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DOI:

10.4103/jdras.jdras_318_23

Evaluation of the effects of *Vishesha Shodhit Guggulu* and its marketed formulation on the intestinal mucosa in experimental rats

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Abstract

BACKGROUND: *Shodhit Guggulu* and its formulations are commonly used in the long-term Ayurvedic management of osteoarthritis. To date, no studies have reported the effects of *Vishesha Shodhit Guggulu* (VSG) and marketed formulation (MF) containing VSG on the gastrointestinal mucosa, and therefore, this study was conducted to evaluate the effects of *Triphala* VSG (TVSG), *Gomootra* VSG (GVSG), and MF on the histopathology of stomach, small intestine (SI), and biochemical parameters of SI in comparison with those of diclofenac in the experimental model.

METHODS: After obtaining the permission of the Institutional Animal Ethics Committee, 90 Wistar rats were randomized into five groups ($n = 18/\text{group}$). The vehicle control group (Group I) received orally (p.o.) 5 ml/kg bw of 0.5% Sodium Carboxymethyl Cellulose (Na CMC). Group II received p.o. diclofenac (6.75 mg/kg) for 4.5 days. Groups III, IV, and V received p.o. TVSG (135 mg/kg), GVSG (135 mg/kg), and MF (180 mg/kg), respectively, for 15 days. Their effect on gastric and intestinal mucosae was evaluated by histopathology, myeloperoxidase (MPO) activity, and barrier mucus content. The data were analyzed using the GraphPad InStat software with one-way analysis of variance (ANOVA), followed by Tukey's test and Kruskal–Wallis test, followed by *post hoc* Dunnett's test. The level of significance was considered as $P < 0.05$.

RESULTS: Histopathological scores of gastric mucosae showed no statistically significant difference ($P > 0.05$). Histopathology of the SI of animals receiving Ayurvedic drugs showed lesser damage when compared to the diclofenac group. MPO activity observed in the intestinal mucosa of animals receiving Ayurvedic drugs was comparable ($P > 0.05$) with that in the vehicle control group and was significantly lower than that in the group receiving diclofenac ($P < 0.001$). The mucus content of jejunum and ileum of rats receiving Ayurvedic drugs was comparable ($P > 0.05$) with the vehicle control group and significantly ($P < 0.05$) higher compared to the group receiving diclofenac.

CONCLUSION: TVSG, GVSG, and MF showed lesser damage to SI mucosa and preserved mucosal barrier compared to diclofenac.

Keywords:

Ayurveda, Barrier mucus content, Diclofenac, Myeloperoxidase, *Shodhit Guggulu*

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed as analgesic and anti-inflammatory agents

for various inflammatory conditions such as osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis, which may require prolonged administration of NSAIDs.^[1] Such prolonged intake may lead to gastric mucosal damage, and hence, NSAIDs are

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How to cite this article: Dabba S, Uchil D, Athavale C, Chawda M, Narvekar S, Nalawade M, *et al.* Evaluation of the effects of *Vishesha Shodhit Guggulu* and its marketed formulation on the intestinal mucosa in experimental rats. *J Drug Res Ayurvedic Sci* 2025;10:155-63.

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Submitted : 04-Sep-2023

Revised : 10-Jan-2025

Accepted : 15-Jan-2025

Published : 04-Jun-2025

often co-prescribed with anti-secretory drugs like H₂ blockers or proton pump inhibitors (PPIs). However, for many years, the ability of PPIs to cause small intestinal mucosal damage has remained unrecognized. This is mainly because of vague symptoms experienced by patients; nonspecific signs like iron deficiency anemia; occult blood-positive stool, malabsorption, etc.; lack of diagnostic facilities to detect NSAID-induced enteropathy; and lack of awareness that PPIs can exacerbate small intestinal mucosal damage caused by NSAIDs.^[1-3] Though enteropathy and subsequent bleeding are present in 70% of treated individuals (even in those with low risk), it is subclinical.^[1] Metronidazole, misoprostol, and sulfasalazine have shown some beneficial effects in animal models of NSAID-induced enteropathy, but they have not been found to have promising potential in clinical practice.^[1,2] Selective coxibs (COX-2 inhibitors) improved upper and lower GI safety according to the results of clinical trials, but coxibs are still capable of triggering GI adverse events and are still concerned to be associated with cardiovascular toxicity issues.^[4] Novel NSAIDs, for example, NO-releasing NSAIDs and H₂S-releasing NSAIDs, have also been shown to cause negligible damage in the small intestine (SI) of experimental animals by decreasing the small intestinal permeability and by exhibiting anti-inflammatory activity.^[1-3] However, human studies are yet to take place. Hence, till date, there are no proven effective NSAIDs that do not cause enteropathy. This motivated to explore the time-tested medicines from the treasury of Ayurveda for the treatment of inflammatory conditions.

