SKM VAIDHYA AMIRTHAM

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Effective relief from JOINT PAIN AND INFLAMMATION

- Relieves the stiffness and improve the mobility
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Ushna jala - (Warm water)

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हिध्माध्मानानिलश्लेष्मसद्यः शुद्धिनवज्वरे
कासामपीनसश्वासपार्श्वरुक्षुच शस्यते ॥ १७॥"

"Warm water stimulates hunger, aids digestion, soothes the throat, is easy to digest, cleanses the urinary bladder, and relieves hiccups, flatulence, and the aggravation of Vata and Kapha. It is also ideal during purification therapy and for those suffering from early-stage fevers, coughs, Ama (the accumulation of undigested materials), nasal congestion, shortness of breath, and pain in the flanks."

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Gastroprotective effect of SKM-Gelcocid suspension on indomethacin induced gastric ulcer in female Wistar rats

INTRODUCTION:

A peptic ulcer is distinguished by painful sores or lesions on the stomach or duodenal wall. It causes damage and irritation to the stomach wall or, in some cases, the lower parts of the esophagus known as gastric ulcer (Biomed Biol Sci and Bereda, 2022). Gastric ulcers are the most common kind of peptic ulcer in humans, affecting more than 10% of the global population (Kuna et al., 2019). The most common etiology for stomach ulcers includes overuse of non-steroidal anti-inflammatory medications (NSAIDs), alcohol, anxiety, tobacco use, and a helicobacter pylori infection (Osadchuk et al., 2020). Among the risk variables, alcohol use can have a direct impact on stomach mobility and metabolism. In addition, all of the previously mentioned harmful variables, including NSAIDs, can promote the development of ulcers by producing various reactive oxygen species (ROS). This action damages the mucosa and causes ulcers in the stomach (B. et al., 2016). A growing amount of evidence implies that Indomethacin-induced stomach ulcers are directly related to the formation of reactive oxygen species (ROS). Under oxidative stress, excessive ROS generation causes cellular damage in the stomach (Lauridsen, 2019). As a result, gastric cells induce multiple endogenous antioxidant enzymes, including glutathione peroxidase (GPx), and glutathione to maintain gastrointestinal homeostasis via ROS scavenging (Fusco et al., 2020).

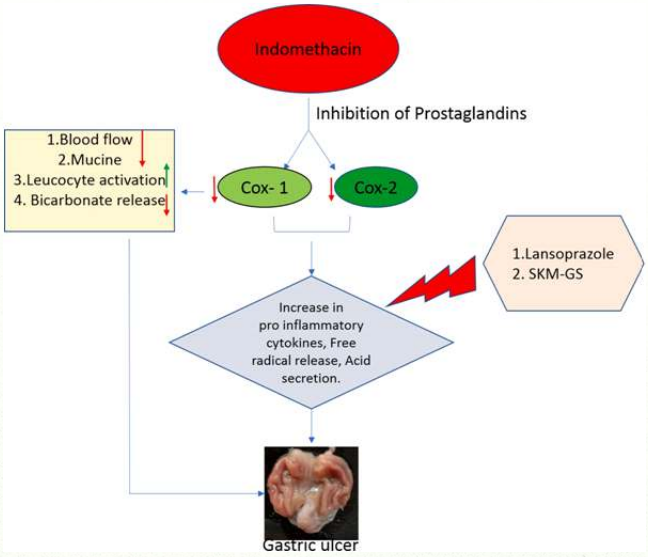

Fig. 1. Mechanism of ulcer induction by Indomethacin: Figure 1. shows the mechanism by which indomethacin causes damage to the stomach walls and in- duces ulcers.

Mechanism of indomethacin in ulcer induction

A more powerful non-selective COX inhibitor is indomethacin, a derivative of acetic acid. It engages in reversible competition at the COX1 and COX-2 active sites with the arachidonic acid (AA) substrate (Attiqet al., 2018). Additionally, it depresses the formation of mucopolysaccharides, decreases the movement of polymorphonuclear leukocytes, and may have a direct vasoconstrictor action that is independentof COX. On the other hand, it causes strong ulcerogenic potential in humans as well as animals (Simoes ˜ et al., 2019). Through several mechanisms, including increased gastric acid secretions, inhibition of PGE-2 synthesis, production of free radicals, decreased gastric nitric oxide levels, invasion by activated neutrophils, and induction of gastric cell apoptosis, indomethacin causes gastrointestinal toxicity (Suleymanet al., 2010) (Fig. 1).

ROS generation by indomethacin

Studies reveal that antioxidant parameters are decreased in gastric tissue with indomethacin-induced damage. Indomethacin causes gastric damage by raising mucosal myeloperoxidase (MPO) and malondialdehyde (MDA) levels (Turkyilmaz et al., 2019). Two hours after indomethacin administration, there is an acute increase in the production of toxic oxygen radicals (superoxide and hydrogen peroxide) in the gastric mucosa, indicating that toxic radicals are the cause of gastric damage. MPO, which is found in phagocytic cells (PNL), catalyzes the production of toxic hypochlorous acid (HOCl) from hydrogen peroxide (H2O2). These cells cause excessive and uncontrolled production of reactive oxygen species, including superoxide anions (O2-) and hydroxyl radicals (OH-) (Pineda-Pena ˜ et al., 2020). Excessive MPO and other reactive radicals lead to oxidative damage. Cell membrane damage is largely caused by lipid peroxidation, of which MDA is the byproduct and a useful indicator of lipid peroxidation degree. Traditional anti-ulcer medications such as omeprazole and lansoprazole have been shown to mitigate the effects of indomethacin-induced elevations in mucosal MPO and MDA levels (Bhattacharyya et al., 2014).

In the allopathic system, many medications, such as H2 receptor antagonists, anti-H. pylori, proton pump inhibitors, and others with rapid therapeutic activities, are utilized to treat peptic ulcers (Namdev and Jain, 2019). Proton pump inhibitors like lansoprazole prevent the production and secretion of acid into the stomach lumen. However, the mucosal protective mechanisms in the stomach usually offset any potential negative effects of lansoprazole. The indomethacin-induced ulceration model likely compromises this protective mucosal barrier, leaving the exposed mucosa vulnerable to the acid's damaging effects. This prevents the injured tissues from repairing and regenerating and enlarges the lesions that have already occurred. Lansoprazole's effects are contingent upon the body's absorption and subsequent activity at the level of the gastric mucosa's proton pump (Simoes ˜ et al., 2019). In the Indomethacin induction model, where ulceration is accomplished in just one hour, this process most definitely does not take place in time for lansoprazole to exhibit anti-ulcer activity. Hence, lansoprazole treatment is started before ulcer induction by indomethacin. The anti-ulcer activity of lansoprazole is independent of acid generation and secretion.

