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RESEARCH ARTICLE

Assessment Of Nutritional Potential And Food Safety Evaluation In Mice And Rat By The Fruit Of Mangrove Plant, *Sonneratia apetala* Of Sundarban, India

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Abstract

The Study was under taken on human edible fruit of mangrove plant of Sundarban. Within 94 mangal species reported from Sundarban only seven species produce human edible fruits as confirmed by local use. So far, no study has been under taken on the nutritive value as well as on toxicity levels of these fruit. This is why they did not find the place in the ICMR food list.
The work was taken up for the fruit *Sonneratia apetala*. It was revealed that this fruit contain several beneficial nutrients. A Hepato-toxicity study was under taken which indicated no mortality of mice even in the acute toxicity tests. During sub-acute toxicity test increased Haemoglobin level was noticed for *S.apatala*, & decreased serum urea levels, increased HDL level, SGPT level decreased.
Very interesting compound squalene pick was detected by GC-MS analysis which is valuable for tumor depressant, anti-diabetic. The fruit is rich in anti-oxidants. The above study indicates a strong case for inclusion of this mangrove fruit in ICMR food list.

INTRODUCTION

Sundarban ecosystems harbour several types of mangrove plants. *Sonneratia apetala* locally called as Keora among other mangrove species (Xavier, 1999). The distribution of the plant is restricted in both the coast from Mumbai to Sundarban. The stem is used for paper pulp, matches, and as poles, leaves as fodder. Its fruits are used as vegetable and pickles. The antimicrobial activity of the mangrove plant extract of *Sonneratia apetala* on the various test microorganisms, including clinical multiple antibiotic resistant bacteria and phytopathogens have already been investigated. Antimicrobial activities of the extracts were determined by the well diffusion method. In vitro screening of *S.* apetala mangrove plant extracts showed species specific activity in inhibiting the growth of bacteria and fungi. Hexane, chloroform and methanol extract showed good activity against all the pathogens, where as only methanolic extracts were active against most of the pathogens (Bobbarala et al., 2009).

There are several studies have already been carried out on the nutritive values and presence of potent micronutrient in the fruits of different plant species (Bellec e.tal., 2006; Jaafar et.al., 2009; Zainoldin and Baba, 2009; Hor et al., 2012; Halder et al., 2013;) as well as safety evaluation by acute and chronic toxicity studies after in-vivo exposure in animals (Rhiouani et.al.,1999; 2001; Rhiouani et al., 2008) but less studies have been documented with fruits of mangrove plants (Halder et al., 2013). Recently, the fruit of *S. apetala* has increased in popularity by local people in and around Sundarban, not only because of its green coloration and economic value as a food product, but also because of its health benefits. The fruit of *S. caseolaris* has already been reported a potent anti-diabetic and anti-nephric as medicinal benefits (Halder et al., 2013). In Malaysia, the fruit of *Hylocereus polyrhizus* is rich in ascorbic

acid (vitamin C) and lycopene. Lycopene is associated with a reduction in cancer risk and heart disease, and a lowering of blood pressure (Bellec et.al., 2006; Jaafaretal., 2009; Zainoldin and Baba, 2009). Furthermore, *H. polyrhizus* and H. undatus seeds contain high levels of essential fatty acids, namely linoleic and linolenic acids (Ariffin et.al., 2009). According to Hor et al. (2012), safety assessment of methanol extract of red dragon fruit (*Hylocereus polyrhizus*) acute and subchronic toxicity studies revealed that the lethal oral dose of the fruit extract is more than 5000 mg/kg and the no-observed-adverse-effect level (NOAEL) of the extract for both male and female rats is considered to be 5000 mg/kg per day for 28 days.

Despite its local use, potent nutritional values and toxicological data is only available on the fruit of other species *Heritiera fomes*, locally called Sundari (Halder et al., 2013), but no one has attempted earlier regarding the assessment of nutritional contents and safety evaluation by acute and subacute exposure to *Sonneratia apetala* fruit.

The present study aims to determine nutritive values and a safety evaluation of *S. apetala* fruit by analysis of nutritive contents and toxicological study of this fruit in mice for acute and in Sprague–Dawley rats for chronic toxicity studies.

2. Materials and Methods

2.1 Experimental sample

Sonneratia apetala fruits are collected from the Sundarban, in the month of August - November 2011. Fresh fruits, dried sample, methanol extracted sample, solvent extracted sample, perchloric acid extracted sample were taken for the estimation of moisture content and gross calorific value, fiber content, protein content, glucose content and fat content respectively.