According to Ayurveda, the Indian system of medicine, *Guggulu* and its formulations have been recommended for the treatment of inflammatory conditions such as arthritis, metabolic disorders like obesity, and skin disorders.^[5] It has been mentioned in Ayurvedic texts that administration of raw *Guggulu* may lead to skin rashes, irregular menstruation, diarrhea, headache, mild nausea, and, with very high doses, liver toxicity. Ayurveda advocates a number of *Shodhana Vidhi* (purification processes) using different *Dravyas* (fluids) for *Guggulu* before its usage as a medicine to make it free of adverse effects and to improve its therapeutic efficacy.^[6] When *Guggulu* is boiled only with water and filtered to get a clear slurry, it is called as *Samanya shodhana*,^[7] while further *Shodhana* of *Samanya Shodhit Guggulu* carried out using *Dravya* other than water is termed as *Vishesha Shodhana*.^[8] The *Vishesha Shodhana* of *Samanya Shodhit Guggulu* using *Triphala Kwath* [decoction of *Haritaki* (*Terminalia chebula* Retz.), *Bibhitaka* (*Terminalia bellirica* Roxb.), and *Amalaki* (*Phyllanthus emblica* L.)] yields *Triphala Shodhit Guggulu*, while use of *Gomootra* (cow's urine) yields *Gomootra Vishesha Shodhit Guggulu* (GVSG).^[8,9] In this study, two variants of *Vishesha Shodhit Guggulu* (VSG), i.e., *Triphala VSG* (TVSG; *Guggulu* purified using

Triphala) and GVSG (*Guggulu* purified using *Gomootra*) were used.

Shodhit Guggulu, especially TVSG, is an important ingredient of marketed formulation (MF), a tablet which is a proprietary polyherbal formulation indicated in the management of joint disorders like osteoarthritis, rheumatoid arthritis, tenosynovitis, frozen shoulder, gout, lumbago, cervical spondylosis, and lumbar spondylosis.^[10] MF also offers the benefits of *Shallaki* (*Boswellia serrata* Roxb.), *Ashvagandha* (*Withania somnifera* (L.) Dunal), *Haridra* (*Curcuma longa* L.), *Guduchi* (*Tinospora cordifolia* (Willd.) Miers), *Shunthi* (*Zingiber officinale* Roscoe), *Kulanjana* (*Alpinia galangal* (L.) Willd.), *Musta* (*Cyperus rotundus* L.), *Nirgundi* (*Vitex negundo* L.), etc., which have anti-inflammatory and anti-arthritis effects.^[11-18] A randomized controlled pilot study in patients with osteoarthritis of the knee demonstrated a steady reduction in symptoms of osteoarthritis, particularly pain.^[19] Both MF and *Guggulu* have exhibited anti-inflammatory and chondroprotective effects in *in vivo* and *in vitro* experimental studies.^[20-22]

Long-term use of VSG and its formulations may be required to manage certain chronic inflammatory conditions. To date, no studies have reported the effects of *Vishesha Shodhit Guggulu* and MF on the gastrointestinal mucosa; therefore, it was felt necessary to evaluate their potential to cause gastroenteropathy. Thus, the present study was undertaken to assess the effects of TVSG, GVSG, and MF on histopathological changes in the stomach (gastric mucosa) and small intestinal mucosa, as well as biochemical changes in the SI as compared to diclofenac, a commonly used anti-inflammatory drug.^[23]

Materials and Methods

This preclinical study was conducted in 90 male Wistar rats weighing 180–200 gm. Prior to initiation of the study, permission of the Institutional Animal Ethics Committee (60/PO/ReBi/S/99/CPCSEA) was sought under reference number IAEC GSMC/06/2018. The study procedures and maintenance of the study animals were carried out as per the guidelines by Committee for Control and Supervision of Experiments on Animals (CCSEA). Animals were housed in an air-conditioned area with 12–15 filtered fresh air changes, temperature 22 ± 3°C, relative humidity 30%–70%, and a 12-h light–dark cycle. Cages had a stainless-steel top grill having facilities for food and drinking water in polypropylene bottles with a stainless steel sipper tube. Standard rat feed and Aquaguard pure drinking water were supplied to the experimental animals *ad libitum*. All the Ayurvedic drugs, namely, TVSG, GVSG, and MF, were provided by M/s. Shree Dhootapapeshwar Limited, Mumbai, Maharashtra, India. Diclofenac was procured from Sigma Aldrich [Catalog no. 93484].