However, due to the limitations of allopathic treatments (most notably the concern of safety) and for various other reasons, people are interested in herbal formulations. The use of herbal formulations to treat various ailments has found widespread use in the pharmaceutical health care system. The global herbal medicine market is projected to grow from USD 165.66 billion in 2022 to USD 347.50 billion by 2029 (Fayiah et al., 2023). Phytotherapy has been used for thousands of years and was a major component of ancient civilization in nations such as India, China, and Egypt. The usage of phytotherapeutics has expanded dramatically among patients and physicians in the last decade, as evidenced by a surge in the market for herbal medications (Cossio et al., 2012). Another factor contributing to the growth in market share is the introduction of herbals in the form of dietary supplements and nutraceuticals. Herbal medications are in significant demand for basic healthcare in both developed and developing nations because of their broad biological activity, better safety margins, and cheaper prices (Hishe et al., 2016). A large number of people use medicinal herbs as remedies for various diseases as they are safe, effective, and better tolerated. The efficacy of herbal medications is determined by the overall function of a range of active components, since all ingredients generate synergistic action and so increase therapeutic value (Yuanet al., 2017). Nature provides the precise proportion of all elements for diverse illnesses in a single species. Each active ingredient has a crucial role to play, and they are all interconnected. Plant-based drugs are thought to be safer and have fewer negative effects. The ancient traditional systems of medicine (TSM) Ayurveda, Siddha, and Unani describe a variety of single and compound medication compositions of plant origin for the treatment of various illnesses (Ansari, 2020). TSM compound formulations are commonly employed in therapy because they have a synergistic therapeutic impact and help reduce the undesirable effects of the primary medications (Ansari, 2020).

Gelcocid suspension is a polyherbal Ayurveda formulation that is popular for treating peptic ulcers. It is composed of different ingredients, like Emblica officinalis, Hemidesmus indicus, Terminalia chebula, Mentha viridis, and Cuminum cyminum. Despite the widespread use of gelcocid suspension in peptic ulcer maladies, there is a lack of scientific evidence to substantiate its use. So, the point of this study was to check the levels of anti-oxidant enzymes (GSH, GPx, and MDA) and see how well Gelcocid suspension protects the stomach, then compare its effectiveness to Lansoprazole.

MATERIALS AND METHODS:

The following chemicals were used to carry out the study: SKM Gelcocid suspension (SKM Siddha and Ayurveda Pvt. Ltd., India), Indomethacin, Lansoprazole, ROS scavenging assay kit, Alcian blue, EDTA, Ketamine, Xylazine, Sodium Acetate, Tris buffer, and dithiol-bis (2-nitrobenzoic acid) (DTNB). We purchased all the analytical grade chemicals from Sigma Aldrich (Merck, India).

Screening of antiulcer activity of SKM-Gelcocid suspension on indomethacin-induced ulcer mode

Animals

The rats were kept in typical laboratory settings at 50±15 % humidity and 25±2 C, with a 12-hour alternate cycle of light and dark. Before the experiment, they had a week to get used to the animal house's environment. They also had unrestricted access to the usual lab supplies of water and food. We conducted the experiment in accordance with the standards set by the Committee for Control and Supervision of Experiments on Animals (CCSEA). All procedures were approved by the IAEC. CPCSEA approval No. 118/PO/ReBiRc/S/1999/CPCSEA.

Induction of gastric ulcers

Indomethacin-induced gastric ulcer in rats

Thirty rats were divided into five groups (n = 6). The vehicle, SKM Gelcocid suspension, and Lansoprazole (Lanso) were administered orally every day for seven days before ulcer induction. The rats were subsequently starved for 24 h.

Gastric ulcers developed after giving INDO (40 mg/kg BW) orally, and the animals were euthanized 6 h later. Group 1 received the vehicle as a normal control (NC group). Group 2 received only INDO (negative control). Lanso, a 30 mg/kg proton pump inhibitor, was a positive control in group 3. Rats in Groups 4 and 5 were treated with SKM Gelcocid suspension at two different dosage levels: INDO+SKM-GS (400 mg/kg) and INDO+SKM-GS (200 mg/kg). The two dose levels of SKM GS used in this study were chosen according to the acute oral toxicity studies (OECD 423 TG). Xylazine (10 mg/kg) and ketamine (100 mg/kg) were used to anesthetize the rats as per CCSEA guidelines.

Gastric ulcer area measurement

The stomach was removed, and the larger curvature was opened. The mucosal surface was facing up while it was stretched out on a piece of filter paper, cleaned with cold normal saline (0.9 % NaCl solution), wiped dry, scored for ulcers, photographed, and stored on a computer. The ulcer area (mm2) in the imaged stomach was quantified using Image J software (Version 1.5.3) (Lim et al., 2014). The following formula was used to get the protection index:

[(Ulcer area (negative control)-Ulcer area(treated))/Ulcer area (negative control)] × 100.

Histological and biochemical sampling of gastric tissue

One half of each stomach was maintained at − 80 ◦C for biochemical examination, while the other half was immersed in a 10 % formalin solution for histological study (Mousa et al., 2019).

Estimation of gastric wall mucus

Corne et al. (1974) developed a method for measuring gastric wall mucus. The 0.5 g of stomach glandular tissue was dyed in 10 ml of a 1 % Alcian blue solution in 0.16 M sodium acetate (pH 5.8) for two hours. Using an Alcian blue standard curve, the dye complex was removed with 0.5 M MgCl2, centrifuged, and spectrophotometrically measured at 580 nm (Asuzu and Onu, 1990).

