DISCUSSION
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5.1 Phytochemical studies:

Phytochemical screening indicated that the stem bark of *Soymida febrifuga* Adr. Juss was rich in proanthocyanidins, with other flavonoids, saponins and terpenoids as minor constituents. It was found to contain 6.03% of phenolics and 8.18% of proanthocyanidins. After extraction and acid hydrolysis, the resulting anthocyanidins when chromatographed by TLC on cellulose F (Merck) using HoAc : HCl : H₂O 30:3:10 (Forestal) as mobile phase, gave one zone with Rf 0.45 which was identified as cyanidin (UV/VIS λ max nm 273, 536) by comparison with authentic material. This indicated that the proanthocyanidins (Flavan-3-ols) contained catechin or epicatechin units exclusively. The two dimensional TLC (2D-TLC) of extract SA₃ of the bark showed as its main component a large conspicuous zone in the centre of the chromatogram and a tailing elliptical zone only mobile in 6% HOAc. After spraying with vanillin – HCl, these zones gave intense red colour characteristic of oligomeric (Pn = 2-8) and polymeric (Pn>8) proanthocyanidins. The type of flavan-3-ol units was also confirmed from the absorption maxima. In the present study, U.V. maxima was found to be at 279 nm which is characteristic of catechin and epicatechin (number of flavan-3-ol units does not affect this parameter). HPLC and HPTLC finger printing: the efficacy of a crude drug / extract could be evaluated as the sum of additive, synergistic and antagonistic effects of all the ingredients. Keeping this in view, HPLC and HPTLC finger printing profile was developed and optimized to give a distinct chemoprofile to the pharmacologically active fraction SA₃. The data suggested that the active fraction SA₃ was rich in proanthocyanidins, with flavonoids and other phenolics.

5.2 Pharmacological studies:

Peptic ulcers which are often considered as a minor condition by patients, kill a few but trouble many. Initial management for complication of peptic ulcer disease was surgical but the development of newer H₂ antagonists (Cimetidine) and proton pump inhibitors e.g. Omeprazole has changed the scenario and significantly lowered the mortality rate. The importance of bacterium *Helicobacter pylori* has again dramatically
changed the peptic ulcer treatment. Despite of such significant contribution of modern medicine, one should not forget the traditional system of medicine like Ayurvedic system.

Ayurveda, the science of life, offers a wide array of plants for keeping the animals and human beings hale and healthy. One such plant – *Soymida febrifuga* – used as antiulcer in Ayurveda has been investigated pharmacologically in various experimentally-induced gastric ulcer models. It is not possible to study the genesis of ulcers as well as to understand the exact mechanism of antiulcer effect from a single model. Novel drug discovery alone would be futile unless it is complemented by the simultaneous introduction of newer experimental methods to evaluate the drugs and to elucidate their possible mode of action.

5.2.1 Antiulcer activity:

The ulcer study was initiated by evaluating extracts SA, SA₁, SA₂, SA₃ and SA₄ for their protective actions against aspirin-induced gastric lesions (Table 4 & 4a). Extracts SA, SA₁, SA₂, SA₃ and SA₄ were assessed further for their effect on ethanol-induced gastric lesion (Table 5). The results of these two studies revealed that the most potent activity lies with extract SA₃, which was then tested on other experimental models (ethanol + indomethacin, pylorus ligation) for evaluation of its mechanism of action.

Extracts SA, SA₁, SA₂ and SA₃ significantly inhibited the formation of gastric lesion and protected the gastric mucosa from the gastric lesions induced by ethanol with complete suppression of ulcerogenesis; extract SA₃ being strongest of all. Necrotising agents (ethanol, ethanol – HCl) induced gastric lesions are not inhibited by suppression of gastric acid secretion but rather by cytoprotective effect⁴⁵⁴, suggesting that the effect of these extracts was on site and direct. It has been reported that once the gastric mucosal barriers are disrupted by agents like ethanol and aspirin, the luminal acidity decreases while Na⁺ concentration increases. Net flux of Na⁺ from the mucosa to lumen is a sensitive index of the gastric mucosal barrier⁸⁴. Studies have also indicated the involvement of increased vascular permeability and lipid peroxidation products in the pathogenesis of ethanol-induced gastric lesions⁴⁵⁶.
Extract SA₃ not only prevented gastric mucosal damage but also reduced the vascular permeability (Table 5c) and extent of lipid peroxidation (Table 5b) as seen from its ability to reduce the malondialdehyde content and thereby promoting the healing of this kind of acute gastric mucosal injury.

