

Evaluation of *Shivaksharpachan Churna* for its Gastroprotective Activity

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Summary

Shivaksharpachan Churna is a popular marketed herbal formulation, composed of herbs and spices, used for treatment of gastrointestinal disorders by traditional healers. But there is no scientific evidence for its gastroprotective activity. So we have taken attempt to evaluate *Shivaksharpachan Churna* for its gastroprotective activity along with effect on the anti oxidant enzymes to justify its anti-ulcer action. Ethanolic extract of *Shivaksharpachan Churna* (ASE) (50, 100 and 200 mg/kg body weight) were administered orally, twice daily for 5 days for prevention from pylorus ligation (PL) and ethanol (EtOH)-induced ulcers followed by estimation of anti-oxidant enzymes i.e. LPO, SOD and catalase. ASE showed dose dependent inhibition of ulcer index in both models. ASE prevents the oxidative damage of gastric mucosa by blocking lipid peroxidation and significant increase in superoxide dismutase and catalase activity. Results shows that ASE possesses significant gastroprotective activity which might be due to gastric defense factors and phenolics might be the main constituents responsible for this activity.

Key words: *Shivaksharpachan Churna*, Anti-ulcer, anti-oxidant enzymes, pylorus ligation, ethanol induced.

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Introduction

Plants and plant-derived products are part of health care system since ancient civilizations. In India, the texts like '*Charak Samhita*' and '*Sushruta Samhita*' were documented in about 1000 years B.C., where applications of plants and polyherbal formulations were highlighted for health care. According to Ayurvedic text, a combination of substances were used to enhance the desired action and eliminate unwanted side effects [1]. In recent years, there has been an increased inclination towards the herbal formulations due to the trend towards the natural sources and a healthy life style. Moreover, the complexity, side effects and costly treatment associated with the allopathic medicines have caused both the health care practitioners and the majority of world populations to turn towards alternative therapies, more likely towards the herbal medicines [2].

Herbal medicines found applications in several disorders like diabetes, hypertension, asthma, ulcers etc. The growing use of botanicals by the public is forcing scientists to evaluate the health claims of these agents and to develop standards of quality and manufacture [3].

Shivaksharpachan Churna is a popular polyherbal formulation, composed of herbs and spices, used for managing all the digestive disturbances and improves the functioning of liver [4]. Further it has been investigated that there is presence of ulcer protective plant materials in this formulation [5-7]. With the above aspects and use of this novel formulation as gastroprotective agent by traditional healers compels us to evaluate scientifically for its anti-ulcer activity.

Gastric ulcers is the most common gastrointestinal disorder in clinical practice, arises due to various factors [8]. Even though the etiology of gastric ulcers is still debated, it is accepted that ulcers are caused due to net imbalances in mucosal offensive and defensive factors [9]. Ulcer therapy is now mainly focused on limiting the deleterious effects of offensive acid secretion, but the search for new safer drugs have rekindled the interest in natural drugs possessing this activity. Considering the several side effects (arrhythmias, impotence, gynaecomastia, and haematopoeitic changes) of modern medicine, traditional drugs possess fewer side effects and should be looked as a better alternative for the treatment of peptic ulcer.

There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and gastric ulcer [10]. A great number of spices and aromatic herbs contain chemical compounds exhibiting antioxidant properties. These properties are attributed to a variety of active phytochemicals including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, simple phenols and phenolic acids, etc [11].

Thus, the aim of the present study was to evaluate this formulation for its ulcer protective activity in vivo along with effect on the antioxidant enzymes to justify its anti-ulcer action.

Materials and methods

Composition and extraction

Shivaksharpachan Churna contains the fine powders of *Zingiber officinale* Roscoe (Zingiberaceae), *Piper nigrum* Linn. (Piperaceae), *Piper longum* Linn. (Piperaceae), *Cuminum cyminum* Linn. (Apiaceae), *Ferula foetida* (Apiaceae), *Trachyspermum ammi* Linn. (Apiaceae), *Terminalia chebula* Retz. (Combretaceae) and Sarji-kshara in one part each.

Air-dried powder of *Shivaksharpachan Churna* (30 g) was extracted with 100ml of ethanol for 20 minutes with the help of a sonicator at room temperature and concentrated under reduced pressure to yield 8.34% w/w of extract.

Preliminary phytochemical screening

ASE was subjected to qualitative chemical screening for the identification of the various major classes of active chemical constituents. Test for flavonoids: 2ml of the extract was filtered and 1ml of the filtrate was mixed with dilute NaOH, golden yellow precipitate confirmed the presence of flavonoids. Test for phenols: 2ml of the extract was mixed with 3ml 5% ferric chloride and five drops of potassium ferricyanide, dark green precipitate confirmed the presence of phenols. Test for steroids/saponins: 1 g of the extract was mixed with 10 ml of warm distilled water, frothing persistent indicated the presence of saponins. An additional test was performed by Liebermann–Burchard test. To 100mg of extract, 2ml of acetic anhydride was added; the mixture was thoroughly stirred, heated for 2 min on a water bath and allowed to stand at room temperature. When 2ml of sulfuric acid was gently added to 0.7 ml of a supernatant acetic anhydride layer, the upper layer gave a blue to green colour confirming the presence of steroidal saponins [12].