Study procedure

The animals were randomly allocated to five experimental groups ($n = 18/\text{group}$), as shown in Table 1. The animals in the vehicle control group (Group I) were administered per orally (p.o.) 5 ml/kg bw of 0.5% Sodium Carboxymethyl Cellulose (Na CMC). Group II received p.o. diclofenac.^[24] The animals in Groups III, IV, and V received p.o. TVSG, GVSG, and MF, respectively. The doses of drugs administered to the animals (Group II, III, IV, and V) were equivalent to the human doses (average human dose of TVSG and GVSG 1500 mg/day and MF 2000 mg/day; Rat dose calculation by using Paget and Barnes table i.e. multiplying human dose by 0.018 to get dose for 200 gm bw rat then multiplying by 5 to get the rat dose per kg bw) used in clinical practice.^[6-8,19]

Each group had 18 animals, which were further subdivided into three sets, each comprising six rats. The animals from Group I received 5 ml/kg bw vehicle i.e. Na CMC and the animals from groups III, IV, and V received the respective drugs once a day for 15 days. The animals in the Group II received diclofenac twice a day for the last 4.5 days.^[25] At the end of the treatment period, the animals were made to fast overnight. From the Set 1 ($n = 6$), the animals were exposed to carbon dioxide gas followed by euthanization by cervical dislocation. The mesentery was trimmed off. An intestinal segment from the jejunum starting 10 cm below the ligament of Treitz and extending to the ileum leaving last 10 cm proximal to the ileo-cecal junction was isolated. After weighing, the segment was cut open longitudinally and washed with normal saline. Then, two to three segments measuring 2 cm (especially if gross abnormal changes like erosions, ulcers, hemorrhagic spots, or mucosal sloughing were observed) were cut from the jejunum as well as the ileum and were transferred to 10% buffered formalin. Similarly, specimens were cut from the stomach and transferred to 10% buffered formalin. They were stored till all the specimens were sent for histopathology. From set 2 ($n = 6$), the SI was isolated as described above

Table 1: Experimental groups

Group no. ($n = 18/\text{group}$)	Drug administered	Dose (mg/kg), frequency	Duration and route of administration (Days; per oral)
I.	0.5% Na CMC	5 ml/kg bw, once a day	15
II.	Diclofenac	6.75, twice a day	4.5
III.	TVSG	135, once a day	15
IV.	GVSG	135, once a day	15
V.	MF	180 once a day	15

n, number of animals; Na CMC, Sodium Carboxymethyl Cellulose

and was placed in the physiological buffer [ice cold 0.1 mol/L phosphate-buffered saline (PBS) having pH 7.4]. This piece was used for biochemical estimation, which included myeloperoxidase (MPO) activity. From set 3 ($n = 6$), the SI was isolated as above, the jejunum and ileum were cut, weighed, and used for estimation of mucosal barrier content.

Variables assessed in the study

The following variables were assessed on day 16:

- Histopathological assessment of gastric and small intestinal mucosa
- Myeloperoxidase (MPO) activity of the SI
- Barrier mucus content of the SI

The detailed methodology for the assessment of each variable is as follows:

- Histopathological assessment

Specimens of the stomach and intestine (jejunum and ileum separately) fixed in formalin were processed for histopathological examination. The paraffin block-embedded specimens were cut longitudinally into 4- μm sections, and slides were prepared. They were stained with hematoxylin and eosin and observed under a light microscope. Histopathological changes in the stomach and SI were assessed using following scoring systems.

Evaluation of gastric mucosal injury^[26]

Depending on the damage to the stomach, a grade was assigned to each specimen. The higher the grade, more is the damage, with the maximum damage being 3.

Grade 0 – No increase in mononuclear cells, infiltration, or glandular disruption.

Grade 1 – Occasional mononuclear cells present in a patchy distribution with no glandular disruption.

Grade 2 – Moderate-to-severe infiltration with mononuclear cells. Glandular architecture maintained, but mild regenerative changes.

Grade 3 – Severe inflammatory infiltration with mononuclear cells and considerable glandular disruption, marked regenerative changes, or ulceration.

Evaluation of intestinal mucosal injury^[25,27]

Depending on the damage to the intestine, a grade was assigned to each specimen. The higher the grade, more is the damage, with the maximum damage being 3.

Grade 0 – Absence of inflammatory reaction, ulceration, or tissue destruction.

Grade 1 – Slight inflammatory reaction but no ulceration or tissue destruction.

Grade 2 – Moderate inflammatory infiltration with mild tissue destruction of villous crypts but no ulceration.

Grade 3 – Intense inflammatory reaction, presence of ulceration, and extensive tissue destruction.