Estimation of NP-SH (non-protein sulfhydryl groups)

Sedlak and Lindsay (1968) described a technique for measuring gastric mucosal NP-SH. Control and treatment rats' glandular stomachs were extracted and homogenized in ice-cold 0.02 M EDTA (ethylene diamine tetraacetic acid). The homogenate was centrifuged after mixing with distilled water and 50 % TCA; the supernatants were mixed with Tris buffer; 5,5′-dithiol-bis (2-nitrobenzoic acid) (DTNB) was added; and the sample was shaken. Within 5 min of adding DTNB, the absorbance was measured at 412 nm against a reagent blank (Sedlak and Lindsay, 1968).

Assessment of antioxidant enzyme markers

To assess the antioxidative capabilities of SKM-GS, we assessed gastric malondialdehyde (MDA), antioxidative enzyme activity, and protein expression. Worldwide, people recognize MDA as a measure of metabolic oxidative stress during inflammation. The MDA level is an indicator of lipid peroxidation and was evaluated using the method of Ohkawa et al. with minor modifications. Hu et al. previously found that reduced glutathione (GSH) is one of the non-enzymatic antioxidants that helps the body get rid of xenobiotics. GSH also works with glutathione peroxidase (Gpx), which is an enzyme that is very important in how enzymes react to oxidative stress. GPX can scavenge H2O2 in the mitochondrial matrix. GPX converts GSH, a tripeptide consisting of glutamate, cysteine, and glycine, into oxidized glutathione (also known as glutathione disulfide, GSSG). This process reduces H2O2 to H2O and lipid hydroperoxides (ROOH) to corresponding stable alcohols. (Nabilet al., 2021).

Assessment of antioxidant enzyme markers

The stomach tissues were infused with paraffin wax and fixed in 10 % neutrally buffered formalin for a day before being transversally sectioned into 5 m-thick sections using a sled microtome. Hematoxylin and eosin staining were employed for histological evaluation under the light microscope to detect microscopic stomach damage (Ortaç et al.,2018).

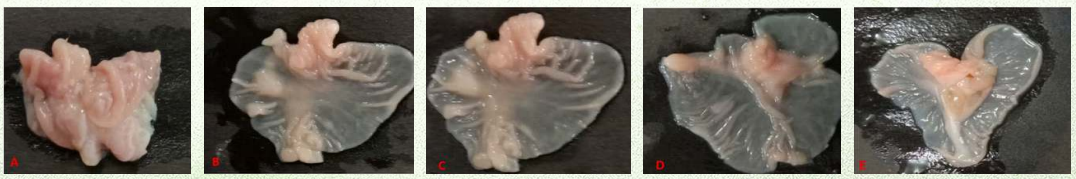

Fig. 2. Macrographic representation of stomachs: Figure 2. Photo macrographs of representative rat stomach that were sliced along the larger curvature.

(A) Normal Control, (B) Indomethacin group, (C) Lansoprazole, (D) SKM-GS (200 mg/kg), and (E) SKM-GS treated (400 mg/kg) show the presence of numerous circular and linear gastric ulcers in the indomethacin group, which were significantly reduced by Lansoprazole treated and the two tested doses of SKM-GS.

Table 1

The effect of SKM-GS on Ulcer Index, Ulcer area, and Preventive Index.

S. No.	Groups	Ulcer Index	Ulcer area (mm2)	Preventive Index (%)
I	Normal Control (1 ml/ kg)	0	0	100
II	INDO (40 mg/kg)	4.75 ±0.15 [a]	6.54 ± 0.09 [a]	0
III	Lanso (30 mg/kg) + INDO (40 mg/kg)	1.45 ±0.07[b]	1.23±0.04[b]	69.47
IV	SKM-GS (200 mg/kg) + INDO (40 mg/kg)	2.58 ±0.08 [b, c]	1.63 ± 0.01 [b, c]	45.68
V	SKM-GS (400 mg/kg) + INDO (40 mg/kg)	2.10 ±0.06 [b, c]	1.52 ± 0.08 [b, c]	55.78

INDO- Indomethacin, Lanso- Lansoprazole, SKM-GS – Gelcocid suspension. Data are presented as mean ±SEM, n = 6. Statistical analyses were carried out using one-way ANOVA followed by Dunnett's test.

[a] Significantly different from the control group at p > 0.001.

[b] Significantly different from the indomethacin group at p > 0.001.

[c] Significantly different from Lansoprazole and SKM-GS (200 mg/kg and 400 mg/kg) groups at p > 0.001.

Statistical analysis

All data were given as means ± SEM by using GraphPad Prism 8.0. The statistical significance of variations between groups for each parameter was determined using a one-way ANOVA. The significance level was set at p > 0.0001 for antioxidant parameters and p<0.001 for gastric wall mucus and NP-SH levels.

RESULTS:

Indomethacin-induced gastric ulcer model

Effects of SKM-GS on ulcer index, preventive index, and ulcer area

The stomach mucosa in the normal control group showed a regular structure (Fig. 2A), but the INDO group showed widespread gastric ulcers (Fig. 2B). SKM-GS at 200 mg/kg and 400 mg/kg doses decreased the severity of stomach ulcers (Fig. 2D and E). Lanso at a dosage of 30 mg/kg (Fig. 2C) decreased the severity of the stomach ulcers. Ulcer index and preventative indexes (Fig. 2A and B) Table 1 were 0 and 100 %, respectively in normal control. In the INDO group, these values substantially (P>0.001) reached 4.750±0.15 and 0 %. SKM-GS at 200 mg/ kg and 400 mg/kg doses substantially reduced the ulcer index while increasing the preventative index produced by INDO. Lanso at 30 mg/kg significantly decreased ulcer index (P > 0.001) when compared to INDO.

Table 2

The effect of SKM-GS on Gastric wall mucus, and NP-SH.

S. No.	Groups	Gastric wall mucus (µg Alcian blue of wet glandular tissue)	NP-SH (mmol/ 100 mg wet tissue)
I	Normal Control (1 ml/kg)	456.8 ± 3.49	9.23±0.04
II	INDO (40 mg/kg)	289.7 ± 2.45[a]	6.33±0.12 [a]
III	Lanso (30 mg/kg) + INDO (40 mg/kg)	396.8 ± 2.97[b]	8.16±0.10 [b]
IV	SKM-GS (200 mg/kg) + INDO (40 mg/kg)	314.2 ± 3.02 [b, c]	7.31 ± 0.03 [b, c]
V	SKM-GS (400 mg/kg) + INDO (40 mg/kg)	341.1 ± 2.06 [b, c]	7.82 ± 0.02 [b, c]

INDO- Indomethacin, Lanso- Lansoprazole, SKM-GS – Gelcocid suspension. Data are presented as mean ±SEM, n = 6. Statistical analyses were carried out using one-way ANOVA followed by Dunnett's test.

