Chapter 4

ORGANOPROTECTIVE ACTIVITY

INTRODUCTION

Human beings are exposed to various environmental, occupational and xenobiotics challenges due to modern life style. Gastrointestinal tract, liver and kidney are the major target organs because they are involved in the digestion, metabolism and excretion of xenobiotics. In allopathic system of medicine, the drugs prescribed for treating various ailments may cause side effects and toxicities to various organs. Hence researchers start looking at various natural products as an alternative to allopathic system of treatment.

Toxicological studies are very essential to establish the safety and efficiency of a new drug. No drug substance is used clinically without its laboratory safety assessment at preclinical phase. Toxicological studies help to make a decision whether a new drug should be adopted for clinical use or not (Anisuzzaman et al., 2001 and Alam et al., 2006). Bioassays are used to measure the pharmacological activity of new or chemically undefined substances. Bioassay normally involves comparison of the unknown preparation with a standard. It is used to measure drug toxicity and unwanted side effects on the target organs.

GASTROPROTECTIVE ACTIVITY

Peptic ulcer disease is a chronic inflammatory disease characterized by ulceration in the lining of the stomach, duodenum, lower esophagus and Markel’s
diverticulum. It is a common gastrointestinal tract disorder affecting a large number of people worldwide, which causes abdominal discomfort, gnawing, burning or even blood in stools of patients. Ayurveda, the ancient Indian medical science describes it as ‘Amalpitha’ or ‘Parinamasula’. However, peptic ulcer was established in autopsy for the first time in the 16th century (Dabburu et al., 2012). Since then, there have been continuous efforts to unearth the pathophysiology of ulcer production and to discover newer remedies for ulcer treatment.

Even though the etiology of gastric ulcers is still debated, it is accepted that ulcers are caused due to net imbalances between mucosal offensive factors like acid, pepsin secretion, *Helicobacter pylori*, bile salts, ethanol, excessive ingestion of NSAIDs (like indomethacin, aspirin etc.,), lipid peroxidation, nitric oxide and defensive mucosal factors like prostaglandins, gastric mucus, cellular renovation, blood flow, mucosal cell shedding, glycoproteins, mucin secretion, proliferation and glutathione level (Venkateswararao and Venkataramana, 2013). Gastric mucosal layers acts like a barrier that limits an exposure of the gastric mucosal cells from these factors.

Ulcer therapy is now mainly focused on limiting the deleterious effects of gastric secretion to promote gastroproctection, block apoptosis and stimulate epithelial cell proliferation for effective healing. The modern antisecretary therapeutic agents such as proton pump inhibitors (omeprozole, lansoprazole etc.,), histamine H₂ receptor blocker (ranitidine, famotidine etc.) and antacids are extensively used to inhibit gastric acid secretion, boost mucosal defence mechanisms and acid related disorders caused by stress, smoking, alcohol, nutritional deficiencies, NSAIDs and *H. pylori* infection. However, post marketing surveillance
of these drugs has shown development of tolerance, relapses and side effects such as arrhythmias, impotence, gynaecomastia and haematopoietic changes (Ariyoshi et al., 1986). Considering these effects of modern medicines, indigenous drugs with fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer and many of them have been reported to strengthen mucosal defensive factors and reduces the offensive factors. Moreover pretreatment with different substances could effectively prevent gastric mucosa from the development of erosions and ulceration to account for gastroprotection by various irritants and ulcerogens.

HEPATOPROTECTIVE ACTIVITY

Liver is the vital organ regulating homeostasis in the body. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamins. Because of its important role in metabolism and relationship to gastro intestinal tract and being the detoxification centre liver is an important target for toxicity produced by the drugs, xenobiotics, environmental pollutants, chemotherapeutic agents, viral infection, chronic alcoholism and oxidative stress which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease (Sharma et al., 1991 and Subramonium and Pushpangadan, 1999). Liver damage is always associated with cellular necrosis, increase in tissue liquid peroxidation and elevation of serum biochemical markers like Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminse (SGPT), Serum Alkaline Phosphatase (ALP) and bilirubin (Mossa et al., 1991 and Mascola et al., 1998).
Liver diseases still remain as one of the worldwide serious health problem. About 20,000 deaths are found every year due to liver disorders. Drug induced liver injury is one of the most common causative factor that poses a major clinical and regulatory challenge (Russman et al., 2009). More than 900 drugs, toxins and herbs have been reported to cause liver injury. Paracetamol (Acetaminophen) is a well known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses (Boyd and Bereczky, 1966). Paracetamol (PCM) toxicity is due to the formation of toxic metabolites by cytochrome P-450 activity (Dahlin et al., 1984). Introduction of cytochrome P-450 or depletion of glutathione is a prerequisite for PCM induced hepatotoxicity (Moron et al., 1979 and Gupta et al., 2006).

Pharmaceutical market is growing rapidly and continuously. But still the demand for new drug discovery is encouraged. The phenomenal growths of modern allopathic medicines have little to offer for alleviation of hepatic diseases. It is chiefly the plant based preparations which are employed for the management of various liver disorders (Karan et al., 1999). Among this silymarin is safe and effective in protecting the liver from viral hepatitis, poisoning by ethanol, PCM and carbon tetra chloride. Many studies have demonstrated the beneficial hepatoprotective effects when treatment with silymarin (Fraschini et al., 2002). But there are meager marine based drugs available for the treatment of liver disorders. This may be due to lack of ethnomedical history and the difficulties involved in the collection of marine organisms (Jha and Xuzi-rang, 2004). Thus a concerted effort is needed to evaluate the scientific basis of the marine organisms and their product for therapeutic applications including hepatoprotective action.
NEPHROPROTECTIVE ACTIVITY

Kidney is an important excretory organ. The function of kidney is not only to excrete metabolic waste but also to maintain the acid base balance, regulate blood pressure and release erythropoietin (Jain and Agrawal, 2008). At present, renal failure is a common clinical syndrome and approximately 19 million adults have chronic kidney disease and it is estimated that 80,000 persons have diagnosed chronic kidney failure annually in India. Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin that causes damage to kidneys. It involves constriction of the kidney blood vessels, reduction of renal blood flow and glomerular filtration rate. It is the third most common problem of the renal system with an estimated lifetime risk of 2-5% in Asia, around 20% in the Middle East and 8-15% in Europe and America (Priyadarsini et al., 2012).

Our modern lifestyle is much more responsible for creating nephrotoxicity. Persons having high blood pressure, diabetes, having habits of eating junk food and taking excessive pain killer medicines, antibiotics, anticancer drugs and NSAIDs like PCM have high risk. It involves acute renal insufficiency, reduction of renal blood flow and glomerular filtration rate (Musu et al., 2011). Kidney is the second target organ of PCM toxicity. PCM overdose can leads to acute renal failure even in the absence of liver injury (Palani et al., 2010). PCM is metabolized primarily in the liver where 60-90% is converted to inactive compounds by conjugation with sulfate and glucuronide and then excreted by the kidney.

Humans have long been used the ocean as a source of food and minerals. The Indians, Chinese and Sumerians are just a few civilization that have provided
evidence suggesting the food can be effectively used as medicine to prevent and treat diseases. The Japanese believed that the regular consumption of sea urchin roe strengthens the immune system and could reduce heart diseases, leukemia oncological diseases and atherosclerosis. But still no scientific and methodological investigation has so far been reported in literature regarding its action on organ protection. Therefore, the present investigation has been designed to evaluate the possible organ protective role of *S. variolaris* ethanolic extracts against NSAIDs induced organ damage in wistar albino rats with reference to stomach, liver and kidney.
MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Healthy albino wistar rats of either sex, approximately of the same age (6 months) were used for all the experiments in this study. They were housed in mesh bottomed cages to minimize coprophagia and maintained under ambient laboratory conditions (Temperature 25 ± 1°C, relative humidity 60-70%, normal light dark cycle and good ventilation). All animals were allowed free access to water and fed with standard commercial pelleted rat chaw (Sai Durga Feeds and Foods, Bangalore). The experiments have been performed in the laboratory of K. M. College of Pharmacy, Madurai, approved by the Committee for the Purpose of Control and Supervision of Experimental Animals (Reg. No: 661/ 02/ C/ CPCSEA & 19/07/2002) with the permission of Institutional animal ethics committee (Reg. No: 4248/ MSU/ KMCP/ IAEC/ 71/2013). The animals were acclimatized for two weeks before the commencement of experiment.

Rats were fasted for 24 h prior to the experiment but were allowed for the free access to water except the last hour. All experiments were performed during the same time of the day to avoid diurnal variations. The rats were divided randomly into seven groups. Each rat was placed individually in separate cages. All the experimental procedures were performed in accordance with the care and use of experimental animals prescribed by the IAEC, constituted under the guidelines of the CPCSEA, India. Throughout the experiments, all animals received human care according to the criteria outlined in the “Guide for the care and use of laboratory
animals” prepared by the national Academy of Sciences and published by the National Institute of Health.

ACUTE TOXICITY STUDIES

The acute toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD- 423) received from CPCSEA. Three rats of the same age group and weight received a single dose of 2000 mg/Kg, b.w S. variolaris extracts orally. Doses were prepared by dissolving the extracts in normal saline (5ml/Kg) and the freshly prepared solutions were administered to fasted rats. Food was withheld for 3-4 hours after administration.

Animals were observed individually after dosing atleast once during the first 30 minutes, for the first 24 hours. Special attention was given for the first 4 hours and thereafter daily for a total of 14 days for any mortality and gross behavioral changes. It was observed that the ethanolic extract of S. variolaris gonad (SVGE) and exoskeleton (SVEE) induced no mortality. Hence, 1/10th (200 mg/Kg, b.w) and 1/5th (400 mg/Kg, b.w) of the dose were selected for further in vivo organoprotective studies.