Oral administration of necrotizing agents like ethanol, stimulates release of prostaglandins by the stomach to prevent gastric lesions through adaptive cytoprotection. This cytoprotection is inhibited by pretreatment with indomethacin, an inhibitor of COX-1 and COX-2. The cytoprotective action of extract SA₃ against ethanol-induced gastric lesion was not abolished by pretreatment with indomethacin (Table 6) indicating that the effect was mainly a direct mucosal protective action and not mediated through endogenous prostaglandins. Flavonoids like catechins, rutin, quercetin, silymarin etc possessing anti-inflammatory and anti-oxidant properties are reported to offer gastroprotection. These types of compounds interfere with the production of arachidonic acid cascade through lipoxygenase enzyme inhibition and reduce concentration of leukotrienes (powerful inflammogens) in different biological systems. Extract SA₃ being rich in flavonoids (catechins) might be acting through a prostaglandin independent mechanism probably involving anti-inflammatory (maintaining capillary integrity) and free radical scavenging actions.

The extracts SA, SA₁ SA₂ SA₃ and SA₄ also showed significant reduction in ulcer index in aspirin-induced gastric lesions, the extract SA₃ being most potent. The mechanism of this ulcerogenic activity could be correlated to that of pylorus ligated gastric ulcer model where the extract SA₃ was administered both orally and intraperitoneally. Gastric acid and Pepsin are important factors for the formation of ulcers in pylorus ligated rats. Since extract SA₃ (500 mg kg⁻¹, po and i.p.) significantly inhibited lesion formation in the glandular stomach and decreased both acid concentration and pepsin activity together with increase in pH values, it is suggested that the extract can suppress gastric damage induced by aggressive factors (Table 7). The incubation of the gastric juice of control rats with extract SA₃ ruled out the hypothesis that the extract exhibited a direct acid neutralization effect as well as direct inhibition of peptic activity and thus indicated that action was related to
the influence on secretory mechanism of pepsin. The possibility of only local action of 
the extract SA₃ was ruled out by the results of the intraperitoneal administration of the 
extact which provoked marked reduction in total gastric acidity and ulcer index. Thus, 
the antiulcer activity shown by the extract SA₃ might be through its systemic 
effect rather than a local one. Here the extract SA₃ might be providing protection by 
preventing back diffusion of H⁺, thus strengthening gastric mucosal barrier which is 
evident from inhibition of gastric acid secretion and increase in pH by the extract SA₃. 
Further, extract SA₃ augmented mucin secretion as evident from significant increase 
in ratio of total carbohydrates to proteins (TC / PR) in gastric juice, which reflects the 
functional integrity of the mucosal barrier and has been accepted as a reliable index of 
mucosal resistance⁴⁶⁰. In the present study, extract SA₃ not only altered the total 
carbohydrates and the proteins and so the TC / PR ratio but also increased the alcian 
blue binding to the gastric mucosa which represents the amount of adherent mucus to 
the surface of gastric mucosa (Table 7a). Thus the results of present study suggest 
that the major mechanism of gastric mucosal protection offered by extract SA₃ is the 
reinforcement of resistance of mucosal barrier in addition to its antisecretory activity 
on acid and pepsin. Also direct cytoprotection may be involved as indicated by 
increased TC / PR ratio. Extract SA₃ is rich in procyanidins and procyanidins have 
been reported to suppress both invivo and invitro peptic activity⁴⁶¹. The fact that 
extact SA₃ reduces the acidity as well as pepsin secretion, suggests that leukotrienes 
may be playing an important role in controlling gastric secretion⁴⁶². It has also been 
suggested that tannins protect the gastric mucosa through their property of 
astriction⁴⁶³. Certain flavonoids are known to increase both the amount of mucus and 
its glycoprotein content⁴⁶². It is therefore possible that gastroprotective action of 
extact SA₃ could also be due to enhanced mucus production. In conclusion our data 
are suggestive of mucoprotective action of extract SA₃ probably through a non-PG 
dependent mechanism involving potent antisecretory properties and enhancement of 
quality and production of gastric mucus.
5.2.2 Hepatoprotective activity:

Hepatoprotective activity of extract was evaluated in chemically-induced acute liver injury caused by treatment with CCl₄ in mice. When mice were pretreated with these extracts for three days before and three days after treatment with CCl₄, liver injuries were significantly suppressed at the dose of 500mg kg⁻¹ per day with extract SA₃ (Table 9).