[a] Significantly different from the control group at p > 0.001.

[b] Significantly different from the indomethacin group at p > 0.001.

[c] Significantly different from Lansoprazole and SKM-GS (200 mg/kg and 400 mg/kg) groups at p > 0.001.

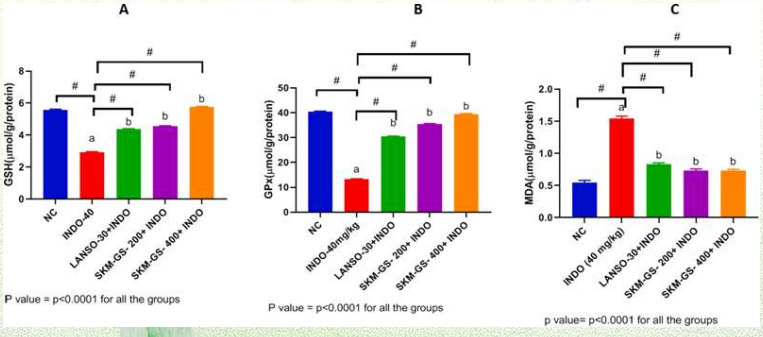

Fig. 3. Assessment of anti-oxidant enzyme markers: Figure 3. Effect of Indomethacin alone and Lansoprazole and SKM-GS (at a dose of 200 mg/kg and 400 mg/kg) on A. Gastric reduced glutathione (GSH), B. Gastric glutathione peroxidase activity (GPx), and C. Malondialdehyde (MDA) levels in rats. Statistical analyses were carried out by one-way ANOVA, n = 6, mean ±SEM.

[a] Significantly different from the control group.

[b] Significantly different from indomethacin alone group at p > 0.0001.

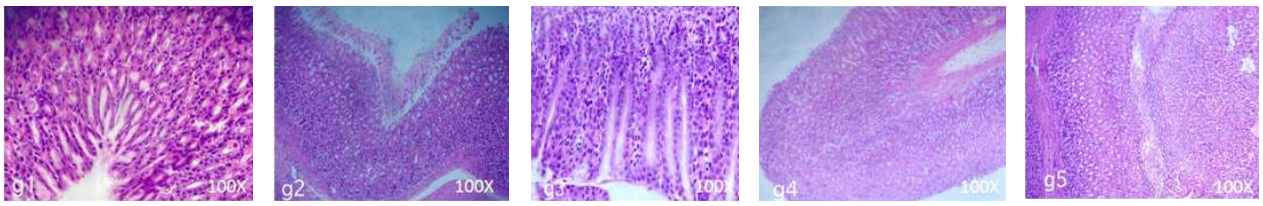

Fig. 4. Histopathological Examination of gastric ulcer induced by Indomethacin in Wistar rats : Fig. 4. An image showing histopathological modifications using 100X magnification: g1. In the control group, normal gastric mucus indicates normal surface mucous cells; g2. Indomethacin group, severe necrotic changes within the gastric mucosa are present, along with the deposition of acid hematin and inflammatory cell infiltration; g3. lansoprazole group, the degenerative and necrotic changes within the gastric mucosa were significantly reduced. g4. The SKM-GS (200 mg/kg)-treated group showed a significant decline in the degenerative alterations in the gastric glands, which suggests minor capillary congestion and few lymphocytes in the surface mucosa; g5. The group treated with SKM-GS (400 mg/kg) exhibited foci of degenerative gastric mucosa and exposed few necrotic gastric glands.

Effects of SKM-GS on gastric wall mucus and NP-SH

In normal control, gastric wall mucus secretion was 456.8±3.49, whereas the decrease in gastric mucus was 289.7±2.45 in the INDO group. SKM-GS at 200 mg/kg and 400 mg/kg increased gastric wall mucus to 314.2±3.02 and 341.1±2.06, respectively. Lanso at 30 mg/kg increased gastric wall mucus to 396.8±2.97. SKMGS at 200 mg/kg and 400 mg/kg significantly increased gastric wall mucus. Lanso at 30 mg/ kg increased mucus in the gastric wall significantly (P > 0.001) compared to INDO. In normal control, gastric wall mucus NP-SH was 9.23±0.04, whereas the decrease in NP-SH was 6.33±0.12 in the Indomethacin group. SKM-GS at 200 mg/kg and 400 mg/kg increased NP-SH to 7.31±0.03 and 7.82±0.02, respectively. Lanso, at a dose of 30 mg/kg, increased gastric wall mucus NP-SH to 8.16±0.10. SKM-GS at 200 mg/kg and 400 mg/kg significantly increased NP-SH. Lanso at 30 mg/kg increased NP-SH significantly (P > 0.001) when compared to INDO Table 2.

Assessment of SKM-GS on antioxidant enzyme markers

The gastric mucosa's oxidative stress markers were examined to determine the SKM-GS gastroprotective mechanism, and it was discovered that INDO reduced both glutathione content and gastric glutathione peroxidase activity by 85 % in comparison to the control group (p > 0.0001). Gastric glutathione activity was elevated in rats pre-treated with Lanso or the SKM-GS at high or low doses (p > 0.0001, Fig. 3A), 110.3 %, 157.2 %, and 160.8 %, respectively. However, the SKM-GS had a substantial impact on the stomach glutathione peroxidase concentration at both low and high doses (p > 0.0001), as shown in Fig. 3B. In contrast, the INDO group had higher levels of lipid peroxidation as measured by MDA than the control group by a factor of 152.4 (p >0.001). Lanso and SKM-GS at the two dose levels decreased stomach MDA levels by 50.6 %, 40.9 %, and 40.1 % (p > 0.0001), respectively, in contrast to the INDO group. (Fig. 3C).