EVALUATION OF GASTROPROTECTIVE ACTIVITY

1. EXPERIMENTAL PROCEDURE

Gastric ulcerations were induced experimentally in albino rats according to the modified method of Giri et al. (2009). The albino rats were divided into seven groups and each group contain 6 rats. Group I represented the normal control group, which received normal saline (5 ml/Kg, b.w) orally for 5 days. Group II rats were
similarly treated as group I. Ranitidine (50 mg/Kg, b.w in normal saline) were administered orally for group III as reference drug. Experimental groups IV and V were orally administered with 200 mg/Kg, b.w and 400 mg/Kg, b.w SVGE respectively. Group VI and VII received oral doses of 200 mg/Kg, b.w and 400 mg/Kg, b.w SVEE respectively for five days. On the 5th day, one hour after drug administration, each animal received orally 200 mg/Kg, b.w of aspirin except group I animals. All animals were sacrificed 4 hour later by using chloroform as anesthesia. The stomach was removed and various parameters of gastric juice (volume and pH) and ulcer index were determined.

2. ASSESSMENT OF GASTRIC MUCOSAL LESIONS

The stomach was excised by, cut along the greater curvature and gently rinsed with ice cold saline to remove gastric contents and blood clots. Gastric mucosal lesions were examined microscopically with the help of 10X hand magnifier lens. The gastric mucosal lesions were expressed in terms of Ulcer Index (U. I) and scoring was done as per reported methods of Ajaiyeoba et al. (2001).

\[0 \text{ - Normal stomach; } 0.5 \text{ - Pink or Red coloration of stomach;}
1.0 \text{ - Superficial ulcer; } 1.5 \text{ - Spot ulcer;}
2.0 \text{ - Hemorrhage spot; } 2.5 \text{ - Scattered hemorrhage streak;}
3.0 \text{ - Bleeding ulcer; } 4.0 \text{ - Perforated ulcer.}
\]

The sum of scores were divided by 10 (The magnification of the lens) to obtain the U. I for each rat (Main and Whittle, 1975).

Preventive Index (P. I %) was calculated according to the method of Hano et al. (1976) as follows:
P. I% = $\frac{U. I \text{ of toxin control group} - U. I \text{ of treated group}}{U. I \text{ of toxin control group}} \times 100$

3. MEASUREMENT OF GASTRIC JUICE VOLUME (Deshpande et al., 2003)

The gastric contents of the resected stomach were collected and centrifuged at 2000 rpm for 10 minutes. The supernatant fluid was measured for the volume of gastric juice and expressed as ml/4 hours.

4. DETERMINATION OF GASTRIC JUICE pH

An aliquot of 1 ml of gastric juice was diluted to 10 ml using distilled water. The pH of the solution was measured using digital pH meter.

EVALUATION OF HEPATOPROTECTIVE ACTIVITY

1. EXPERIMENTAL PROCEDURE (Thamizhselvam et al., 2010)

The rats were randomly assigned into seven groups with 3 animals in each group. Group I served as normal control and group II served as PCM control and both received normal saline (5 ml/Kg, b.w) daily for nine days. The IIIrd group consisted of reference control which received a single daily dose of silymarin (25 mg/Kg, b.w) throughout the experiment. Group IV and V received 200 mg/Kg, b.w and 400 mg/Kg, b.w SVGE respectively, once a day for 9 days. Group VI and group VII received a daily dose of SVEE for 9 days (200 mg/Kg, b.w and 400 mg/Kg, b.w respectively). On the 9th day, two hour after the respective treatments all the animals were administered with 750 mg/Kg, b.w PCM (0.5 % in normal saline) orally, except the rat in group I.
2. PREPARATION OF SERUM FROM BLOOD

Prior to termination, the rats were fasted overnight but water was made available *ad libitum*. The rats were sequentially anaesthetized with chloroform for about 35-45 seconds. Blood samples were obtained directly from the heart chamber of the anaesthetized rats with a 21 gauge needle mounted on 5 ml syringe and it was collected into a sample bottle. For separation of serum from coagulated blood, the sample was left at room temperature for 2 hours followed by 20 min centrifugation at 3000 rpm using the table top REMI centrifuge (Tarsons- Cen- Spinwin- MC- 02). The obtained clear sera were stored at -20° C for subsequent measurement of SGOT, SGPT, ALP, total bilirubin and total protein.

3. ASSAY OF SERUM TRANSAMINASES

Activities of SGOT and SGPT were estimated by the method of Reitman and Frankel (1957). 0.05 ml of substrate (0.2 M aspartate and 0.02 M α- ketoglutarate for SGOT; 0.2 M alanine and 0.002 M α- keto glutarate for SGPT in phosphate buffer pH 7.4) was incubated for one hour in case of SGOT and 30 min for SGPT in a water bath. 0.5 ml of Dinitrophenyl hydrazine (DNPH) solution was added to arrest the reaction and was kept for 20 min in room temperature. After incubation 5 ml of 0.4 N NaOH were added. 0.1 ml of 20mM pyruvate (220 mg of sodium pyruvate in 100 ml of phosphate buffer – standard), 0.1 ml of double distilled water (blank) and 0.5 ml DNPH (control) were similarly treated as above. The absorbance was read at 505 nm in UV-VIS spectrophotometer.
Calculations:

Concentration of SGPT (IU/L) = \( \frac{\text{OD of test} - \text{OD of control}}{\text{OD of standard} - \text{OD of blank}} \times 133 \text{ units} \)

Concentration of SGOT (IU/L) = \( \frac{\text{OD of test} - \text{OD of control}}{\text{OD of standard} - \text{OD of blank}} \times 67 \)

4. ASSAY OF SERUM ALKALINE PHOSPHATASE

Based on the method of King and Armstrong (1934) serum ALP activity was assayed using disodium phenyl phosphate as substrate. Buffered substrate (Disodium phenyl phosphate mixed in 0.1 M carbonate buffer, pH10) at the rate of 2 ml for the experimental sample, 1.1 ml for standard and 2.1 ml for blank was taken in three test tubes and incubated in a water bath at 37°C for 10 minutes. 1 ml phenol (100 mg in 100 ml distilled water- Standard) and 1 ml serum was added in test sample and again incubated at 37°C for 15 minutes. 0.8 ml of 0.5 N NaOH and 1.2 ml of 0.5 M sodium carbonate was added to all the test tubes and mixed well. Then 1 ml of 4- aminoantipyrine and potassium ferricyanide solution was added and mixed thoroughly. The absorbance was read at 520 nm.

Serum ALP activity (IU/L) = \( \frac{\text{OD of test} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times 10 \)

5. ESTIMATION OF TOTAL BILIRUBIN IN SERUM

Serum total bilirubin level was estimated based on the method of Lott et al. (1987). To 0.2 ml of serum, 1.8 ml of bilirubin (10 mg bilirubin in 100 ml chloroform) and 0.5 ml diazo reagent was added. 0.2 ml bilirubin (standard) was simultaneously treated as above. An aliquot of 0.5 ml diazo (serum control) was mixed with 1.8 ml distilled water and 0.2 ml serum. Similarly 0.5 ml diazo (blank)
was diluted with 2 ml distilled water. The contents were mixed well and 2.5 ml methanol was added to each tube and kept in dark for 30 minutes and the absorbance was read at 540 nm. Total bilirubin content was calculated using the following equation

$$\text{Total bilirubin} = \frac{\text{OD of test} - \text{OD of control}}{\text{OD of standard} - \text{OD of blank}} \times 10 \text{ (mg/ml)}$$

6. ESTIMATION OF TOTAL PROTEIN

The total protein was determined using the method of Lowry et al. (1951), as described earlier in chapter I.

7. MEASUREMENT OF PHYSICAL PARAMETERS

The weight of the animals was noted on the first and last day of treatment and the mean change in body weight was calculated. Animals were thoracotomized and then the liver was dissected out, rinsed in normal saline, blotted dried on a filter paper and weighed to measure absolute organ weight. The relative organ weight of each animal was then calculated using the following equation.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on the day of sacrifice (g)}} \times 100$$

8. HISTOPATHOLOGICAL EXAMINATION

Small pieces of liver tissues of each group of animals were fixed in Bovin’s solution (mixture of 75 ml saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 12 hour dehydrated in graded alcohol cleaned in xylene, infiltered with melting wax and then embedded in paraffin wax. 5µm thick
sections were cut using microtome and stained with haematoxylin-eosin dye. Stained sections were finally mounted in di-phenyl xylene (Galigher and Kozloff, 1971). Then the sections were observed under microscope for histopathological changes in liver architecture and their photo micrographs were taken.

EVALUATION OF NEPHROPROTECTIVE ACTIVITY

1. EXPERIMENTAL PROCEDURE (Hamid et al., 2012)

The albino rats were randomly divided into seven groups and each group contains 3 rats. Animals in group I served as untreated control and fed orally with normal saline 5 ml/Kg, b.w daily for seven days. Group II rats were similarly treated as group I, while animals in group III received the reference drug cystone at the dose of 5 ml/Kg, b.w. Experimental groups IV and V were treated with 200 and 400 mg/Kg, b.w of SVGE respectively. Experimental groups VI and VII were orally administered with 200 and 400 mg/Kg, b.w SVEE respectively for seven days. On the 6th and 7th day, one hour after sample administration PCM suspension (0.5% in normal saline) was given by oral route at the dose of 750 mg/Kg, b.w to all rats except for group I.

On the 8th day of the experiment rats were anaesthetized by chloroform and the blood was withdrawn through intra cardiac puncture. The animals were fasted 24 hour before the collection of blood. After blood collection, the rats were sacrificed by cardiac puncture.
2. BIOCHEMICAL STUDIES

Serum was separated by centrifugation at 3000 rpm for 20 minutes and the supernatant was utilized for the estimation of various biochemical parameters including serum urea, serum creatinine and serum uric acid.

2.1. ESTIMATION OF SERUM UREA (Wybenga et al., 1971)

To 0.2 ml of serum 6.8 ml of double distilled water was added and mixed thoroughly. 3 ml of 10% TCA was added and mixed again and allowed to stand for 10 minutes and filtered through Whatman No. 1 filter paper into a dry test tube. The protein free filtrate was used for the determination of urea in the given blood serum. To 1 ml of protein free filtrate 1 ml diacetymonoxime reagent was added, followed by 1 ml thio- semicarbazide and 3 ml of mixed acid reagent (Ferric chloride phosphoric acid reagent and 20% sulphuric acid in the ratio 1: 1000) and mixed thoroughly. 1 ml of urea (standard) and 1 ml of double distilled water (blank) were simultaneously treated as above. Top of each test tube was covered with aluminium foil and kept in a boiling water bath for exactly 15 minutes. Test tubes were cooled immediately and the absorbance was read at 540 nm.