Study of Anti-Ulcer Activity

Animals

Albino rats (Wistar strain) of either sex, weighing between 160 and 200g were procured from Central Animal House of the University approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). They were kept in the departmental animal house at $26\pm 2^{\circ}\text{C}$ and relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. The animals described as fasted were deprived from food but allowed free access to water. All the treatments (except ethanol) dissolved in water were administered orally in a volume of 10 ml/kg in all the experiments.

Acute toxicity

Acute toxicity study was performed according to OECD guideline. Different doses (50–2000 mg/kg, p.o.) of ASE were administered to group of rats and observed continuously for 1 h and then at half-hourly intervals for 4 h, for any gross behavior changes further up to 72 h followed 14 days for any mortality. No mortality was observed.

Experimental Procedures

The animals were divided into eleven groups each consisting of six rats. Where for 5 days groups 1 (**Normal**) and 2 (**Control**) received vehicle 10 ml/kg, groups 3, 4 and 5 (**Test**) were given ASE 50, 100 and 200 mg/kg, respectively, and the group 6 (**Reference**) given reference drug ranitidine (RAN) at the dose of 100 mg/kg. All the doses calculated with respective body weights of animals and administered orally. Afterwards, groups were subjected to induction of ulcer by Pylorus ligation, except in group 1, which served as normal group. Group 7 to 11 followed same treatment protocol as followed in group 2 to 6 respectively and ulcer was induced by administering ethanol.

Study of anti-ulcer and antioxidant activity using pylorus ligation method

The method of Shay rat ulcer was adopted [13]. The rats were kept for 48 h fasting and care was taken to avoid coprophagy. After the pretreatment period of 1h animals were

anaesthetized using pentobarbitone (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. After 4 h of pylorus ligation, stomachs were dissected out and cut open along the greater curvature. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach.

The fundic part of the stomach was homogenised (5%) in ice cold 0.9% saline with a Potter–Elvehjem glass homogeniser for 30 s. The homogenate was then centrifuged at 800×g for 10 min followed by centrifugation of the supernatant at 12,000 ×g for 15 min and the obtained homogenate was used for the estimations of LPO, Catalase and SOD [14].

Study of anti-ulcer and antioxidant activity using ethanol-induced ulcer methods

On the 5th day, 1h after final dose of treatment, the gastric ulcers were induced in rats by administering 96% ethanol (5ml/kg) after overnight fasting [15] and after 1h animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers. The stomach was then weighed and processed for antioxidant markers estimations as mentioned in previous section.

Antioxidant assay

LPO was estimated by standard method of Okhawa et al., 1979 [16] and expressed as nmol of MDA formed/min/mg protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitrobluetetrazolium reaction system as adapted by Kakkar et al., 1984 [17] and the results have been expressed as units (U) of SOD activity/mg protein. Catalase (CAT) was estimated by method of Aebi, 1974 [18] and results are expressed as μ mol of H₂O₂ consumed min⁻¹ mg⁻¹ protein.

Results and discussion

Preliminary phytochemical screening

The qualitative phytochemical screening of the extract gave positive results for the presence of flavonoids, Saponins, phenols, bitter principles and steroids. These bioactives could be responsible for the antisecretory, cytoprotective and gastroprotective action of ASE. Therefore, it can be concluded that this formulation have a great potential to be used as a gastroprotective drug.

Acute toxicity

In acute toxicity study no mortality was observed up to dose of 2000 mg/kg, p.o, So a suitable dose has been selected according to OECD guidelines.

Study of anti-ulcer and antioxidant activity

Effects of ASE at dose of 50, 100 and 200 mg/kg body weight, twice a day for 5 days prevented the acute gastric ulcers in a dose related manner. The oral administration of ASE at

50-200 mg/kg in pylorus ligation decreased the index of gastric lesion by 13.30 ± 3.12 - 3.80 ± 1.20 , respectively (24.85-78.53 % protection) in comparison to control 17.7 ± 2.2 . Studies suggest that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular permeability, edema formation and epithelial lifting [19]. Administration of ASE 1 h before the induction of gastric lesions by ethanol decreased the total ulcer index of by 16.6 ± 2.12 - 8.6 ± 1.4 , respectively (23.32- 60.27 % protection). Results for ASE are comparable to RAN at the dose of 100 mg/kg.