Histopathological examination

Fig. 4 (g1 to g5) displays the presence of severe levels of necrotic alterations within the gastric mucosa, as well as the deposition of acid hematin and the infiltration of inflammatory cells, as evidence that INDO treatment produced significant stomach damage. It was identified by histological analysis of the stomachs of different rat groups. Pretreatment with Lanso considerably reduced the pathological changes in the stomach by dramatically lowering the degenerative and necrotic modifications inside the gastric mucosa. Pre-treatment of rats with SKMGS at a high dose level (400 mg/kg) greatly decreased the degenerative abnormalities within the gastric glands by reducing gastric inflammation in the surface mucosal lining. In contrast, foci of deteriorating gastric mucosa that indicated a few necrotic gastric glands suggested that the low dose level of SKM-GS (200 mg/kg) provided less substantial progress than both the high dose and Lanso. INDO increased stomach edema, necrosis, inflammation, and bleeding as compared to the control group.

Discussion

The present investigation is being reported for the for the first time about the impact of SKM-GS on rat stomach ulcers brought on by INDO. SKM-GS produced gastroprotective effects at two dosage levels (200 and 400 mg/kg) equivalent in most regards to the well-known gastroprotective lansoprazole. Our research not only demonstrated the gastroprotective properties of SKM-GS but also suggested that these properties may have an anti-oxidant impact.

The current findings also demonstrated that SKM-GS increased the protective index while decreasing the ulcer area. The commonly used parameters include the ulcer area, ulcer index, and protective index to examine the impact of herbal remedies, anti-ulcer medications, and their active ingredients on the macroscopic alterations in the gastric mucosa caused by indomethacin.

Indomethacin-induced ulceration may also be associated with several events, including the suppression of prostaglandin production, the generation of ROS, and the onset of lipid peroxidation. Abdallah et al. (2011) said that indomethacin-induced stomach ulcers were accompanied by severe oxidative stress in the stomach tissue. This stress damaged important biomolecules like lipids and sped up the oxidation of lipids. This led to an increase in MDA accumulation and a decrease in gastric antioxidant activity. In this study, pre-treatment with SKM-GS lowered MDA in the INDO group, indicating probable antioxidant activity. The gastroprotective action of SKM-GS is likely due to its antioxidant capabilities.

It is generally known that glutathione peroxidase (Gpx), a seleniumdependent enzyme, serves as a protective wall against hydroperoxide attack (Lujan et al., 2018). The GI isoenzyme of GPx activation must raise the level of glutathione (GSH), which protects redox status. (Cheng et al., 2013) The reduced glutathione peroxidase activity aggravated gastric mucosal damage by causing a rapid rise in lipid peroxides and hydrogen peroxide. As a result, overexpression of antioxidant enzymes such as GSH and elevation of gastric GPx content might be an essential preventive mechanism against stomach ulcers caused by an increase in ROS. (Zaghlool et al., 2019). Only a higher dosage increased stomach GSH, indicating a dose-dependent antioxidant effect, but both doses enhanced GPx activity. Histopathological findings revealed a significant improvement in gastric wall regeneration in the Lansoprazole and SKM-GS-treated groups. In our study, SKM-GS and lansoprazole demonstrated comparable gastroprotective effects against indomethacin-induced stomach ulcers. Ayurvedic and Siddha formulations, with their physiologically active components, have frequently been employed to compare anti-ulcer effects.

CONCLUSION:

SKM Siddha and Ayurveda (India) Pvt. Ltd., located in Saminathapuram, Modakkurichi, Erode, Tamil Nadu, India, manufactures SKM-GS, an Ayurveda formulation. The current study established, for the first time, that SKM-GS provided significant gastroprotection against INDOinduced gastric mucosal injury in a dose-dependent manner, with activity comparable to that of Lanso. This study suggests that SKM-GS may be used against gastriculcers, which have the same potency as lanso. Additionally, SKM-GS proved to be an antioxidant without lessening gastric acidity, eliminating the negative effects of the commonly prescribed chemical anti-secretory medications. Plant extracts from Emblica officinalis, Hemidesmus indicus, Terminalia chebula, Mentha viridis, and Cuminum cyminum that are high in polyphenols help with these behaviors. SKM-GS (Gelcocid suspension) showed gastroprotective and anti-oxidant effects against the gastric ulcers that were induced by Indomethacin. The gelcocid suspension demonstrated a good therapeutic effect at higher doses (400 mg/kg). Hence, SKM-GS may be a safe Siddha formulation against gastric ulcers.

CRediT authorship contribution statement

Ganesh Thangavel: Supervision, Resources, Project administration. Tharani Mohanasundaram: Data curation, Formal analysis, Writing – original draft. Vadivelan Ramachandran: Methodology, Investigation, Formal analysis, Conceptualization.

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Kadukkai maathirai (a polyherbal traditional siddha formulation) prevents D-galactosamine induced hepatic necrosis in rats

INTRODUCTION:

This study evaluated the prophylactic effect of Kadukkai maathirai in D-galactosamine (D-gal) induced hepatotoxicity in rats. D-galactosamine (D-gal) 400 mg/kg intraperitoneally was used to induce liver damage in rats. To assess the hepatoprotective effect of KM, three different doses of KM (36, 72 and 144 mg/kg body weight) were used. The hepatoprotective effect of KM was compared with standard drug silymarin (50 mg/kg). The biochemical parameters such as AST, ALT, ALP and total bilirubin were estimated. The livers were dissected out to look for histological changes. KM 144 mg/kg and silymarin showed a significant decrease in AST, ALP and total bilirubin. Both KM and silymarin significantly prevented decrease in liver weight. In KM treated groups, the liver did not show necrosis of hepatocytes, and apoptotic bodies with mild to moderate inflammatory infiltrate in the lobules and portal tracts. Hence, the results of this study confirms the hepatoprotective effect KM in rats.

Fulminant hepatitis can be caused by various factors including viruses, alcohol and chemicals.This disease is associated with a high mortality rate as there are no effective prophylaxis or treatment. Liver transplantation is the only option in case of fulminant hepatitis which is very expensive. Hence newer agents need to be developed to treat various liver ailments. Extensive research is ongoing around the world to understand the pathogenesis of fulminant hepatitis. Different animal models are being used to test new chemical entities and herbal drugs.One of the models commonly used to mimic fulminant hepatitis in rats is D-galactosamine (D-gal) induced acute liver injury[1].