Concentration of urea in serum (mg/100ml) = \[
\frac{\text{OD of test} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times 50
\]

2.2. ESTIMATION OF SERUM URIC ACID (Buchanan et al., 1965)

1.5 ml of serum, 1.5 ml of distilled water and 1.5 ml of 20% TCA solution were added into a centrifuge tube one by one. The contents were mixed well and allowed to stand for 5 minutes. The mixture was centrifuged at 3000 rpm for 10 minutes. 3 ml of supernatant was thoroughly mixed with 1.5 ml or saturated
solution of sodium carbonate and 1 ml diluted folin’s reagent. To 1 ml uric acid (2 mg/100 ml- Standard), 1 ml of 20% TCA, 1 ml distilled water, 1.5 ml of saturated solution of sodium carbonate and 1 ml diluted folin’s reagent was added. 2 ml distilled water (blank) was taken and treated like the same way as mentioned earlier. All the contents were mixed thoroughly and all the tubes were allowed to stand for 10 minutes and the absorbance was read at 540 nm.

\[
\text{Serum uric acid concentration (mg/100ml)} = \frac{\text{OD of test} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times 2
\]

2.3. ESTIMATION OF SERUM CREATININE (Varley et al., 1991)

1 ml serum was mixed well with 1 ml distilled water. To this 1 ml of 5% sodium tungstate and 1 ml of 2/3 N sulfuric acid was added. The contents were mixed well and centrifuged at 3000 rpm for 5 to 10 minutes. 2 ml creatinine (20 mg/Kg) and 2 ml distilled water were similarly treated as above. An aliquot of 2 ml supernatant was mixed with 0.5 ml picric acid solution and 0.5 ml of 0.75 N sodium hydroxide solutions. This was allowed stand at room temperature for 20 minutes and the absorbance was read at 520 nm.

\[
\text{Concentrations of creatinine in serum (mg/ml)} = \frac{\text{OD of test} - \text{OD of blank}}{\text{OD of standard} - \text{OD of blank}} \times 4
\]

3. MEASUREMENT OF PHYSICAL PARAMETERS

The body weight of rats in each group was measured before starting and at the end of the experiment. Kidneys were carefully dissected out and weighed in grams.
4. HISTOPATHOLOGICAL STUDIES

A portion of the kidney tissue from each group was fixed immediately in 10% neutral formalin for a period of at least 24 h to fix the tissue. The tissue was then dehydrated in graded (50-100%) alcohol and was embedded in paraffin wax. It was then processed by microtome into 4-5μm thick sections, stained with hematoxylin-eosin and observed under a computerized light microscope to evaluate the details of renal architecture.

STATISTICAL ANALYSIS

The values were expressed as mean ± standard deviation
RESULTS

ACUTE TOXICITY STUDIES

Acute toxicity test was carried out for all the extracts using OECD guidelines number 423. Animals were observed for 14 days with special attention for first 4 hours after administration. All treated animals were slightly sedated within first hour of administration and returns to normal and active life within two hours of post treatment. All animals treated with ethanolic extract of *S. variolaris* gonad and exoskeleton were survived beyond 14 days of post treatment upto the dose of 2000 mg/Kg, b.w without any signs of toxicity. So the dose selected for the evaluation of organoprotective activity was 200 mg/Kg, b.w and 400 mg/Kg, b.w (1/10\(^{th}\) and 1/5\(^{th}\) of 2000 mg/Kg, b.w) of ethanolic extract of *S. variolaris* gonad and exoskeleton.

GASTROPROTECTIVE ACTIVITY

Table 4. 1 and figure 4. 1 showed the status of gastric juice volume, pH, ulcer index and preventive index of the wistar albino rats, an aspirin induced ulcer model, treated with ranitidine and different concentrations of *S. variolaris* gonad and exoskeleton extract. When compared to normal control group (2.96 ± 0.21 ml) and ranitidine group (1.42 ± 0.33 ml) gastric juice volume was increased in aspirin treated group (4.91 ± 0.07 ml). Whereas *S. variolaris* gonad extract treated rats showed gastric juice volume of 3.73 ± 0.22 ml and 2.66 ± 0.08 ml at 200 mg/Kg, b.w and 400 mg/Kg, b.w respectively. SVEE showed gastric juice volume of 4.22 ± 0.26 ml at 200 mg/Kg, b.w and 4.25 ± 0.13 ml at 400 mg/Kg, b.w respectively.
In the present investigation, the aspirin control group (1.87 ± 0.10) exhibited a low level of pH compared with normal control (3.59 ± 0.37). The data depicted that with standard drug ranitidine pH of the gastric juice increased to 4.78 ± 0.17 and the SVGE extracts showed gastric juice pH of 3.18 ± 0.24 and 3.83 ± 0.16 at 200 mg/Kg, b.w and 400 mg/Kg, b.w respectively. While SVEE treated groups showed very little change in the pH level (2.31 ± 0.15 with 200 mg/Kg, b.w and 2.73 ± 0.26 with 400 mg/Kg, b.w concentration) when compared with that of aspirin control.

Aspirin administration resulted in remarkably high ulcer index (61.66 ± 3.25) when compared to normal control group (9.83 ± 1.52). Pretreatment with ranitidine produced higher gastroprotective effect as compared to group II and the ulcer index reduced to 11.33 ± 1.25 providing 81.62% prevention against gastric mucosal injury. Similarly SVGE at the concentration of 200 mg/Kg, b.w reduced the ulcer index to 21.16 ± 1.52 showing 65.68% prevention, whereas SVGE 400 mg/Kg reduced ulcer index to 16.66 ± 1.75 showing 72.98% prevention. SVEE 200 mg/Kg, b.w showed the ulcer index of 45.66 ± 1.25 and preventive index of 25.94% compared with the aspirin control, similarly SVEE 400 mg/Kg, b.w concentration revealed the ulcer index of 46.16 ± 1.52 with a preventive index of 25.94%.

HEPATOPROTECTIVE ACTIVITY

The effects of S. variolaris extract on serum transaminases, ALP, total bilrubin and total protein levels in rats with PCM induced liver damage rats were summarized in table 4. 2 and figure 4. 2. When the rats are administered with paracetamol at the rate of 750 mg/Kg, b.w, a significant increase in the serum SGOT
(102.38 ± 1.02 IU/L), SGPT (72.86 ± 1.02 IU/L), ALP (174.01 ± 0.28 IU/L) and total bilirubin (3.86 ± 0.11 mg/dl) levels were noticed when compared with the normal control (SGOT = 60.76 ± 0.36 IU/L; SGPT = 57.43 ± 0.40 IU/L; ALP = 119.89 ± 0.94 IU/L and total bilirubin = 2.65 ± 0.11 mg/dl). Administration of hepatoprotective drug silymarin resulted in the reduction of SGOT (44.01 ± 0.26 IU/L), SGPT (36.78 ± 0.55 IU/L), ALP (103.13 ± 1.68 IU/L) and total bilirubin (1.48 ± 0.46 mg/dl) in the serum when compared with the PCM intoxicated and normal control rats.

The *S. variolaris* gonad extract at 200 mg/Kg, b.w and 400 mg/Kg, b.w concentration significantly reversed the levels of SGOT (55.06 ± 0.74 IU/L; 50.54 ± 1.39 IU/L), SGPT (41.88 ± 0.72 IU/L; 39.65 ± 1.25 IU/L), ALP (111.20 ± 0.82 IU/L; 108.44 ± 1.15 IU/L) and total bilirubin (2.7 ± 0.37 mg/dl; 2.21 ± 0.21 mg/dl) respectively, when compared to PCM alone treated rats. The activity of SGOT, SGPT, ALP and total bilirubin in the serum of rat treated with *S. variolaris* exoskeleton at the concentration of 200 mg/Kg, b.w groups was 68.45 ± 1.03 IU/L, 62.99 ± 0.29 IU/L, 131.83 ± 0.89 IU/L and 3.22 ± 0.15 mg/dl and the values of 65.13 ± 0.31 IU/L, 60.67 ± 0.84 IU/L, 127.93 ± 0.45 IU/L and 2.93 ± 0.11 mg/dl were noticed with exoskeleton extract at 400 mg/Kg, b.w concentration.

PCM treated animals showed decreased level of total protein (3.46 ± 0.32 mg/dl) compared with normal control (4.87 ± 0.23 mg/dl) and silymarin treated rats (7.44 ± 0.21 mg/dl). Whereas a total protein value of 6.46 ± 0.43 mg/dl and 5.69 ± 0.33 mg/dl were noticed with *S. variolaris* gonadal extract at a concentration of 200 and 400 mg/Kg, b.w and a value of 4.05 ± 0.03 mg/dl and 4.09 ± 0.01 mg/dl were noticed in rats treated with exoskeleton extract at the same concentration.
The results of physical parameters of *S. variolaris* extracts on PCM treated rats were shown in table 4.3 and figure 4.3. The animals treated with toxic doses of PCM showed reduced level of body weight (-9.09 ± 3.08 g) and elevated liver weight (7.509 ± 0.418 g) as compared to normal rats of group I (bodyweight = 14.072 ± 1.128 g; liver weight = 4.951 ± 0.099 g). The rats treated with the reference drug silymarin indicated increased body weight (10.09 ± 0.270 g) and decreased liver weight (5.441 ± 0.5008 g) when compared to the toxin control. The SVGE extract at one dose of 200 mg/Kg, b.w and 400 mg/Kg, b.w showed increased body weight (6.916 ± 0.478 g and 7.285 ± 0.704 g respectively) and decreased liver weight (6.557 ± 0.339 g and 6.102 ± 0.123 g respectively) when compared with toxin control. Rats treated with 200 mg/Kg, b.w and 400 mg/Kg, b.w SVEE extracts showed a significant decrease in body weight (-2.451 ± 1.295 g and -0.446 ± 1.388 g) and increase in liver weight (6.77 ± 0.467 g and 6.83 ± 0.233 g) as compared to control group, but these values are reversed when compared with PCM intoxicated group.