CCl₄ is one of most widely studied hepatotoxin. This hepatotoxin is converted into highly reactive hepatotoxic product such as trichloromethyl radical primarily by the liver microsomal mixed function oxidase system which plays an important role in CCl₄-induced liver lesions. This radical binds covalently to neighbouring components in the endoplasmic reticulum and may initiate lipid peroxidation followed by various dysfunctions such as loss of permeability, inactivation of enzymes and abnormal flux of ion between blood and liver. This observation is in accordance with the earlier report suggesting involvement of free radicals in the propagation of peroxidation process. The antihepatotoxic action of number of natural products as well as many flavonoids like quercetin, catechin and hypericin in chemically-induced hepatocyte injuries is attributed to their antiperoxidative property. The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic mechanism which has been disturbed by a hepatotoxin is the index of its protective effect. From the results obtained in the present study it can be seen that extract SA₃ has the ability to prevent the changes mediated by CCl₄. This activity of extract SA₃ is evident from the reduced hepatotoxicity induced by simultaneously administered CCl₄, as judged by pentobarbitone sleeping time.

The fact that anesthesia induced by short acting barbiturates is significantly prolonged in the event of any hepatic damage is a measure of hepatic microsomal drug metabolizing enzymes. In the present study the extract SA₃ significantly reduced the normal pentobarbitone-induced sleep time (Table 9) which was prolonged with CCl₄ administration. Liver cells participate in a variety of metabolic activities and therefore contain a host of enzymes. Estimation of serum levels of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) is
one of the most widely used measures of hepatocellular injury. An increase in transaminase activity in serum following CCl₄ administration is attributed to increased release of enzymes from damaged liver parenchymal cells and due to increased synthesis and/or decreased destruction of these enzymes in hepatic cells. Thus CCl₄-induced damage to the liver raises the serum levels of these enzymes by releasing them in the bloodstream. This is related to the extent of liver damage in animals. Also increased malondialdehyde (MDA) levels in hepatic tissue by CCl₄ has been reported.

Treatment with extract SA₃ in mice seems to preserve the integrity of the liver cell membrane as documented by the reduction of CCl₄-induced rise of s-GOT and s-GPT levels and MDA levels (Table 9a) in hepatic tissue. These results show the ability of extract SA₃, to maintain the normal functional status of liver by reducing the hepatic disorder induced by CCl₄ intoxication. It is possible that extract SA₃ inhibits the early activation of CCl₄ in addition to the subsequent peroxidation process. Thus it can be speculated that extract SA₃ could serve as an antioxidant in the chemically induced hepatitis. Since, extract SA₃ mainly contains flavan-3-ols with other flavonoids, our study is well in agreement with the report that flavan-3-ols like epicatechin, epigallocatechin, catechin and gallocatechin show liver protective activity.

5.2.3 Analgesic activity:

The analgesic effect of different extracts of bark was investigated. The findings indicated that oral administration of extract SA, SA₂ and SA₃ reduced the number of abdominal constrictions in mice induced by 0.6 % acetic acid. All three extracts showed protective effect in dose dependent manner (Table 10). The order of potency was SA₃>SA₂>SA. Diclofenac sodium (100 mg kg⁻¹ p.o.) elicited 90.43 % protection against acetic acid – induced writhing. Further, the potent extract SA₃ also exerted significant protective effect on thermal painfull stimuli in tail flick test as evidenced from increase in latency period or in the reaction time of mice at higher dose (500 mg kg⁻¹, p.o.) (Table 12). It is known that peripherally acting components reduce or inhibit writhings induced by acetic acid and centrally acting drugs elicit protective effect on thermal painfull stimuli. Also, it has been reported that narcotic agents like morphine...
and centrally acting analgesics, could inhibit thermally-induced nociceptive responses in mice\(^{481,482}\). The activity of extract SA\(_3\) in both acetic acid-induced writhing and tail flick model reflects the effect on both peripheral as well as central components of the pain. Previous studies have also indicated the ellagitannins, flavones and biflavonoid to be responsible for antinociceptive action of crude extracts of plants\(^{483,484}\). However, it is paradoxical to find anti-inflammatory, analgesic and antiarthritic effects in a compound possessing antiulcer activity, since anti-inflammatory agents are often ulcerogenic.

