL-6 levels in gastric tissue were elevated in indomethacin induced ulcerated rats through activating neutrophil infiltration [23]due to withdrawal of down regulated control of prostaglandin as prostaglandin were decreased in ulcerated groups. AT extract has also exhibited an anti-inflammatory effect that causes significant decrease in TNF-α and IL-6 levels by stimulating prostaglandin. From histo-architectural point of view it may be stated that significant damage and erosion was noted in mucosa and submucosa layer of stomach tissue in all the indomethacin treated groups and severe lesion observed in severe

ulcerated groups [48]. Hydro-methanol extract of AT treatment gives significant protection. Serum GOT and GPT levels also restored towards control in AT treated rats which indicate the AT has no toxic effect in general.

CONCLUSION: From this result it may be concluded that hydro-methanol extract of AT shows potent gastro-protective activity in mild, moderate and severe ulcerated groups by stimulating the PGE_2 level and by scavenging the free radicals generation. Most protective effect was observed in the mild ulcerated group. This study was conducted on crude extract further research will be required for identification of active ingredient(s) of leaf extract of *Ayapana triplinervis* for the protection of gastric ulcer.

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