In the present investigation, when compared with toxic control group (3.684 ± 0.315 g), decreased level of relative organ weight was observed in the groups administered with normal saline (2.91 ± 0.047 g), silymarin (2.654 ± 0.276 g), SVGE 200 mg/Kg, b.w (3.15 ± 0.214 g), SVGE 400 mg/Kg, b.w (3.14 ± 0.056 g), SVEE 200 mg/Kg, b.w (3.53 ± 0.179 g) and SVEE 400 mg/Kg, b.w (1.296 ± 0.168 g).

Haemotoxylin and eosin stained section of the control rat liver showed normal liver architecture with central vein and hepatic sinusoids. Liver parenchyma showed the presence of hepatocytes with normal nucleus (Plate 4.1 and 4.2).
Whereas the liver of paracetamol treated rat revealed degenerative changes in the hepatocytes with cytoplasmic vacuolation and the presence of pyknotic nuclei. Blood filled necrosed areas are also prominent (Plate 4.3, 4.4 and 4.5). When the rats are treated with paracetamol and silymarin, liver showed hepatocyte regeneration and enlarged sinusoidal space. Hepatocytes also showed mild cytoplasmic vacuolation and few pyknotic nuclei (Plate 4.6 and 4.7).

Administration of rats with both the concentration (200 mg/Kg, b.w and 400 mg/Kg, b.w) of *S. variolaris* gonadal extract revealed normal hepatocytes and central vein. Mild cytoplasmic vacuolations are also noticed in this treatment (Plate 4.8 to 4.11).

Rats treated with paracetamol and *S. variolaris* exoskeleton extract, showed normal liver architecture with central vein and normal nucleated hepatocytes (Plate 4.12 to 4.15) with both the concentration of exoskeleton extract.

**NEPHROPROTECTIVE ACTIVITY**

Table 4.4 and figure 4.4 displayed the effect of *S. variolaris* gonad and exoskeleton extracts on serum urea, serum creatinine and serum uric acid levels in PCM induced nephrotoxic rats. Serum urea (52.54 ± 0.89 mg/dl), serum creatinine (3.93 ± 0.05 mg/dl) were found to be increased in the rats treated with PCM as compared to normal control group (serum urea = 19.04 ± 0.90 mg/dl; serum creatinine = 1.68 ± 0.16 mg/dl). Serum uric acid level decreased in PCM treated group (4.23 ± 0.07 mg/dl) when compared with normal control (8.87 ± 0.08 mg/dl). There was a decrease in serum urea (21.02 ± 1.39 mg/dl) and serum creatinine levels (1.11 ± 0.18 mg/dl) in cystone treated reference group when compared with paracetamol treated rats. Serum uric acid levels in cystone treated rats were
increased (9.44 ± 0.18 mg/dl) when compared with normal control and paracetamol treated rats.

When the rats are treated with *S. variolaris* gonad extract at 200 mg/Kg, b.w concentration there is reduction in serum urea (25.95 ± 0.83 mg/dl) and creatinine (2.51 ± 0.13 mg/dl) level. Similar trend was noticed in rats treated with 400 mg/Kg, b.w concentration (serum urea = 24.30 ± 0.86 mg/dl; serum creatinine = 1.88 ± 0.002 mg/dl). Moreover, both the concentration of *S. variolaris* gonadal extracts showed a significant nephroprotective effect as evidenced by an increase in the uric acid level (6.47 ± 0.12 mg/dl and 7.39 ± 0.36 mg/dl) when compared with PCM treated group. Serum urea level decreased in rats treated with *S. variolaris* exoskeleton at 200 mg/Kg, b.w concentration (37.71 ± 0.57 mg/dl) and 400 mg/Kg, b.w concentration (34.71 ± 0.64 mg/dl) when compared with PCM induced rats. However SVEE decreased creatinine levels in the serum samples both at 200 mg/Kg, b.w (3.62 ± 0.07 mg/dl) and 400 mg/Kg, b.w (3.13 ± 0.10 mg/dl) and the serum uric acid level increased in both 200 mg/Kg, b.w (5.07 ± 0.05 mg/dl) and 400 mg/Kg, b.w concentration (5.97 ± 0.016 mg/dl).

Effect of the *S. variolaris* ethanolic extracts on change in body weight and kidney weight in PCM induced nephrotoxic rats were summarized in table 4. 5 and figure 4. 5. The body weight of the rats treated with PCM were found to be reduced (-11.01 ± 0.76 g) as compared to normal control group (16.62 ± 1.38 g). Rats treated with PCM showed an increase (1.062 ± 0.054 g) in kidney weight compared to normal control group (0.889 ± 0.079 g). Increased body weight (15.04 ± 1.24 g) and decreased kidney weight (0.881 ± 0.071 g) was noticed in cystone treated rats.
Like cystone administration, the SVGE extracts at all the doses showed significant increase in rat body weight (14.18 ± 0.75g at 200 mg/Kg, b.w and 14.43 ± 0.90 g at 400 mg/Kg, b.w). There was dose dependent decrease of kidney weight in animals treated with SVGE 200 mg/Kg, b.w (1.024 ± 0.10 g) and 400 mg/Kg, b.w (0.915 ± 0.085 g). There was a slight dose dependent increase of body weight in animals treated with SVEE at 400 mg/Kg, b.w concentration (0.35 ± 3.34 g) but with 200 mg/Kg, b.w concentration there was reduction in body weight (-2.37 ± 1.16 g). Kidney weight also slightly increased with both the concentration of *S. variolaris* exoskeleton extract (1.008± 0.019 g at 200 mg/Kg, b/w and 1.070 ± 0.027 g at 400 mg/Kg, b.w).

The results also indicated that the relative organ weight of normal control (0.439 ± 0.042 g), reference drug cystone (0.422 ± 0.033 g), SVGE 200 mg/Kg, b.w (0.463 ± 0.057 g), SVGE 400 mg/Kg, b.w (0.439 ± 0.049 g), SVEE 200 mg/Kg (0.519 ± 0.012 g), and SVEE 400 mg/Kg (0.493 ± 0.017 g) were low when compared with PCM treated group (0.520 ± 0.025 g).

Histopathological examination of rat kidney sections was depicted in plate 4. 16 to 4. 33. Normal control group showed intact architecture of renal parenchyma, normal renal tubules, intact glomeruli and Bowman’s capsule (Plate 4. 16 and 4. 17). Whereas PCM control group showed glomerulus with loss of surrounding Bowman’s capsule, severe necrosis, haemorrhages and degeneration of renal tissue. Inflammatory cells were also seen in kidney section from the PCM treated groups (Plate 4. 18 to 4. 22). Groups treated with cystone revealed regenerative changes in kidney histology induced by PCM and showed normal renal tubules, normal glomeruli with encapsulated Bowman’s capsule (Plate 4. 23 to
4. 25). Moderate necrosis, tubular degeneration and cellular infiltration were observed in groups treated with SVGE 200 mg/Kg, b.w (Plate 4. 26 and 4. 27). The SVGE 400 mg/Kg, b.w group showed almost normal glomerular and tubular arrangements with only few inflammatory cells (Plate 4. 28 and 4. 29). The rats treated with SVEE 200 mg/Kg, b.w showed normal tubular pattern with a mild degree of swelling, necrosis and inflammation (Plate 4. 30 and 4. 31). Pretreatment with the SVEE 400 mg/Kg, b.w extract minimized the toxic manifestations in the kidney, although few minor changes still persisted (Plate 4. 32 and 4. 33).
### TABLE 4.1

**Effects of *S.variolaris* ethanolic extracts (gonad and exoskeleton) on gastric content changes in rats with aspirin induced gastric mucosal damage**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Gastric juice volume (ml)</th>
<th>pH</th>
<th>U. I</th>
<th>P. I (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline (5 ml/Kg, b.w)</td>
<td>2.96 ± 0.21</td>
<td>3.59 ± 0.37</td>
<td>9.83 ± 1.52</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Normal Saline (5ml/Kg, bw) + Aspirin (200 mg/Kg)</td>
<td>4.91 ± 0.07</td>
<td>1.87 ± 0.10</td>
<td>61.66 ± 3.25</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>Ranitidine (50 mg/Kg)</td>
<td>1.42 ± 0.33</td>
<td>4.78 ± 0.17</td>
<td>11.33 ± 1.25</td>
<td>81.62</td>
</tr>
<tr>
<td>IV</td>
<td>SVGE (200 mg/Kg, b.w) + Aspirin (200 mg/Kg)</td>
<td>3.73 ± 0.22</td>
<td>3.18 ± 0.24</td>
<td>21.16 ± 1.52</td>
<td>65.68</td>
</tr>
<tr>
<td>V</td>
<td>SVGE (400 mg/Kg, b.w) + Aspirin (200 mg/Kg)</td>
<td>2.66 ± 0.08</td>
<td>3.83 ± 0.16</td>
<td>16.66 ± 1.75</td>
<td>72.98</td>
</tr>
<tr>
<td>VI</td>
<td>SVEE (200 mg/Kg, b.w) + Aspirin (200 mg/Kg)</td>
<td>4.22 ± 0.26</td>
<td>2.31 ± 0.15</td>
<td>45.66 ± 1.25</td>
<td>25.94</td>
</tr>
<tr>
<td>VII</td>
<td>SVEE (400 mg/Kg, b.w) + Aspirin (200 mg/Kg)</td>
<td>4.25 ± 0.13</td>
<td>2.73 ± 0.26</td>
<td>46.16 ± 1.52</td>
<td>25.94</td>
</tr>
</tbody>
</table>
## TABLE 4.2