(b) Evaluation of myeloperoxidase (MPO) activity of the SI^[28]

The intestinal segments isolated were placed in ice-cold 0.1 mol/L phosphate-buffered saline (PBS) of pH 7.4 and then homogenized in an ice container with 5 mL of 0.5% of hexadecyl trimethyl- ammonium bromide (HTAB) in 50 mM phosphate buffer on ice by using a glass homogenizer. The homogenized sample was used for measurement of myeloperoxidase activity on Spectronic-20 (Milton Roy) spectrophotometer. The MPO activity was expressed as $\delta A / \text{min} / \text{g}$ of O-dianisidine hydrochloride.

$$\text{MPO activity} = \frac{\delta A / \text{min}}{\text{Weight of the intestine (gm)}}$$

(c) Evaluation of barrier mucus content of the SI^[29]

Alcian blue 8GX, a histological dye which forms complex with acidic mucins, was used for the quantitative estimation of soluble polysaccharides in the gastric mucus. The portions of the jejunum and ileum from set 3 were immersed in 0.1% Alcian blue 8GX dissolved in sucrose-sodium acetate (pH 5.8). The uncomplexed dye was removed by successive washes of 0.25 M sucrose. The dye complexed with mucus was eluted by immersion in 0.5 M MgCl_2 . The resulting blue solutions were shaken briefly with equal volumes of diethyl ether and the optical density of the aqueous phase measured at 605 nm. The amount of dye measured is directly proportional to the mucus content of the intestine. The barrier mucus content was expressed as μg of Alcian blue content per gram of tissue.

Statistical analysis

The data generated in the present study were analyzed using the GraphPad InStat software version 3.06. The numerical data were expressed as mean \pm SD for each group. Inter-group comparison of myeloperoxidase activity and barrier mucus content was done using one-way analysis of variance (ANOVA), followed by Tukey's test. The histopathological findings were in the form of scores and were analyzed using the Kruskal–Wallis test, followed by *post hoc* Dunnett's test. The level of significance was considered $P < 0.05$.

Results

The observed findings were as follows:

Histopathological assessment

Histopathology of the stomach

None of the animals from the vehicle control group (Group I) had any change in the mucosa. It was found that four out of six rats (66.6%) receiving diclofenac had grade 1. Similar changes were observed in three out of six rats (50%) receiving TVSG and GVSG. Only two out of six

rats (33.3%) which received MF had grade 1. The number of rats showing the changes were lesser in the groups receiving Ayurvedic drugs as compared to the diclofenac group. However, no statistically significant difference ($P > 0.05$) was found in the histopathological scores of these various groups. The average score assigned for each group is presented in Table 2.

Histopathology of the small intestine

The histopathological score in the vehicle control group (Group I) was 0 for both the jejunum and ileum of rats. In the Group II which received diclofenac, three out of six animals had grade 3 damage in the jejunum, and four out of six animals had grade 3 damage in the ileum. This damage was statistically significant ($P < 0.001$) when compared with the vehicle control group. Two rats in each of the *Vishesha Shodhit Guggulu* receiving groups showed grade 2 damage in the jejunum and ileum. In Group V which received MF, only one rat in the group had grade 2 damage in the jejunum, and two animals had grade 2 damage in the ileum. None of the animals in any of the groups receiving Ayurvedic drugs (Groups III, IV, and V) exhibited grade 3 damage in the jejunum and ileum, and the scores were lesser than the group receiving diclofenac. However, no statistically significant difference ($P > 0.05$) was observed in the scores as compared to diclofenac. The histopathological scores of small intestinal mucosae in all experimental study groups are depicted in Table 3. The histopathological

Table 2: Histopathological scores for gastric mucosal damage occurring in various experimental groups

Group no. (n = 6/group)	Drug administered	Histopathological scores for the stomach
		Mean \pm S.D.
I.	0.5% Na CMC	0
II.	Diclofenac	0.66 \pm 0.52
III.	TVSG	0.50 \pm 0.55
IV.	GVSG	0.50 \pm 0.55
V.	MF	0.33 \pm 0.52

n, number of animals; S.D., standard deviation. Kruskal–Wallis test with *post hoc* Dunnett's test for comparison vs Group I and vs Group II

Table 3: Histopathological scores for small intestinal mucosal damage occurring in various experimental groups

Group no. (n = 6/group)	Drug administered	Histopathological scores	
		Mean \pm S.D.	
		Jejunum	Ileum
I.	0.5% Na CMC	0	0
II.	Diclofenac	2.50 \pm 0.55*	2.60 \pm 0.55*
III.	TVSG	1.33 \pm 0.52	1.33 \pm 0.52
IV.	GVSG	1.33 \pm 0.52	1.33 \pm 0.52
V.	MF	1.16 \pm 0.41	1.33 \pm 0.52

n, number of animals; S.D., Standard Deviation. Kruskal–Wallis test with *post hoc* Dunnett's test:
* $P < 0.001$ vs vehicle control Group I