Hepatic injury by D-galactosamine (D-gal)resembles human viral hepatitis in its morphological and functional features[2]. The intermediary toxic metabolites (UDP-galactosamine and UDP-glucosamine) of D-gal trap uracil nucleotides required for the biosynthesis of nucleic acids and proteins3. Subsequently, necrosis of hepatocytes occurs. Galactosamine induces activated mast cells to release histamine leading to increase in the permeability of the gut. Damage by D-galactosamine is also due to release of tumor necrosis factor-alpha (TNF-α) from Kupffer cells which causes cell death by oxidative stress and by triggering inflammation4-6.

Many Indian traditional medicines have been used in the treatment of liver disease, and there are plenty of reports demonstrating hepatoprotective effects of various herbs[8]. Among the traditional Indian medicines, the Siddha system of medicine is practiced mainly in South Indian states and other South East Asian countries. Kadukkai maathirai (KM), one of the polyherbal Siddha preparation is often used to prevent and treat liver diseases[9,10].

KM consists of four herbs namely Terminalia chebula (Retz.), Piper nigrum (Linn.), Ecliptaalba (Linn.), Citrus limon (Linn.), and ferrous sulfate11. Each herb contained in KM has been proved to exhibit hepatoprotective effect individually against different hepatotoxin induced liver disease animal models like -isoniazid, rifampicin, and pyrazinamideinduced liver toxicity; carbon tetrachloride as well as thioacetamide-induced liver toxicity. Studies with KM alone has been shown to have hepatoprotective effect in CCl_4-as well as alcohol induced liver toxicity in rats[12-18].

The current experiment was undertaken to investigate whether Kaddukai maathirai could protect against D-galactosamine induced hepatotoxicity.

MATERIALS AND METHODS:

Animals and reagents

Adult female Sprague Dawley rats weighing about 150-200 g were used in this study. They were housed individually in polypropylene cages at 27 ± 3°C, humidity of 60 ± 10%- and 12-hours' light /dark cycle. The study was in accordance with standards laid down by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines following clearance from the Institutional Animal Ethics Committee, Manipal. (IAEC/KMC/19/2016 dated 16.03.2016).

D-galactosamine was procured from TCI, chemicals industry, Co.Ltd, Tokyo, Japan. All other chemicals were obtained from SPINREACT chemicals, Spain. Silymarin (standard drug) was procured from a local pharmacy. Kadukkai maathirai was procured from SKM Siddha and Ayurveda Company (India) Ltd, Saminathapuram, Modakkurichi, Erode District- 638 104, Tamilnadu, a GMP certified company.

Experiment design

Nine groups with six rats in each was taken for the study. D-galactosamine was used to induce liver damage. Group I received the vehicle (2% gum acacia) and served as control. Groups II, III, IV test drug control, received KM 36, 72,144 mg/kg, respectively. Group V was kept as toxic (D-gal) control and received 2% gum acacia. Groups VI, VII, VIII received Kadukkai maathirai (36 mg/kg, 72 mg/kg and 144 mg/kg, respectively) along with D-gal.

Group IX received standard drug silymarin (50 mg/kg) with D-gal. Drugs, other than D-gal, were given by oral gavage upto day seven. On day eight, D-galactosamine 400 mg/kg was injected intraperitoneally in rats of groups V, VI, VII, VIII, IX. On the 9th day, body weight was measured, and blood was collected by retro-orbital puncture and the serum was separated in sterile centrifuge tubes. The following serum enzymes were analyzed in serum using commercial assay kits obtained from Agappe Diagnostics Ltd: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total bilirubin. The estimation of biochemical parameters was done by standard procedures using kits. Rats were sacrificed by high dose pentobarbitone, and the liver was weighed and fixed in 10% formalin solution for microscopic examination using hematoxylin-eosin stain.

Statistics

The statistical program SPSS 16.0 was used. Data were expressed as mean and standard deviation. Oneway ANOVA followed by post hoc Tukey's test was used. Statistical significance was set at $p<0.05$.

RESULTS:

Biochemical estimation in serum

Administration of D-galactosamine showed a significant ($p<0.05$) rise in serum AST and ALT, ALP, total bilirubin levels than normal control. Prophylaxis with KM at 36, 72, 144 mg/kg significantly ($p<0.05$) prevented D-galactosamine induced rise in AST and ALT, ALP, total bilirubin levels versus those who were administered Dgalactosamine alone (Table 1). Serum ALT level was significantly ($p<0.05$) lower in rats treated with silymarin than the group which received KM-144 mg/kg ($p<0.05$). Alteration in weight of rats was not significant between groups. The weight of the liver in D-galactosamine group was significantly reduced whereas three different doses KM at 36, 72, 144 mg/kg and silymarin significantly prevented decrease in weight of liver when given prophylactically.

Histopathology

Histopathological findings of the liver sections of normal and test drug treated groups are shown in Figure 1.

Figures 1a-100x, 1b-400x show the liver section of group I (normal) rats where hepatic parenchyma with normal lobular architecture and cell structure. Figures 2a, 2b; 3a, 3b; 4a, 4b- 10× and 40× show liver tissue of groups II, III, IV (KM in 36, 72, 144 mg/ kg body weight) where normal hepatic parenchyma with hepatic lobules and occasional cytoplasmic vacuolation. Figures 5a, 5b-10×, 40× show group V (D-galactosamine) where there were extremely pronounced focal necrosis of hepatocytes, occasional apoptotic bodies, and also showed partially effaced architecture with small clusters of lymphocytes within the lobules and portal tracts. In groups VI, VII, VIII (KM 36, 72, 144 mg/kg along with D-gal) there was better structural appearance of the liver without necrosis of hepatocytes, andapoptotic bodies with mild to moderate inflammatory infiltrate in the lobules and portal tracts (Fig. 6a-10×, 6b-40×; 7a-10×, 7b-40×; 8a-10×, 8b40×) as compared to group IX (Fig 9a-10×, 9b-40×).