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on biochemical parameters in rats with PCM induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (mg/dl)</th>
<th>Total protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline (5 ml/Kg, b.w)</td>
<td>60.76 ± 0.36</td>
<td>57.43 ± 0.40</td>
<td>119.89 ± 0.94</td>
<td>2.65 ± 0.11</td>
<td>4.87 ± 0.23</td>
</tr>
<tr>
<td>II</td>
<td>Normal Saline (5ml/Kg, bw) + PCM (750 mg/Kg)</td>
<td>102.38 ± 1.02</td>
<td>72.86 ± 1.02</td>
<td>174.01 ± 0.28</td>
<td>3.86 ± 0.11</td>
<td>3.46 ± 0.32</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (25 mg/Kg) + PCM (750 mg/Kg)</td>
<td>44.01 ± 0.26</td>
<td>36.78 ± 0.55</td>
<td>103.13 ± 1.68</td>
<td>1.48 ± 0.46</td>
<td>7.44 ± 0.21</td>
</tr>
<tr>
<td>IV</td>
<td>SVGE (200 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>55.06 ± 0.74</td>
<td>41.88 ± 0.72</td>
<td>111.20 ± 0.82</td>
<td>2.7 ± 0.37</td>
<td>6.46 ± 0.43</td>
</tr>
<tr>
<td>V</td>
<td>SVGE (400 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>50.54 ± 1.39</td>
<td>39.65 ± 1.25</td>
<td>108.44 ± 1.15</td>
<td>2.21 ± 0.21</td>
<td>5.69 ± 0.33</td>
</tr>
<tr>
<td>VI</td>
<td>SVEE (200 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>68.45 ± 1.03</td>
<td>62.99 ± 0.29</td>
<td>131.83 ± 0.89</td>
<td>3.22 ± 0.15</td>
<td>4.05 ± 0.03</td>
</tr>
<tr>
<td>VII</td>
<td>SVEE (400 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>65.13 ± 0.31</td>
<td>60.67 ± 0.84</td>
<td>127.93 ± 0.45</td>
<td>2.93 ± 0.11</td>
<td>4.09 ± 0.01</td>
</tr>
</tbody>
</table>
# TABLE 4.3

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on physical parameters in rats with PCM induced hepatotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Change in body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative organ weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline (5 ml/Kg, b.w)</td>
<td>211.87 ± 4.341</td>
<td>225.948 ± 3.526</td>
<td>14.072 ± 1.128</td>
<td>4.951 ± 0.099</td>
<td>2.91 ± 0.047</td>
</tr>
<tr>
<td>II</td>
<td>Normal Saline (5ml/Kg, bw) + PCM (750 mg/Kg)</td>
<td>213.23 ± 3.226</td>
<td>204.14 ± 6.286</td>
<td>-9.09 ± 3.08</td>
<td>7.509 ± 0.418</td>
<td>3.684 ± 0.315</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin (25 mg/Kg) + PCM (750 mg/Kg)</td>
<td>195.086 ± 5.91</td>
<td>205.183 ± 5.765</td>
<td>10.09 ± 0.270</td>
<td>5.441 ± 0.0508</td>
<td>2.654 ± 0.276</td>
</tr>
<tr>
<td>IV</td>
<td>SVGE (200 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>201.456 ± 5.576</td>
<td>208.373 ± 5.612</td>
<td>6.916 ± 0.478</td>
<td>6.557 ± 0.339</td>
<td>3.15 ± 0.214</td>
</tr>
<tr>
<td>V</td>
<td>SVGE (400 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>186.823 ± 6.726</td>
<td>194.108 ± 7.292</td>
<td>7.285 ± 0.704</td>
<td>6.102 ± 0.123</td>
<td>3.14 ± 0.056</td>
</tr>
<tr>
<td>VI</td>
<td>SVEE (200 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>193.933 ± 4.985</td>
<td>191.482 ± 3.785</td>
<td>-2.451 ± 1.295</td>
<td>6.77 ± 0.467</td>
<td>3.53 ± 0.179</td>
</tr>
<tr>
<td>VII</td>
<td>SVEE (400 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>207.743 ± 5.172</td>
<td>207.296 ± 3.832</td>
<td>-0.446 ± 1.388</td>
<td>6.83 ± 0.233</td>
<td>1.296 ± 0.168</td>
</tr>
</tbody>
</table>
### TABLE 4.4

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on renal function parameters in rats with PCM induced nephrotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Serum urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline (5 ml/Kg, b.w)</td>
<td>19.04 ± 0.90</td>
<td>1.68 ± 0.16</td>
<td>8.87 ± 0.08</td>
</tr>
<tr>
<td>II</td>
<td>Normal Saline (5ml/Kg, bw) + PCM (750 mg/Kg)</td>
<td>52.54 ± 0.89</td>
<td>3.93 ± 0.05</td>
<td>4.23 ± 0.07</td>
</tr>
<tr>
<td>III</td>
<td>Cystone (5 ml/Kg) + PCM (750 mg/Kg)</td>
<td>21.02 ± 1.39</td>
<td>1.11 ± 0.18</td>
<td>9.44 ± 0.18</td>
</tr>
<tr>
<td>IV</td>
<td>SVGE (200 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>25.95 ± 0.83</td>
<td>2.51 ± 0.13</td>
<td>6.47 ± 0.12</td>
</tr>
<tr>
<td>V</td>
<td>SVGE (400 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>24.30 ± 0.86</td>
<td>1.88 ± 0.002</td>
<td>7.39 ± 0.36</td>
</tr>
<tr>
<td>VI</td>
<td>SVEE (200 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>37.71 ± 0.57</td>
<td>3.62 ± 0.07</td>
<td>5.07 ± 0.05</td>
</tr>
<tr>
<td>VII</td>
<td>SVEE (400 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>34.71 ± 0.64</td>
<td>3.13 ± 0.10</td>
<td>5.97 ± 0.016</td>
</tr>
</tbody>
</table>
### TABLE 4.5

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on physical parameters in rats with PCM induced nephrotoxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Change in body weight (g)</th>
<th>Kidney weight (g)</th>
<th>Relative organ weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline (5 ml/Kg, b.w)</td>
<td>186.03 ± 4.587</td>
<td>202.65 ± 3.20</td>
<td>16.62 ± 1.38</td>
<td>0.889 ± 0.079</td>
<td>0.439 ± 0.042</td>
</tr>
<tr>
<td>II</td>
<td>Normal Saline (5ml/Kg, bw) + PCM (750 mg/Kg)</td>
<td>215.03 ± 5.352</td>
<td>204.05 ± 4.876</td>
<td>-11.01 ± 0.76</td>
<td>1.062 ± 0.054</td>
<td>0.520 ± 0.025</td>
</tr>
<tr>
<td>III</td>
<td>Cystone (5 ml/Kg) + PCM (750 mg/Kg)</td>
<td>193.98 ± 5.07</td>
<td>208.47 ± 5.06</td>
<td>15.04 ± 1.24</td>
<td>0.881 ± 0.071</td>
<td>0.422 ± 0.033</td>
</tr>
<tr>
<td>IV</td>
<td>SVGE (200 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>207.43 ± 5.604</td>
<td>221.62 ± 6.351</td>
<td>14.18 ± 0.75</td>
<td>1.024 ± 0.10</td>
<td>0.463 ± 0.057</td>
</tr>
<tr>
<td>V</td>
<td>SVGE (400 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>193.98 ± 5.07</td>
<td>208.80 ± 6.53</td>
<td>14.43 ± 0.90</td>
<td>0.915 ± 0.085</td>
<td>0.439 ± 0.049</td>
</tr>
<tr>
<td>VI</td>
<td>SVEE (200 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>196.43 ± 5.881</td>
<td>194.053 ± 7.039</td>
<td>-2.37 ± 1.16</td>
<td>1.008 ± 0.019</td>
<td>0.519 ± 0.012</td>
</tr>
<tr>
<td>VII</td>
<td>SVEE (400 mg/Kg, b.w) + PCM (750 mg/Kg)</td>
<td>214 ± 5.24</td>
<td>216.79 ± 5.819</td>
<td>0.35 ± 3.34</td>
<td>1.070 ± 0.027</td>
<td>0.493 ± 0.017</td>
</tr>
</tbody>
</table>
FIGURE 4.1

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on gastric content changes in rats with aspirin induced gastric mucosal damage.

![Graph showing effects of *S. variolaris* extracts on gastric content changes](image-url)
FIGURE 4.2

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on biochemical parameters in rats with PCM induced hepatotoxicity.
FIGURE 4.3

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on physical parameters in rats with PCM induced hepatotoxicity.
FIGURE 4.4

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on renal function parameters in rats with PCM induced nephrotoxicity.

![Graph showing effects of S. variolaris ethanolic extracts on renal function parameters in rats with PCM induced nephrotoxicity. The x-axis represents experimental groups (I to VII), and the y-axis represents renal function parameters (serum urea, serum creatinine, and serum uric acid). The graph compares Group I (control), Group II (PCM group), and Groups III to VII (treated groups with varying levels of S. variolaris extract).](image-url)
FIGURE 4.5

Effects of *S. variolaris* ethanolic extracts (gonad and exoskeleton) on physical parameters in rats with PCM induced nephrotoxicity

![Graph showing the effects of *S. variolaris* extracts on physical parameters in rats.](image)
HISTOPATHOLOGICAL EVALUATION OF LIVER

Plate 4. 1: Photomicrograph of liver tissue of control rats showing normal hepatocytes with central vein and sinusoidal space (H & E, 100 X)

Plate 4. 2: A representative section of control liver showing normal hepatocyte architecture with nucleus (H & E, 400 X)
Plate 4.3: Photomicrograph of liver tissue of rats treated with paracetamol showing degenerative changes in the hepatocytes with necrosis (H & E, 100 X)

Plate 4.4: A sectional representation of liver tissue of rats treated with paracetamol showing total loss of hepatocyte architecture, nuclear disintegration and accumulation of blood in the sinusoidal space and other areas (H & E, 400 X)

Plate 4.5: A representative section of rat tissue treated with paracetamol showing nuclear pyknosis, hepatocyte degeneration and cytoplasmic vaculation and collection of blood (H & E, 400 X)
Plate 4.6: Photomicrograph of liver tissue treated with silymarin showing hepatocyte regeneration with dilated central vein and enlarged sinusoidal space (H & E, 100 X)