### 5.2.4 Barbiturate sleeping time:

Administration of extract SA\(_3\) by i.p. route potentiated pentobarbitone-induced hypnosis in dose dependent manner in mice (Table 14). Although this indicated probability of central mechanism contributing towards the antiulcer activity, the potentiation could be due to the direct action of extract on some CNS site. At the same time the possibility of inherent depressant activity or reduced metabolism of pentobarbitone due to inhibition of liver microsomal enzyme system responsible for degradation of pentobarbitone by the extract can not be ruled out. Consequently it is difficult to state, based on present data the exact mechanism of potentiation of pentobarbitone hypnosis. To know whether or not the central depressant action is associated with peptic ulcer, requires further studies.

### 5.2.5 Antidiarrhoeal activity:

Diarrhoea is considered as a consequence of altered motility and fluid accumulation. Since a number of flavonoids have been reported to inhibit intestinal motility and secretion\(^{485}\), they may presumably exert an antidiarrhoeal action. The results of the present study showed that extract SA\(_2\) which is rich in procyanidins with flavonoids (condensed tannins) significantly reduces the incidence and severity of castor oil-induced intraluminal accumulation of fluid. The extract at the doses of 100, 300 and 500 mg kg\(^{-1}\) p.o., like the standard antidiarrhoeal agent loperamide, significantly inhibited the frequency of defecation and wetness of faecal droppings evident by increase in the initiation time of the excretion of first diarrhoeic feaces and decrease in
faecal weight compared to castor oil-treated mice (Table 16). The reference standard loperamide which acts through calcium channel blockade\(^{486}\) (smooth muscle relaxing effect) also produced potent inhibition of accumulation of intestine fluid. It has been proposed that the action of number of flavonoids like quercetin, rutin, kaemferol, apigenin etc is mediated through interaction with \(\alpha_2\) – adrenergic and calcium channel system\(^{485,487}\). Since extract SA\(_2\) produced no clear dose dependent action, it suggests a more unspecific effect of extract on intestinal function, probably due to likely interference with the cellular enzymes and neurotransmitter system and / or due to its interaction with calcium channel system\(^{488}\). In conclusion, the inhibitory effect of the extract SA\(_2\) justifies the use of the plant in folk medicine and its use as a non-specific antidiarrhoeal agent. The underlying mechanism appears to be spasmolytic by which plant extract produces relief in diarrhoea. Tannic acid and tannins present in many plants denature proteins forming protein tannate which makes the intestinal mucosa more resistant and reduces secretion by virtue of which so many different plant species have been reported to possess antidiarrhoal potential\(^{489,490}\). Thus the tannins present in the extract SA\(_2\) may be responsible for the antidiarrhoeal activity.

5.2.6 Antimicrobial activity:

Tannins and related compounds have long been recognized to possess quite potent antibacterial activities, reflected by documented uses of traditional herbal medicines rich in polyphenols as effective antiseptic drugs\(^{491 - 493,297}\). In the present study, the antibacterial activity of the tannin rich fraction SA\(_2\) evaluated against eight microorganisms was found to possess only weak to moderate antibacterial activity evident from its high MIC values (Table 18) against the microorganisms tested compared to that of standard erythromycin and nystatin. A plausible explanation of moderate antimicrobial activity of tannin rich extract SA\(_2\) may be that tannins are present in plants and herbal preparations in large concentrations and the chemical heterogenicity of tannins could counterbalance their low antimicrobial activity.

In conclusion, the extracts of stem bark of *Soymida febrifuga* possess significant beneficial effects in gastric ulcers. The results of this study show that out of all the extracts tested, extract SA\(_3\) of stem bark of *Soymida febrifuga* is most effective
against various experimentally - induced gastric ulcers in rats. The mechanism of this can be attributed to decrease in gastric acid secretory activity along with strengthening of gastric mucosal defensive barrier. Extract SA₃ also possesses significant hepatoprotective and analgesic action. Former may be due to the ability of extract to prevent the changes mediated by chemically - induced hepatitis and later may be due to the presence of both peripheral and centrally active components in the extract. Extract SA₂ possesses significant but unspecific antidiarrhoeal action that may be attributed to its spasmolytic action and its likely interaction with calcium channel system. SA₂ also possesses moderate antibacterial action.

Our study offers evidence that *Soymida febrifuga* might play an important role in protection against gastric lesions and thereby offers insight into its mechanism of gastric ulcer inhibition. These results justify the use of the plant in gastrointestinal disorders including ulcers and diarrhoea. The study also confirms the bioapplications of polyphenols or condensed tannins as liver protective, antidiarrhoeal and antimicrobial agents. This study might prove important in the development of new and improved therapies for the treatment and prevention of peptic ulcers.