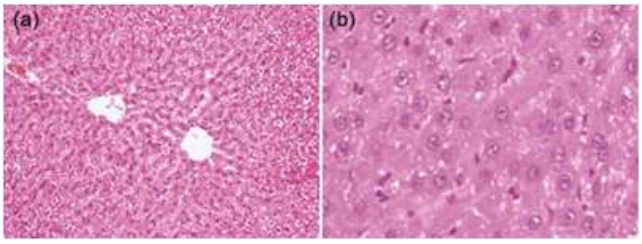

Fig. 1 — Histopathological findings of the liver in control group

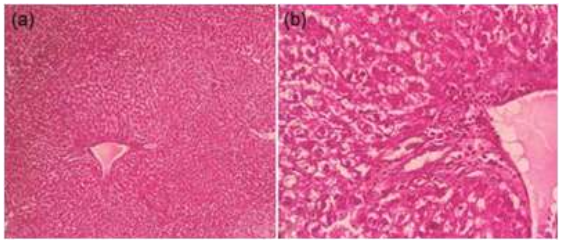

Fig. 3 — (a) 10x Photomicrograph of liver tissue of group III (KM in 72 mg/ kg body weight) showing normal hepatic parenchyma with hepatic lobules and occasional cytoplasmic vacuolation; low power view (10x), H&E stain; & (b) Photomicrograph of liver tissue of group III (KM in 72 mg/ kg body weight) showing normal hepatic parenchyma with hepatic lobules and occasional cytoplasmic vacuolation; high power view (40x)

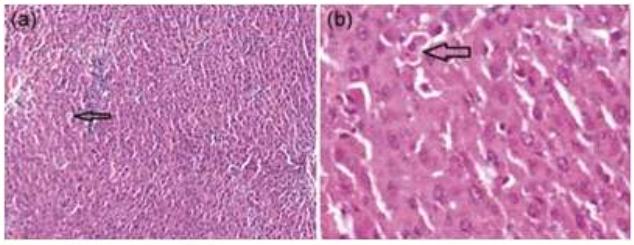

Fig. 5 — (a) Liver tissue of group V(D-galactosamine) showed extremely pronounced focal necrosis of hepatocytes, occasional apoptotic bodies, and also showed partially effaced architecture with small clusters of lymphocytes within the lobules and portal tracts; low power view (10x) , H&E stain; & (b) Photomicrograph of liver tissue of group V(D-galactosamine) showed extremely pronounced focal necrosis of hepatocytes, occasional apoptotic bodies, and also showed partially effaced architecture with small clusters of lymphocytes within the lobules and portal tracts ; high power view (40x) , H&E stain

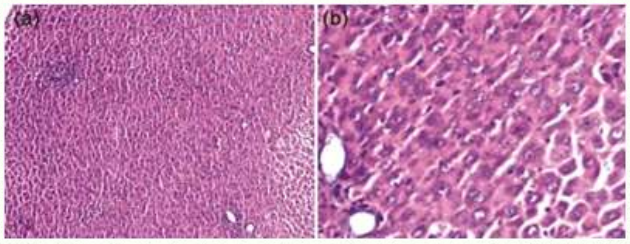

Fig. — 7 (a) Photomicrograph of liver tissue showing of group VII(KM 72 mg/kg along with D-gal)better structural appearance of the liver without necrosis of hepatocytes, and apoptotic bodies with mild to moderate inflammatory infiltrate in the lobules and portal tracts; low power view (10x), H&E stain; & (b) Photomicrograph of liver tissue showing of group VII(KM 72 mg/kg along with D-gal)better structural appearance of the liver without necrosis of hepatocytes, and apoptotic bodies with mild to moderate inflammatory infiltrate in the lobules and portal tracts ; high power view (40x) , H&E stain

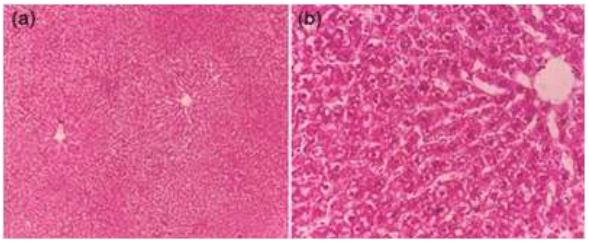

Fig. 2 — (a) Photomicrograph of liver tissue of group II (KM in 36 mg/ kg body weight) showing normal hepatic parenchyma with hepatic lobules and occasional cytoplasmic vacuolation; low power view (10x) , H&E stain; &(b) - Photomicrograph of liver tissue of group II (KM in 36 mg/ kg body weight) showing normal hepatic parenchyma with hepatic lobules and occasional cytoplasmic vacuolation ; high power view (40x) , H&E stain

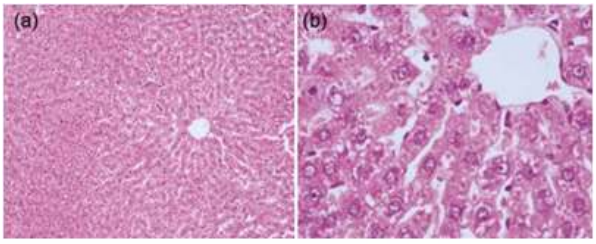

Fig. 4 — (a) Photomicrograph of liver tissue of group IV (KM in 144 mg/ kg body weight) sowing normal hepatic parenchyma with hepatic lobules and occasional cytoplasmic vacuolation; low power view (10x), H&E stain; & (b) Photomicrograph of liver tissue of group IV (KM in 144 mg/ kg body weight) showing normal hepatic parenchyma with hepatic lobules and occasional cytoplasmic vacuolation; high power view (40x), H&E stain

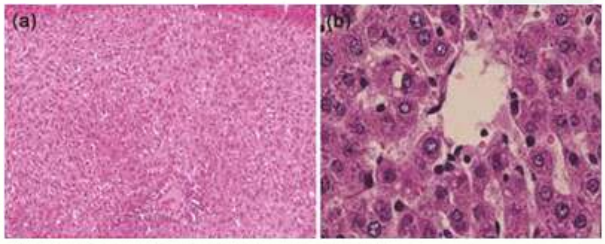

Fig. 6 — (a) Photomicrograph of liver tissue showing of group VI(KM36 mg/kg along with D-gal) better structural appearance of the liver without necrosis of hepatocytes, and apoptotic bodies with mild to moderate inflammatory infiltrate in the lobules and portal tracts; low power view (10x), H&E stain; & (b) Photomicrograph of liver tissue showing of group VI(KM 36 mg/kg along with D-gal) better structuralappearance of the liver without necrosis of hepatocytes, and apoptotic bodies with mild to moderate inflammatory infiltrate in the lobules and portal tracts; high power view (40x), H&E stain