Plate 4.7: A sectional representation of rat liver tissue treated with silymarin showing mild degenerative changes in the hepatocytes with few pyknotic nuclei and mild inflammatory areas (H & E, 400 X)
**Plate 4. 8:** Photomicrograph of liver tissue of rats treated with SVGE (200 mg/ Kg, b.w) showing mild degeneration and mild chronic inflammation (H & E, 100 X)

![Plate 4. 8: Photomicrograph of liver tissue of rats treated with SVGE (200 mg/ Kg, b.w) showing mild degeneration and mild chronic inflammation (H & E, 100 X)](image)

**Plate 4. 9:** A representative section of liver tissue of rats treated with SVGE (200 mg/ Kg, b.w) showing variable size of hepatocytes with mild increase in fibrous connective tissue (H & E, 400 X)

![Plate 4. 9: A representative section of liver tissue of rats treated with SVGE (200 mg/ Kg, b.w) showing variable size of hepatocytes with mild increase in fibrous connective tissue (H & E, 400 X)](image)
Plate 4.10: Photomicrograph of liver tissue of rats treated with SVGE (400 mg/Kg, b.w) showing normal hepatocyte architecture with central vein and normal hepatic sinusoids without inflammatory changes (H & E, 100 X)

Plate 4.11: A sectional representation of liver tissue of rats treated with SVGE (400 mg/Kg, b.w) showing normal liver hepatocytes and liver tissue with typical lobular arrangement (H & E, 400 X)
Plate 4.12: Photomicrograph of rat liver tissue treated with SVEE (200 mg/Kg, b.w) showing mild degree of necrosis with mild inflammation (H & E, 100 X)

Plate 4.13: A representative section of rat liver tissue treated with SVEE (200 mg/Kg, b.w) showing almost normal hepatocytes with mild degeneration (H & E, 400 X)
Plate 4.14: Photomicrograph of liver tissue of rats treated with SVEE (400 mg/ Kg, b.w) showing almost normal hepatocytes with central vein and traces of blood in the sinusoidal space (H & E, 100 X)

Plate 4.15: A sectional representation of liver tissue of rats treated with SVEE (400 mg/ Kg, b.w) showing typical lobular arrangement and few hepatocytes shows steatotic accumulation (H & E, 400 X)
HISTOPATHOLOGICAL EVALUATION OF KIDNEY

Plate 4. 16: Photomicrograph of normal control rat showing intact architecture of renal parenchyma (H & E, 100 X)

Plate 4. 17: A section of control rat kidney showing normal renal tubules, intact glomeruli and Bowman’s capsule (H & E, 400 X)
Plate 4.18: Photomicrograph of paracetamol treated rat renal tissue showing severe tubular necrosis and swollen glomeruli and reduced capsular space (H & E, 100 X)

Plate 4.19: A representative section of paracetamol intoxicated rat kidney showing tubular degeneration (H & E, 400 X)

Plate 4.20: A sectional representation of paracetamol treated rat kidney showing swollen glomeruli with blood infiltration and glomerular degeneration (H & E, 400 X)
Plate 4.21: A representative section of paracetamol treated rat kidney showing glomerular degeneration and blood filled spaces (H & E, 400 X)

Plate 4.22: A sectional representation of paracetamol intoxicated rat kidney showing severe tubular damage (H & E, 400 X)
**Plate 4. 23:** Photomicrograph of rat kidney pretreated with cystone (5 ml/Kg, b.w) showing normal renal tubules with glomeruli and Bowman’s capsule (H & E, 100 X)

**Plate 4. 24:** A sectional representation of cystone (5 ml/Kg, b.w) treated rat kidney showing mild tubular degeneration (H & E, 400 X)

**Plate 4. 25:** A representative section of cystone (5 ml/Kg, b.w) treated rat kidney showing normal glomeruli with encapsulated Bowman’s capsule (H & E, 400 X)
Plate 4.26: Photomicrograph of rat kidney pretreated with SVGE (200 mg/Kg, b.w) showing normal glomeruli and moderate glomerular and tubular changes (H & E, 100 X)

Plate 4.27: A representative section of SVGE (200 mg/Kg, b.w) treated rat kidney showing mild degree of necrosis and degeneration (H & E, 400 X)
Plate 4. 28: Photomicrograph of SVGE (400 mg/Kg, b.w) treated rat kidney showing normal glomeruli, encapsulated by normal Bowman’s capsule (H & E, 100 X)

Plate 4. 29: A sectional representation of SVGE (400 mg/Kg, b.w) treated rat kidney showing moderate tubular degeneration with normal glomeruli (H & E, 400 X)
Plate 4.30: Photomicrograph of SVEE (200 mg/Kg, b.w) treated rat renal tissue showing moderate tubular necrosis and degeneration (H & E, 100 X)

Plate 4.31: A representative section of SVEE (200 mg/Kg, b.w) treated rat renal tissue showing mild degree of swelling, degranulation and thinning out of Bowman’s capsule (H & E, 400 X)
Plate 4.32: Photomicrograph of SVEE (400 mg/Kg, b.w) treated rat kidney showing normal tubular pattern with a mild degree of necrosis and degranulation (H & E, 100 X)

Plate 4.33: A sectional representation of SVEE (400 mg/Kg, b.w) treated rat kidney showing normal glomeruli and mild tubular degeneration (H & E, 400 X)
DISCUSSION

Acute toxicity studies are the first step in the pharmacological assessment of an unknown substance (OECD/OCDE, 2001). In the present study, rats administered with *S. variolaris* gonad and exoskeleton extracts were monitored continuously for 14 days and the treated rats did not show any toxic symptoms and no mortality was noticed throughout the observation period. Therefore *S. variolaris* extracts found to be safe at a dose of 2000 mg/Kg, b.w and may be considered for long term treatment at normal therapeutic doses.

GASTROPROTECTIVE ACTIVITY

The pattern of changes in the gastric juice volume, pH, ulcer index and ulcer inhibition percentage normally indicate the response of gastric system to various therapeutic agents used for ulcer and gastric disorders. The risk of gastric attacks is significantly increased due to the exposure of human to many noxious substances and chemicals. In the present study, aspirin administration caused a remarkably significant increase in gastric juice volume (4.91 ± 0.07 ml) and decrease in pH (1.87 ± 0.10) in the experimental animals when compared with normal control. Aspirin is a salicylate drug often used as analgesic, antipyretic and anti-inflammatory drug. It also has antiplatelet effects and long term use at low doses helps to prevent heart attacks, strokes and blood clots.

Recent studies showed that long term use of aspirin enhance the gastric ulcer formation (Giri *et al.*, 2009; Khatib *et al.*, 2010 and Takawale *et al.*, 2011). The ulceration induced by aspirin is attributed by various processes including their topical irritant effect, impairing the defense ability of gastric mucosal barrier and
reduced gastric mucosal blood flow (Fiorucci et al., 2003). Exposure of unprotected lumen of the stomach to accumulating acid could facilitate ulceration (Olsen, 1988). The volume of acid present in gastric secretion encompasses HCl, pepsinogen, mucus, bicarbonate, intrinsic factor and protein.

The present study revealed that the ranitidine administration reduced the gastric juice volume and increased the pH level in wistar albino rats. Normally ranitidine, a histamine 2- blocker is used to reduce the acid secretion by the stomach and to reduce ulcer and heart burn. Gastric acid dissemination by ranitidine is attributed to its ability to antagonize the binding of histamine to the H$_2$ receptor on the parietal cells (Banji et al., 2010).

The results of this comprehensive study also reveals that oral administration of SVGE extract at 200 mg /Kg, b.w and 400 mg/Kg, b.w decreased the gastric juice volume and increased the pH level when compared with toxin control. The effects on gastric contents and pH suggest that the SVGE extract exerted antispasmodic activity and acid neutralizing effects and at 400 mg/Kg, b.w concentration its activity was almost equivalent to ranitidine treatment. However, SVEE extract administration at 200 mg/Kg, b.w and 400 mg/Kg, b.w produced no significant change in gastric volume and pH when compared with control.

The ulcer index parameter was used for the evaluation of gastroprotective activity. In this study, oral administration of aspirin to albino rats produced characteristic mucosal lesions. Aspirin administration can easily produce mucosal damage interfering with prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the re epithelization of the
ulcer crater (Levi et al., 1990). When compared with aspirin treated rats, ranitidine administered rats significantly reduced the ulcer index (11.33 ± 1.25) and increased the ulcer inhibition percentage. Ranitidine can therefore counter the effect of aspirin.

As evidenced from the present study, the *S. variolaris* gonad extract reduced the ulcer index and increase the ulcer inhibition percentage. In the present study, the flattening of the mucosal folds suggests that gastroprotective effect of *S. variolaris* gonad extract might be due to the decrease in gastric motility. It is reported that the changes in the gastric motility may play a role in the development and prevention of experimental gastric lesions (Garrick et al., 1986 and Takeuchi et al., 1986). Relaxation of circular muscles may protect the gastric mucosa through flattening of the folds. This will increase the mucosal area exposed to necrotizing agents and reduce the volume of the gastric irritants (Takeuchi and Nobuhara, 1985) and thus increase the ulcer inhibition percentage. Such protection was shown to be dose dependent and in the present study protection was most prominent at a dose of 400 mg/Kg, b.w of gonad extract.

**HEPATOPROTECTIVE ACTIVITY**

Liver is the most important accessory digestive gland of the animal body and it is highly affected by toxic agents. The study of different liver function parameters such as SGOT, SGPT, ALP, total bilirubin and total protein have been found to be of great value in the assessment of clinical and experimental liver damage (Vaishwana and Kowale, 1976). PCM the mild analgesic produced toxic metabolites which induced chemical hepatic injury in rats, mice and humans at higher doses. Even though safe for use at recommended doses, small over dose can be fatal. For
screening of hepatoprotective agents, PCM induced hepatotoxicity has been used as a reliable model (Wendel et al., 1979; Gupta and Misra, 2006 and Dash et al., 2007). The hepatotoxicity that was induced by PCM resembled naturally occurring liver diseases (Sherlock and Dooley, 2002). An obvious sign of hepatic injury is the leakage of cellular enzymes into the plasma due to the disturbances caused in the transport functions of hepatocytes.