Gastrointestinal ulcer is a common disease in clinic. The imbalance of aggressive (gastric juice, pepsin) and protective factors include mucosal blood flow, bicarbonate secretion, the secretion of prostaglandin and other hormones, mucosa integrity of cellular membrane, cell regeneration are considered as the major mechanism. The traditional and allopathic anti-ulcer drugs inhibit the acid secretion, protect the mucosa, or inhibit the *Helicobacter pylori*. We designed two different experimental models to investigate the effect and mechanism of ASE on gastric ulcer, ethanol-induced gastric ulcer and pylorus ligation induced gastric ulcer. Alcohol can cause the lesion of gastric mucosa, reinforcement of the aggressive factors while weakness of the protective factors, so the ulcer was formed. Pylorus ligation can lead to the accumulation of gastric juice in the stomach, damaging the balance of aggressive and create protective factors, therefore, ulcer is formed. Our results clearly demonstrate that ASE is in possession of good preventive and therapeutic action on the gastric ulcers. It was a dose-dependent protection against gross damaging action of ethanol and pylorus ligation on gastric mucosa of animals. Pylorus ligation-accumulated secretions and the related ulcers confirm gastric acid output to be the root cause of gastric ulcers [20]. The treatment with ASE was found to inhibit the Pylorus ligation-accumulated secretions. ($P < 0.01$)

Studies have shown alterations in the antioxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus ligation-induced [21] and ethanol-induced [22] ulceration in rats. In the present study administration of ASE, at the doses of 50, 100 and 200 mg/kg in pylorus-ligation was found to decrease in lipid peroxidation and increase in SOD and catalase as compared to control group, thus leading, to oxidative stress (Table 1). Ethanol administration was found to decrease in lipid peroxidation and increase in SOD, and catalase, as compared to control group (Table 2) ($P < 0.01$).

Table 1. Effect of ASE on the antioxidant parameters in stomach of pylorus ligated rats.

	Normal	Control	50mg	100mg	200mg	RAN
CAT	7.95 ± 0.25	4.87 ± 0.32	5.76 ± 0.27^{ns}	$6.45 \pm 0.41^*$	$7.1 \pm 0.38^{**}$	$7.54 \pm 0.45^{***}$
SOD	6.12 ± 0.15	2.23 ± 0.37	3.78 ± 0.26^{ns}	$4.48 \pm 0.52^{**}$	$5.56 \pm 0.43^{***}$	$5.75 \pm 0.62^{***}$
LPO	3.56 ± 0.28	11.2 ± 0.34	10.12 ± 0.48^{ns}	$9.5 \pm 0.53^*$	$4.12 \pm 0.16^{***}$	$3.81 \pm 0.22^{***}$

All values are expressed in mean \pm SEM (n=6), ns $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 2. Effect of ASE on the antioxidant parameters in stomach of ethanol-treated rats.

	Normal	Control	50mg	100mg	200mg	RAN
CAT	7.95±0.25	5.02±0.44	6.58±0.18*	6.97±0.52**	7.32±0.23***	7.62±0.35***
SOD	6.12±0.15	2.43±0.46	4.32±0.33*	5.08±0.74**	5.25±0.35***	5.86±0.29***
LPO	3.56±0.28	6.8±0.39	4.85±0.18 ^{ns}	3.92±0.68**	3.73±0.41***	3.47±0.60***

All values are expressed in mean ± SEM (n=6), ns P>0.05, * P<0.05, ** P<0.01, *** P<0.001

Results in the present study indicate similar alterations in the antioxidant status after pylorus ligation and ethanol induced ulcers. Preventive antioxidants, such as superoxide dismutase and catalase enzymes are the firstline of defence against reactive oxygen species. Administration of ASE resulted in a significant increase in the SOD and catalase levels as compared to the control animals, which suggests its efficacy in preventing free-radical-induced damage. Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids which eventually results in destruction of membrane lipids. Biological membranes are often rich in unsaturated fatty acids and bathed in oxygen-rich metal containing fluid. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack [23]. The study has revealed a significant decrease in lipid peroxidation by ASE in both experimental models, which suggests its protective effect.

Conclusion

The present work confirmed that *Shivaksharpachan Churna* contain a number of active compounds which may be responsible for their therapeutic activity. Chemical analysis of ASE by HPTLC co-chromatography and our previous study for the quantification of eugenol, piperine, trans-caryophyllene and euclyptol reveals there is more content of more content of piperine as it is present in more number of ingredients of the formulation. Except these four marker compounds ASE also contain a number of active constituents which is shown in chromatogram. It might be a useful contribution to the selection of an appropriate formulation in the clinical practice and hence effective rational therapy. Further ASE prevents pylorus ligation and ethanol induced ulcers, in view of the fact that the action of *Shivaksharpachan Churna* due to antioxidant action, and that *Shivaksharpachan Churna* contain relatively high amount of anti-oxidant substances, it is possible that the mechanism of the gastroprotective action is via an oxidant action, however, other mechanisms cannot be excluded. Work is in progress to isolate and purify the active principle responsible for the gastro protective activity.

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