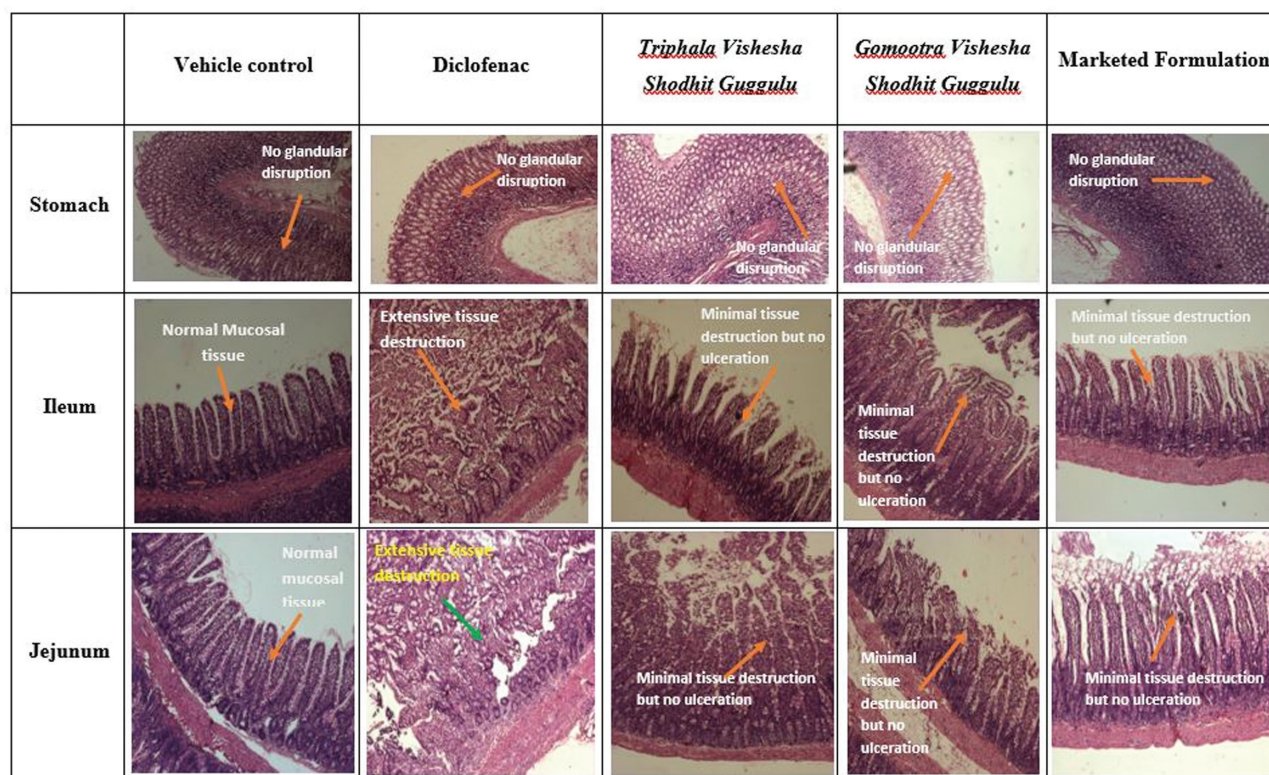


Figure 1: Histopathological images of different groups, H&E stain, 5x magnification

Table 4: Myeloperoxidase activity ($\delta A/\text{min}/\text{gm}$) in small intestinal mucosa from various experimental groups

Group no. (n = 6/group)	Drug administered	Myeloperoxidase activity $\delta A/\text{min}/\text{gm}$ Mean \pm S.D.
I.	0.5% Na CMC	0.11 \pm 0.01
II.	Diclofenac	0.16 \pm 0.01 [*]
III.	TVSG	0.13 \pm 0.01 [§]
IV.	GVSG	0.13 \pm 0.01 [§]
V.	MF	0.12 \pm 0.01 [§]

n, number of animals; S.D., standard deviation. One-way ANOVA with *post hoc* Tukey's test,

^{*}P < 0.001, vs Vehicle Group I,

[§]P < 0.001 vs Diclofenac Group II

changes observed in gastric and small intestinal mucosae are depicted in Figure 1.

Myeloperoxidase activity in the small intestine

The MPO activity increased significantly ($P < 0.001$) in the group of animals receiving diclofenac as compared to the animals from the vehicle control group. As seen in Table 4 and Figure 2, no statistically significant difference ($P > 0.05$) was observed in the MPO activity among the animals from the vehicle control group and animals receiving TVSG, GVSG, and MF. The MPO activity in the intestinal mucosa of the animals in Groups III, IV, and V was significantly lower than the MPO activity observed in the mucosae of rats receiving diclofenac ($P < 0.001$).

Barrier mucus content in the small intestine

As seen in the Table 5 and Figure 3, the rats receiving diclofenac showed a significant ($P < 0.001$) reduction in the mucus content in both the jejunum and ileum when compared to the vehicle control group (Group I). However, the values of mucus content of the jejunum and ileum of rats receiving TVSG, GVSG, and MF were comparable with the mucus content of the jejunum and ileum of animals from the vehicle control group. These values were significantly higher ($P < 0.05$) when compared to the mucus content of animals who received diclofenac.