In the present study, the damage of liver due to PCM over dosage increased the serum concentration of SGOT, SGPT and ALP than the normal control rats, whereas the total serum protein level reduced than the control rats. The rise in serum levels of SGOT and SGPT has been attributed to the damaged structural integrity of the liver hepatocytes because they are cytoplasmic in location and released into circulation after cellular damages. Likewise, ALP levels in plasma will rise when large bile duct obstruction is present. In the present study, when silymarin a potent antioxidant and liver protector is co administered with paracetamol protects liver from the hepatotoxicity of PCM. Silymarin correct liver damages by means of several mechanisms, including free radicals scavenging, increased glutathione synthesis and increasing the levels of superoxide dismutase. It also inhibits the synthesis of mediators of inflammation (Hussain et al., 2009). Comparable results were previously explained and reported (Pandey et al., 2012 and Sahu et al., 2012).

In the present investigation, the reduction of PCM induced elevated enzyme levels in animals treated with the S. variolaris extracts was found to be significant and the gonadal extract at 400 mg/Kg, b.w concentration works in par with silymarin and reduced the serum SGOT, SGPT and ALP level almost to the level reduced by silymarin. The above changes are in agreement with the commonly accepted view
that serum level of transaminase returns to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew et al., 1987 and Shukla et al., 1992). Esmat et al. (2013) reported that the coadministration of H. atra extract attenuated the hepatotoxic effect of thiocetamide as evident by the normalization of serum SGOT, SGPT and ALP levels, which is consistent with the present findings. Gonzalez et al. (2000) studied the ALP level in rats treated with sea urchin based diets. They suggested that the sea urchins P. lividus and E. esculentus meals modify the intestinal enzymatic activity.

Serum bilirubin is considered as an index for the assessment of hepatic function and any abnormal increase indicates hepatobiliary disease and severe disturbance of hepatocellular architecture (Martin and Friedman, 1992). In the present study, the rats fed with PCM showed significant increased levels of bilirubin as compared to normal control rats suggesting severe hepatic injury and confirming the hepatotoxic nature of PCM. Similarly Parmar et al. (2010) reported that the hepatic damage induced by PCM overdose, could raise the level of total serum bilirubin in experimental animals. Decrease in serum bilirubin after treatment with the S. variolaris extracts in liver damage induced by PCM indicated the effectiveness of the extract in normal functional status of the liver.

The results also showed that rats exposed to PCM significantly decreased the serum total protein levels. The reduction can be assigned to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P-450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides resulting in fatty liver (Recknagel and Glande, 1973; Azri et al., 1992 and Takate et al., 2010). Stabilization of serum protein levels in the
pretreatment groups administered with *S. variolaris* extracts is further a clear indication of the stabilization of the function of endoplasmic reticulum leading to normal protein synthesis.

Paracetamol induced hepato toxicity of the experimental animals and their corrective action by the plant based commercial antioxidant silymarin and *S. variolaris* gonadal and exoskeleton extracts were well supported by histopathological observations in the present study. Liver parenchyma consists mainly of cuboidal cells called hepatocytes arranged in the form of hepatic lobe and each lobe has a central vein passing through its core. Liver of rats administered with paracetamol showed severe degenerative changes in the liver with loss of hepatocytes and blood filled spaces revealing the paracetamol induced hepatotoxicity. But when the test animals are co administered with *S. variolaris* gonadal and exoskeleton extract at different concentration, liver regeneration occurs depends upon the concentration of the extract used. With 400 mg/Kg, b.w concentration, gonad extract showed almost normal architecture of the liver indicating its prominent hepatoprotective action. Normally the drugs administered for liver diseases, increases protein synthesis in the liver which in turn causes the increased production of liver cells to replace the damaged one. In the present study also the specific hepatoprotective compounds found in the *S. variolaris* extracts stimulated the liver cell to rapid protein synthesis for cell regeneration like the commercial drug silymarin and resulted in hepatocyte replacement.

Generally, the reduction in body weight and internal organ weight is a simple and sensitive index of toxicity in animals after exposure to toxic substances (Raza *et al.*, 2002 and Teo *et al.*, 2002). In the current investigation, there was a reduction
in the body weight of rats after PCM administration. This reduction can be attributed
to reduced intake of food due to the damage of liver cells leading to indigestion and
anorexia. However, the *S. variolaris* extracts ameliorated the harmful effects of
PCM on body weight loss. This may be due to increase in food intake. Similar
observation of an increase in weight was indicated on studies with the ethanolic
extract of the simple ascidian *Microcosmus exasperates* (Meenakshi *et al.*, 2013).
The crude liver weight, which was increased after PCM administration may be due
to haemorrhages, hydropic degeneration and fatty changes associated with necrosis.
The weight of liver were unaltered in the experimental groups compared with the
normal control and silymarin control groups, indicating that *S. variolaris* extract was
not toxic to this vital organ.

In the present investigation, both body parts exert excellent hepatoprotective
activity against PCM. This may be due to the presence of free amino acids such as
methionine, valine and alanine in the *S. variolaris*. Normally, methionine is used to
prevent liver damage in PCM poisoning. In PCM poisoning, methionine prevents
the breakdown products of PCM from damaging the liver. Valine and alanine are
used to treat liver diseases. Valine is also used in the treatment of alcohol or drug
related liver damage. Comparison of the gonad and exoskeleton extracts showed that
the gonad extract was found to be more effective than the exoskeleton extracts. The
probable reason may be due to the fact that the gonads are generally rich in
β-carotene (Garama *et al.*, 2012), the dietary antioxidants capable of preventing the
buildup of free radicals with subsequent reduction in tissue damage.
NEPHROPROTECTIVE ACTIVITY

The kidney disorders are world problem but hitherto, no therapy has successfully prevented the progression of kidney diseases. The newly developed drugs used to treat chronic kidney disorders, often have side effects. In clinical practice drugs like PCM, gentamicin and others used in the treatment of cancer and tuberculosis can cause severe nephrotoxicity. In this study, the administration of 750 mg/Kg, b.w PCM induced nephrotoxicity in rats due to the entry of paracetamol metabolic intermediate in the renal system, that are produced in the liver during paracetamol metabolism. It can cause a significant increase in the serum urea and serum creatinine concentrations in rats, when compared to the normal control group. PCM overdose is often associated with a wide range of metabolic disorders including serum electrolytes, urea and creatinine derangements. Thus elevations in the serum concentrations of these markers after PCM treatment are indicative of renal injury and inability of the renal system to maintain the metabolic waste within the homeostatic level. The signification reduction in the serum urea concentration following the administration of ethanolic extract of *S. variolaris* gonad at various doses may be attributed to the correction of renal tubular filtration system leading to reduced concentration of this toxic metabolic waste product in the serum. Yakubu *et al.* (2003) reported similar findings in rat kidney function following repeated administration of sildenafil citrate.

Creatinine on the other hand, is mostly derived from endogenous sources by tissue creatinine breakdown (Venkatesan *et al.*, 2000). Elevated levels are sometimes seen in kidney diseases with paracetamol administration and two fold increases in serum creatinine was noticed in rats, owing to the inflammation and
glomerular dysfunction. Kidney tissue of paracetamol administered rats revealed abnormalities in the renal tissue with either inflamed or shrunken glomeruli and distended and widened renal tubules leading to inefficient filtration system. Decrease in creatinine content of the serum following the administration of ethanolic extracts of *S. variolaris* extracts and cystone may be an indication of their ability to restore the glomerular and tubular functions. Co administration of cystone, an herbal medicine with spasmolytic and anti-inflammatory actions along with paracetamol reduces the serum urea and creatinine to the normal level. *S.variolaris* gonad extract co administration also exhibit similar serum urea and creatinine level. Histopathological observation of renal tissue in the present study showed same mode of renal recovery in both cystone and *S.variolaris* gonad extract administration after paracetamol induced nephrotoxicity in rats. Funk *et al.* (2014) reported similar nephroprotective effect of spongean alkaloids against cisplantin induced cytotoxicity in rat kidney.

In the present investigation, decrease in uric acid as observed in paracetamol treated rats may suggest impairment in the renal function. Normally, low levels of uric acid maybe indicative of kidney diseases, malabsorption, poor diet and liver damage. In the present study, the most remarkable increase in uric acid was observed when the ethanolic extract of *S. variolaris* gonad was delivered at 400 mg/Kg, b.w concentration. Histological studies also confirmed the nephroprotective effect of the ethanolic extracts of *S. variolaris*. From the histopathological observation it can inferred that the extracts had protective effect against degenerative injury caused by PCM.
Change in body weight, kidney weight, organ weight and body weight ratio in the control and various experimental groups showed that the rats treated with single dose of PCM reduced the body weight considerably as compared to normal group. This progressive weight loss may be due to the injury of renal tubules and the subsequent loss of the tubular cell to reabsorb water, leading to dehydration and corresponding body weight loss. However, pretreatment with *S. variolaris* ethanolic extracts and the reference drug cystone significantly increased the body weight along with a significant decrease in kidney weight when compared to PCM induced group. This may be due to the increased ability of the kidney to absorb nutrients after *S. variolaris* treatment. Organ- body weight ratio is a marker of cell constriction and inflammation (Moore and Dalley, 1999). The non-significant effect on the rat kidney- body weight ratio following the administration of various doses of the gonad extract suggests that the extract did not induce inflammation or constriction of the kidney cells. This indicates that both extracts had no effects on organ weight and therefore would possibly have no organ related toxicity. Organ weights are widely accepted in the evaluation of test agent associated toxicities. The society of toxicological pathology recommends that organ weights be included routinely in toxicity studies for multidose drugs administered in durations from 7 days to one year (Sellers *et al.*, 2007).