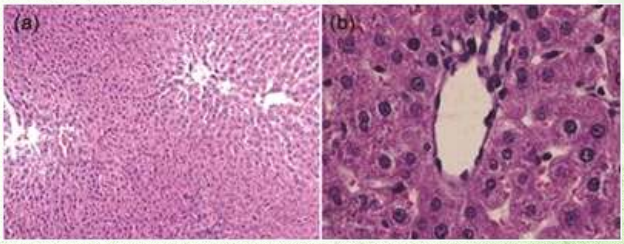

Fig. 8 — (a) Photomicrograph of liver tissue showing of group VIII(KM 144 mg/kg along with D-gal)better structural appearance of the liver without necrosis of hepatocytes, and apoptotic bodies with mild to moderate inflammatory infiltrate in the lobules and portal tracts; low power view (10x), H&E stain; & (b) Photomicrograph of liver tissue showing of group VIII(KM 144 mg/kg along with D-gal)better structural appearance of the liver without necrosis of hepatocytes, and apoptotic bodies with mild to moderate inflammatory infiltrate in the lobules and portal tracts ; high power view (40x), H&E stain

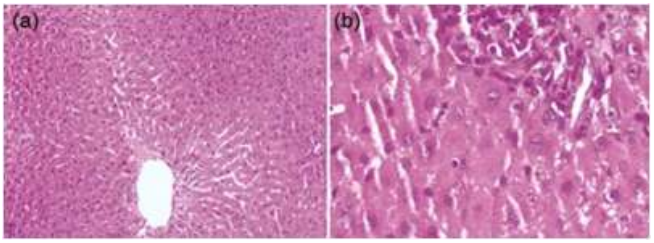

Fig. 9 — (a) Photomicrograph of liver tissue showing of group IX (Silymarin along with D-gal) ; low power view (10x), H&E stain; & (b) Liver tissue showing of group IX(Silymarin along with D-gal); high power view (40x), H&E stain

DISCUSSION:

D-galactosamine-induced liver injury model is commonly used to screen the hepatoprotective effects of new chemical entities and also various herbal drugs. The changes seen in liver pathology with D-galactosamine induced liver damage resembles acute viral hepatitis in human beings19,20. Hence, this model was chosen in the current study. D-galactosamine makes the liver more susceptible to oxidative stress as it alters antioxidant status20. Various studies in the past have shown that D-galactosamine causes a drastic rise in AST, ALT and ALP.D-galactosamine causes fulminant hepatitis by blocking hepatic protein synthesis, and this could be attributed to liver weight loss observed in this study21. D-galactosamine-induced liver injury is associated with the exhaustion of uracil nucleotides leading to inhibition of synthesis of RNA and protein which causes necrosis of liver cells22. Administration of D-galactosamine in a dose of 400 mg/kg body weight i.p. produced extensive liver damage within 24 hours. As a result, the typical architecture of the liver changed to partially affected architecture with small clusters of lymphocytes within the lobules and portal tracts, focal necrosis of hepatocytes along with occasional apoptotic bodies. Subsequently, hepatic enzymes seeped into the plasmawhichresulted in a significant ($p<0.05$) rise in serum levels of AST, ALT and ALP in D - gal treated rats.

Silymarin was used as a standard hepatoprotective compound in this study as it is known to protect the plasma membrane of liver cells23. Silymairn acts by reduction of free radicals. It also hinders the entry of toxic substances into the liver cells. Also, it promotes protein synthesis[24].

In the present study, KM at 36, 72, 144 mg/kg significantly ($p<0.05$) prevented the D-galactosamine induced rise in AST and ALT levels. This was comparable with standard drug silymarin (Table 2). KM drug alone (36, 72, 144 mg/kg) showed a slight elevation in liver enzymes without any structural damage. This elevation might be because of presence of certain minerals or metals in KM as it is a polyherbal preparation. The absence of structural damage was confirmed by histopathological findings. In this study we have not evaluated whether it is selflimiting or spontaneously reversible. However, in future studies this can be evaluated.

Clinically, AST, ALT, ALP and bilirubin levels are elevated in acute hepatitis, and their levels return to normal when the healing process starts23. KM consists of herbs such as T. chebula, P. nigrum, E. alba, and C. limon11. C limon has antioxidants such as Vitamin C and flavonoids which target the free radicals25. P. nigrum exerts antioxidant effect which could be mediated by flavonoids and phenolic constituents. It has been shown to inhibit lipid peroxidation, and generation of superoxide free radicals26,27. Gallic acid and chebulic acid are essential constituents of T. chebula. Gallic acid has antioxidant and anti-inflammatory properties. Chebulic acid is known to be an antioxidant and hepatoprotective agent28-31. Coumestans, present in E. alba, has been shown to exert a protective effect in liver disorders and stimulate liver cell regeneration. KM treatment showed significant restoration of the altered liver enzymes and bilirubin levels towards normal in D-galactosamine intoxicated rats. The hepatoprotective effect of KM at the dose of 144 mg/kg and the standard drug silymarin were comparable which was further confirmed by histopathological findings.

CONCLUSIONS

The Siddha preparation, Kadukkai maathirai exhibited hepatoprotective activity in Dgalactosamine induced liver damage in rats. Hence, this study could be a piece of scientific evidence for traditional medicine practitioners. The hepatoprotective effect exhibited by KM could be attributed to its anti-inflammatory and antioxidant properties reported earlier.

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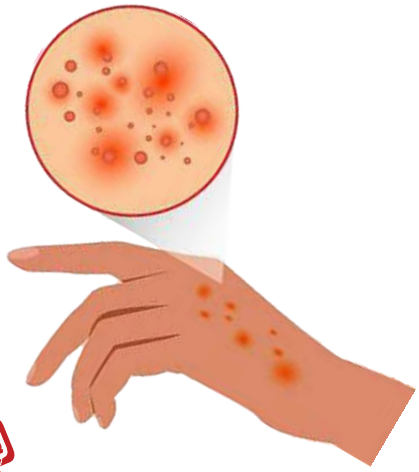

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