Discussion

NSAIDs are the most prescribed drugs for inflammatory conditions. Among nonselective NSAIDs, ibuprofen and naproxen seem preferable to diclofenac, the latter being associated with higher cardiovascular risk.^[30] However, diclofenac being a potent anti-inflammatory agent is still used in clinical practice for osteoarthritis, despite of its serious gastro-intestinal side effects.^[23,31,32] *Vishesha Shodhit Guggulu* is an integral ingredient of many anti-arthritis Ayurvedic formulations, and hence, this study was conducted to evaluate the effects of two variants of *Vishesha Shodhit Guggulu*, viz., TVSG and GVSG, on the gastrointestinal mucosa of rats. MF, a proprietary polyherbal formulation, has been shown to

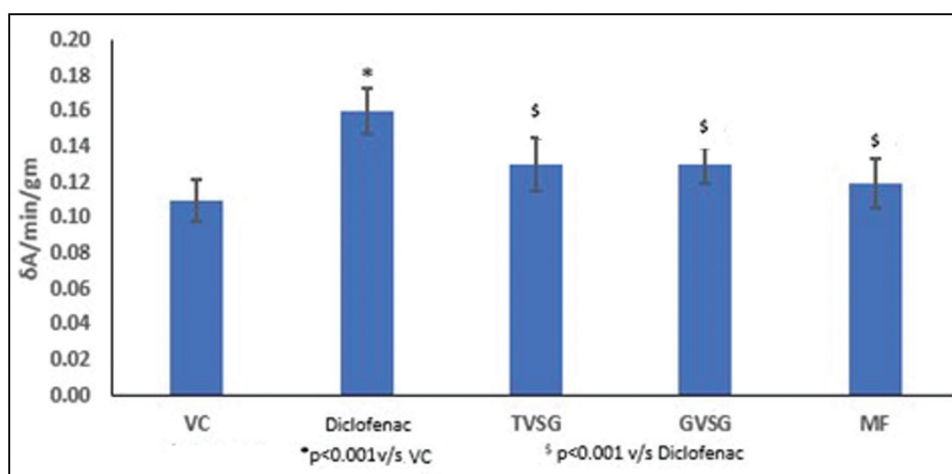


Figure 2: Myeloperoxidase activity in the small intestine (SI)

Table 5: Barrier mucus content of the jejunum and ileum in various experimental groups

Group no. (n = 6/group)	Drug administered	Barrier mucus content µg Alcian Blue/gm	
		Mean ± S.D.	
		Jejunum	Ileum
I.	0.5% Na CMC	449.20 ± 57.30	436.11 ± 81.55
II.	Diclofenac	288.43 ± 40.76*	302.43 ± 20.42*
III.	TVSG	392.68 ± 69.71 [§]	391.83 ± 49.62 [§]
IV.	GVSG	395.12 ± 43.89 [§]	402.86 ± 27.52 [§]
V.	MF	407.22 ± 29.23 ^{§§}	416.33 ± 30.07 ^{§§}

n, number of animals; S.D., standard deviation. One-way ANOVA with post hoc Tukey's test,

*P < 0.001 vs Vehicle Group I,

§§P < 0.01,

§P < 0.05 vs Diclofenac Group II

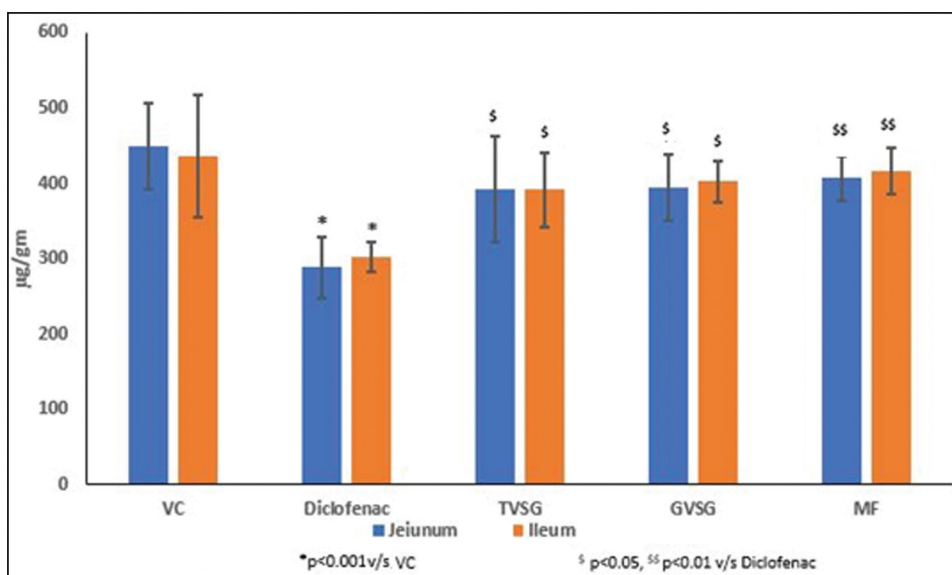


Figure 3: Barrier mucus content in the SI

decrease pain in osteoarthritis and is used by healthcare professionals in the management of osteoarthritis.^[19] Therefore, it was also of interest to compare the effects of MF and diclofenac on gastrointestinal mucosae of rats.