The gonad and exoskeleton extracts of *S. variolaris* possess nephroprotective potential against PCM induced nephrotoxicity as evidenced by the physical parameters, biochemical status and histological findings. This may be due to the presence of arginine in *S. variolaris*. In clinical practice, arginine is used to improve kidney function after a kidney transplant.
In the present investigation, representative of NSAIDs family drugs (aspirin and PCM) were used to induce organotoxic effects. NSAIDs are used primarily to treat inflammation, mild to moderate pain and fever. But chronic use of NSAIDs cause serious side effects such as kidney failure, liver failure, ulcers and prolonged bleeding after an injury or surgery. The present findings indicate that the ethanolic extract of *S. variolaris* gonad display potential organoprotective activity against NSAIDs than the exoskeleton. This supports the traditional belief and use of *S. variolaris* by the coastal people of Kanyakumari district.

The reduction noticed in the serum SGOT, SGPT, ALP, bilirubin, urea, creatinine, uric acid as well as the gastroprotective action exhibited by *S. variolaris* gonad and exoskeleton extract in aspirin induced gastric mucosal damage and paracetamol induced hepatotoxic and nephrotoxic rats were comparable and almost similar to or in the normal range of these parameters in human blood and gastric system. Therefore the therapeutically potential *S. variolaris* gonads may explored further for the discovery of gastro, hepato and nephroprotective drug from this valuable marine species.
SUMMARY

The quest for solutions to the global problem of drug resistance has often focused on the isolation and characterization of new pharmacologically active compounds from a variety of sources including marine organisms. Thousands of bioactive compounds identified in marine organisms revealed that sea creatures constitute a larger reservoir for pharmacologically active drug. In such effort, the present study was undertaken to identify the pharmacologically active compounds from the sea urchin *Stomopneustes variolaris* body parts and to evaluate its organoprotective activity.

Biochemical constituents were estimated in the gonad and exoskeleton of *S. variolaris*. Variations in the biochemical composition of both body parts of *S. variolaris* were observed. Protein was the most dominant biochemical constituent in *S. variolaris* and it was high in gonad (149 ± 0.82 mg/g) and low in exoskeleton (134.33 ± 1.25 mg/g). Similar to protein, carbohydrate, total cholesterol and triglycerides were also maximum in gonads and minimum in exoskeleton.

The analysis of the amino acid composition of the *S. variolaris* revealed the presence of nine amino acids in gonads and ten amino acids in exoskeleton. The essential amino acids methionine, histidine, valine and leucine were observed in both body parts, whereas phenyl alanine was present only in exoskeleton. However, the non-essential amino acids proline, alanine, glutamate and tyrosine were found to be dominant among the free amino acids in *S. variolaris*.
Minerals are micronutrients and are present in small quantities. Among the estimated minerals, potassium ($3.23 \pm 0.04 \text{ mg/g}$) and phosphorous ($1.21 \pm 0.01 \text{ mg/g}$) were high in gonads. On the other hand, sodium ($33.52 \pm 0.03 \text{ mg/g}$) and magnesium ($0.64 \pm 0.01 \text{ mg/g}$) were found to be maximum in exoskeleton and minimum in gonad.

The physicochemical parameters are mainly used in judging the purity and quality of the drug. The moisture content was maximum (75.96%) in gonad and minimum (9.64%) in exoskeleton. Whereas, the exoskeleton recorded the highest ash percentage (81.62%) and the gonad recorded lowest ash percentage (5.58%). The current findings served as a standard data for quality control studies of pharmaceutical preparation from *S. variolaris*.

Extract of *S. variolaris* gonad and exoskeleton extracted with six organic solvents and aqueous medium was screened against eight human pathogenic bacterial (Gram positive isolates- *S. aureus, M. luteus, S. pyogenes, E. faecalis;* Gram negative isolates- *E. coli, K. pneumoniae, P. vulgaris* and *S. typhi*) and four pathogenic fungal (*C. albicans, A. niger, T. gamsii* and *T. flavus*) strains.

Distinct antibacterial activity was observed against almost all the bacterial pathogens. Data indicated that the most active extracts were the exoskeleton extract of *S. variolaris*. Maximum activity was conferred against *S. typhi* ($19.33 \pm 1.52 \text{ mm}$) for hexane and chloroform extracts. Of the four fungal species assayed three species *A. flavus, T. gamsii* and *T. flavus* were resistant to both the extracts but it showed some significant activity against the *C. albicans*.
Based on preliminary antimicrobial screening, the ones with a potentially promising activity (Ethanolic extract) were subjected to HPLC purification. The high peak fraction thus obtained from HPLC purification was further used for the identification of pharmacologically active constituents by using GC-MS analysis. A total of 10 compounds were isolated from the 1st fraction and 7 chemical constituents have been identified from the 2nd fraction of *S. variolaris* gonad extract. Among them 1, 2- Benzenedicarboxylic acid, butyl 2- methylpropyl ester is the dominant component present in the first fraction whereas 1, 2- Benzenedicarboxylic acid, butyl cyclohexyl ester is the dominant component present in 2nd fraction. The studies on the active constituents in the exoskeleton fraction clearly showed the presence of seven compounds. The compound Dimethyl sulfoxide is the major constituent in the exoskeleton fraction. The compounds identified by GC-MS are medicinally valuable and possesses various pharmaceutical applications.

Before screening the crude extracts for organoprotective activity, it was subjected to acute oral toxicity study. The acute toxicity study revealed that *S. variolaris* gonad and exoskeleton extracts at a dose of 2000 mg/Kg, b.w was totally safe to wistar albino rats. So the dose selected for evaluation of organoprotective activity was 200 mg/Kg, b.w and 400 mg/Kg, b.w (1/10th and 1/5th of 2000 mg/Kg, b.w).

For assessing gastroprotective activity, gastric ulcerations were induced experimentally in wistar albino rats by aspirin. The gastro protective efficacy of *S. variolaris* extract was evaluated by measuring gastric juice volume, pH, ulcer index and preventive index.
Increased level of gastric juice volume was noticed in aspirin (200 mg/Kg, b.w) treated group than the control group. But the *S. variolaris* gonad extract (200 and 400 mg/Kg, b.w) and the standard drug ranitidine (50 mg/Kg, b.w) reduced the gastric juice volume, while exoskeleton extract (200 and 400 mg/Kg, b.w) treated groups showed no remarkable change in the gastric juice volume when compared to that of aspirin treated group.

The aspirin control group exhibited a low level of pH compared with normal control. The data depicted that the standard drug ranitidine and the gonad extracts recorded a high pH value, whereas the exoskeleton treated groups showed very little change in the pH level.

Aspirin administration exhibited a remarkably high ulcer index and low preventive index. Pretreatment with ranitidine produced higher gastroprotective effect by decreasing the ulcer index and provide 81.62% prevention against gastric mucosal injury. *S. variolaris* gonad extract at 200 mg/Kg, b.w and 400 mg/Kg, b.w concentration provide 65.68% and 72.98% prevention against mucosal injury.

Hepatotoxicity was induced in albino rats by p. o of paracetamol. The hepatoprotective activity of the ethanolic extract of *S. variolaris* was evaluated by different liver function parameters in the serum (SGOT, SGPT, ALP and total protein), physical parameters (body weight and liver weight) and histopathological examination of the liver.

A significant increase in the serum SGOT, SGPT, ALP and total bilirubin levels were seen in the paracetamol (750 mg/Kg, b.w) intoxicated animals when compared with the normal control (normal saline- 5 ml/Kg, b.w) group and
indicating acute hepatocellular damage. The hepatic parameters of the standard drug silymarin (25 mg/Kg, b.w) treated rats showed reduced level of SGOT, SGPT, ALP and total bilirubin in the serum when compared with the paracetamol intoxicated rats.

The *S. variolaris* gonad extract (200 and 400 mg/Kg, b.w) treatments significantly reversed the levels of SGOT, SGPT, ALP and total bilirubin level, when compared to paracetamol alone treated rats. While the exoskeleton extracts (200 and 400 mg/Kg, b.w) has reduced the elevated levels of SGOT, SGPT, ALP and total bilirubin to lesser extent compared to gonad extracts.

When compared with toxic control group, decreased level of total protein and relative organ weight were observed in the groups administered with normal saline, silymarin, *S. variolaris* gonad and exoskeleton extracts.

Histopathological examination of the liver tissue revealed normal hepatic architecture of the liver up on treatment with *S. variolaris* gonadal and exoskeleton extract in rats with paracetamol induced hepatotoxicity, proving the hepatoprotective effect of *S. variolaris* and the results are comparable to the commercial drug silymarin.

The nephroprotective potential of *S. variolaris* ethanolic extracts against paracetamol induced nephrotoxicity in wistar albino rats was evaluated. Biochemical parameters such as serum urea, uric acid, creatinine and percentage change in body weight and histopathological changes in kidney were studied.
The biochemical parameters such as serum urea and serum creatinine were found to be increased in the group treated with paracetamol (750 mg/Kg, b.w) as compared to normal control group (normal saline- 5 ml/Kg, b.w). While serum uric acid level decreased in paracetamol treated group when compared with control.

Moreover, the cystone (5 ml/Kg) reference drug, and the test drugs (S. variolaris gonad and exoskeleton extracts- 200 and 400 mg/Kg, b.w, respectively) showed a significant nephroprotective effect as evidenced by a decreased serum urea and creatinine level and an increase in the uric acid level when compared with PCM treated group.

The results indicated that the relative organ weight of normal control, reference drug cystone, S. variolaris gonad and exoskeleton was low when compared with paracetamol treated group. The histopathological studies showing renal recovery by S. variolaris ethanolic extracts in a dose dependent manner. The present findings indicate that the ethanolic extract of S. variolaris gonad display potential organoprotective activity against NSAIDs than the exoskeleton.

The present study suggested that S. variolaris possesses many promising compounds with potential pharmaceutical properties. This supports the traditional use of S. variolaris by the coastal people, they believed that this sea urchin have multiple health promoting effects. Hence, the present research provides a good starting point for the identification and isolation of unique compounds from the sea urchin S. variolaris for the treatment of human diseases. Further studies regarding the isolation and characterization of the active principles responsible for
organoprotective properties and preclinical trials are needed before using these compounds as pharmaceuticals for treating human diseases.