Diclofenac used by researchers in various experimental models to induce small intestinal damage were either doses higher than the human equivalent therapeutic dose or were in older rats.^[33-35] It is known to be metabolized by the liver, and the major biliary metabolite, diclofenac acyl

glucuronide, is excreted in the SI. Acyl glucuronide or its oxidative metabolites are responsible for small intestinal mucosal injury. This glucuronide undergoes deconjugation by bacterial beta-glucuronidase, resulting in enterohepatic circulation of liberated diclofenac, causing more injury in the mid and distal part of the ileum.^[36]

In the present study, diclofenac was used in the dose extrapolated from the human dose (i.e., 75 mg twice a day) and frequency of administration was also maintained as twice a day^[24] The doses of diclofenac, TVSG, GVSG, and MF were derived from that used in clinical practice, and the amount equivalent to the human dose was administered to the animals.

The changes in the morphology of SI have remained the gold standard for judging the small intestinal damage because it serves as a direct measure of the damage caused by the responsible agent.^[37] As shown in the results, all the three Ayurvedic drugs prevented the increase in histopathological scores when compared to diclofenac. Statistical significance was not achieved because the nonparametric tests are less sensitive and need larger sample size so that the power of the test is not reduced. Use of a higher number of rats was not possible due to restriction posed by CCSEA on the number of animals in the study.

Although there was no statistical significance in histopathological scores of TVSG, GVSG, and MF as compared to the diclofenac group, the number of animals having a normal mucosa were more in the Ayurvedic drug-treated groups. It has been reported in the literature that the same dose of diclofenac caused a higher degree of small intestinal mucosal damage than gastric mucosal damage.^[34] The results of the present study confirm the difference.

Myeloperoxidase (MPO) activity is enhanced in the ulcerated area of the intestinal wall, whereas its level is reduced during the healing process.^[38] The present results showed that inflammation was induced by diclofenac, as reflected in higher MPO activity. On the other hand, all the three Ayurvedic drugs did not increase MPO activity. The MPO activity following administration of *Vishesha Shodhit Guggulu* and MF, irrespective of *Shodhana* process (*Triphala* or *Gomootra*), remained identical. MPO is an enzyme found abundantly in the azurophilic granules of neutrophils. NSAIDs increase intestinal permeability, which results in low-grade intestinal inflammation. Moreover, their effects are mediated through lipopolysaccharide of gastro-intestinal bacteria, which activates Toll-like receptor 4. These receptor activations, in turn, activate nuclear factor-kB, resulting in neutrophil recruitment. Neutrophils are thus the effector cells for macroscopic damage due to NSAIDs.^[36,39] Estimation of MPO activity is considered a

biomarker of neutrophil infiltration, which measures the degree of inflammation in the gastro-intestinal tissues.^[40]

Diclofenac reduces the barrier mucus content significantly from both the jejunum and ileum. All the three Ayurvedic drugs, viz., TVSG, GVSG, and MF, showed values of the mucosal barrier comparable to those of the vehicle control group, indicating preservation of the mucosal barrier.

Intestinal mucosa contains mucopolysaccharide as its major constituent. It is viscous in nature and has gel-forming properties. It acts as a barrier and is the first line of defense against various intra-luminal toxins.^[41] Therefore, estimation of mucus content was done to find out whether the study drugs disrupt the mucosal barrier.

One of the limitations of the study was the number of animals as explained above. The dose of the diclofenac in our study was selected from the human dose. As it was observed by other researchers that the same dose of diclofenac caused a lesser degree of gastric mucosal damage than small intestinal mucosal damage,^[34] a higher dose should have been selected to induce gastric mucosal damage.

According to Ayurveda scriptures, the *Shodhana Vidhi*, that is, process of purification of herbs like *Guggulu* in different media, is significant as it helps eliminate the impurities present in the raw herb, potentiate its therapeutic action, and ensure its safety and efficacy. Literature suggests that raw *Guggulu* is known to have some gastro-intestinal side effects,^[6] but one of the contributing factors for the protective effect of *Vishesha Shodhit Guggulu* on the small intestinal mucosa can be attributed to the *Shodhana Vidhi*. Further studies are needed to prove the same.

Conclusion

The present study aimed at evaluating the effects of *Triphala Vishesha Shodhit Guggulu* (TVSG), *Gomootra Vishesha Shodhit Guggulu* (GVSG), and the MF, which contains TVSG as the major ingredient with other plant drugs on the intestinal mucosa.

The administration of TVSG, GVSG, and MF to the animals *per se* does not cause any damage to small intestinal mucosa, as evident by lower neutrophil recruitment and preserved mucosal barrier as compared to the administration of diclofenac. Though histopathological findings were not statistically significant, the animals from the treated group showed lower grades. Further, it is essential to conduct clinical studies to confirm these experimental findings.

Acknowledgment

The study drugs were provided by M/s. Shree Dhootapapeshwar Limited, Mumbai, Maharashtra, India.

Financial support and sponsorship

The study was supported by M/s. Shree Dhootapapeshwar Limited, Mumbai, Mumbai, Maharashtra, India.

Conflicts of interest

Dr. Mukesh B. Chawda, Dr. Sangam Narvekar, and Dr. Megha Nalawade are current employees of Shree Dhootapapeshwar Limited, Mumbai, Maharashtra, India.

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हिंदी सारांश

प्रयोगात्मक चूहों में विशेष शोधित गुग्गुलु और उसकी बाजार में उपलब्ध औषधि के आंतों के म्यूकोसा पर प्रभावों का मूल्यांकन

पृष्ठभूमि: शोधित गुग्गुलु और इसके योगों का उपयोग आयुर्वेद में अस्थिसंधिशोथ के दीर्घकालिक प्रबंधन में आमतौर पर किया जाता है। अब तक विशेष शोधित गुग्गुलु (विएसजी) और विशेष शोधित गुग्गुलुयुक्त बाजार में उपलब्ध औषधियों (एम एफ) के जठरांत्र (गैस्ट्रोइंटेस्टाइनल) म्यूकोसा पर प्रभावों का अध्ययन नहीं हुआ था। अतः यह अध्ययन त्रिफला शोधित गुग्गुलु (टीविएसजी), गोमूल शोधित गुग्गुलु (जीविएसजी) और एमएफ के गैस्ट्रिक और छोटी आंत की ऊतक-विज्ञान और जैव-रासायनिक मानकों पर प्रभाव की जाँच के लिए किया गया, जिसकी तुलना डाइक्लोफेनाक से की गई।

पद्धतियाँ: संस्थागत पशु नैतिकता समिति से अनुमति प्राप्त करने के बाद, 90 विस्तार चूहों को पाँच समूहों में विभाजित किया गया (प्रत्येक समूह में 18)। समूह I को 5 मि.ली./किलोग्राम शारीरिक वजन 0.5% सोडियम कार्बोक्सिमिथाइल सेलुलोज (सोडियम सीएमसी) दिया गया। समूह II को डाइक्लोफेनाक (6.75 मि.ग्रा./कि.ग्रा.) 4.5 दिन तक दिया गया। समूह III, IV और V को क्रमशः टीविएसजी (135 मि.ग्रा./कि.ग्रा.), जीविएसजी (135 मि.ग्रा./कि.ग्रा.), और एमएफ (180 मि.ग्रा./कि.ग्रा.) 15 दिन तक दिया गया। उनके गैस्ट्रिक और आंतों के म्यूकोसा पर प्रभाव को ऊतकविज्ञान, एमपिओ गतिविधि, और म्यूकस की मात्रा द्वारा आंका गया। आँकड़ों का विश्लेषण वन-वे-ऐनोवा, टुकी टेस्ट, क्रुस्कल-वाॉलिस परीक्षण और डनेट के पश्च परीक्षण द्वारा ग्राफपैड इन्स्टाट सॉफ्टवेयर के जरिये किया गया। $p < 0.05$ को सांख्यिकीय रूप से महत्वपूर्ण माना गया।

परिणाम: गैस्ट्रिक म्यूकोसा के ऊतक-वैज्ञानिक जांच में कोई सांख्यिकीय रूप से महत्वपूर्ण अंतर नहीं मिला ($p > 0.05$)। छोटी आंत की ऊतक-वैज्ञानिक जांच में आयुर्वेदिक औषधियाँ डाइक्लोफेनाक की तुलना में कम क्षति दिखाती हैं। आयुर्वेदिक समूहों में छोटी आंत की म्यूकोसा के एमपिओ गतिविधि, नियंत्रण समूह के तुल्य ($p > 0.05$) और डाइक्लोफेनाक समूह की तुलना में काफी कम ($p < 0.001$) थी। आयुर्वेदिक दवाओं के प्रयोग से आंत के जेजुनम और इलियम में म्यूकस की मात्रा वहन नियंत्रण समूह के बराबर थी ($p > 0.05$), और डिक्लोफेनाक पाने वाले समूह की तुलना में काफी अधिक (सांख्यिकीय रूप से महत्वपूर्ण) थी ($p < 0.05$)।

निष्कर्ष: टीविएसजी, जीविएसजी और एमएफ ने छोटी आंत की म्यूकोसा को कम क्षति पहुँचाई और म्यूकोसल अवरोध को डाइक्लोफेनाक की तुलना में अधिक संरक्षित रखा।

शब्दकुंजी: आयुर्वेद, अवरोधक म्यूकस सामग्री, डाइक्लोफेनाक, मायेलोपेरोक्सिडेज, शोधित गुग्गुलु