Samples were dried in oven and prepared a powder to estimate micronutrients by using Plasma Spectrophotometer.

The powder of dried fruit was extracted by hydro-methalonic extraction with methanol (64.7°C) Distilled water, Then the mixture are stored in lab temp for 2 days after that filtered out. And then the filtrate material was dried by Rota-evaporator & Water-bath. The temperature should be maintained at 45°-50°C.A brown coloured layer are formed, take out the layer and prepare the dose.

The dried fruit of *Sonaratia apetala*, was dissolved in absolute ethanol (1mg/ml) and extract by the ultrasonicator. Then the extracted fruit samples (each 10µl) were separately injected for gas chromatography- mass spectrometric (GC-MS) analysis.

2.2. Experimental animals

Swiss albino male (5 nos.) and female (5 nos.) adult mice (20-25gm body wt.) were acclimatized in laboratory condition and used for acute toxicity test. For chronic toxicity test, adult male (5 nos.) and female (5 nos.) *Sprague Dawley rat (250-300 gm body weight) were acclimatized in laboratory condition and used for experiment.*

2.3 Nutritive value and micronutrient analysis

To investigate the Nutritive value of this mangrove fruit e.g. total Calorie, carbohydrate, protein, fat, Iron, Calcium, Sodium, Potassium, Copper, Zinc, Manganese, Magnesium, Selenium, moisture content for which following methods are followed**:**

Determination of moisture content was done by the method of Raghuramulu et al. (2003), Gross Calorific Value (Kcal/Kg) was determined by the method of Indian Standard Methods (1970), Protein (%) by Kjeldal method : Nitrogen Estimation X 6.25 (Raghuramulu et al., 2003), Estimation of Carbohydrate, fiber content and vitamin C by the method of Raghuramulu et al. (2003), estimation of fat by Soxhlets method (Vogel, 1964). Determination of micro nutrients by using ICP-OES by the method of Sepctro Arcos (2010).

2.3 Acute toxicity study in mice

For Acute toxicity test (LD_{50} determination) the study was performed as per OECD-423 guidelines (OECD, 2001). Swiss albino mice (20-25gm body wt.) were randomly distributed to four groups and each group is having five animals. The animals were fasted overnight and the various doses of drug were administered orally by feeding needle. The doses are 200 mg/kg, 400 mg/kg, 1000 mg/kg, 2000 mg/kg body weight.

2.4 Chronic toxicity study in rat

For Sub–acute or chronic toxicity test (28 days repeated toxicity study according to the OECD guideline) after getting the LD50 result no mortality was found through 7 days. Then the 400mg/kg dose was taken for sub acute toxicity study. Repeated dose toxicity studies were carried out following OECD guide lines No 407 (OECD, 2008) repeated toxicity studies were conducted on male and female groups of rat for each fruit for 28 days. And distilled water was administered to the control group.

The amounts of food and water consumed were measured daily from the quantity of food and water supplied and the amount remained after 24 hrs.

2.5 Haematological study in rat

Once of a week blood sample were collected from the orbital plexus of each rat and each group into EDTA containing tube for biochemical estimation. Red blood cell, white blood cell and haemoglobin concentration (g/l) estimation were carried out**.**

Haemoglobin estimation was done using Cyanomeath reagent by Haemoglobin cyanide method in after every 14 days during chronic exposure. The Cyanmethemoglobin formed was measured at540nm and the intensity of the colour formed is directly proportional to the haemoglobin concentration. Calibration curve was prepared by using haemoglobin std. 20µl blood sample was added to 5 ml cyanomeath reagent. The sample was mixed thoroughly. And absorbance was measured at 540nm after 3 min incubation at 37°c against blank (cyanomaeth regent). Haemoglobin concentration was determined from the calibration curve (Raghuramulu et al., 2003).

2.6 Biochemical study in rat

The biochemical parameters from blood samples of rat were analyzed in every week during chronic studies. In once of a week blood was collected from each and every animal group by retro-orbital puncture or heart puncture. On every week last day after dosing all animals of all group were euthanized by exsanguinations under diethyl ether anaesthesia, after this blood samples were collected from orbital plexus of each animal by using glass capillary tube. The samples were collected in 2ml ape drop containing 1.5mg k_2EDTA per ml blood. The ape drop were inverted carefully 10 times to makes blood and anticoagulant and stored at room temp until centrifugation. The samples were centrifuged immediately. This was carried out for a minimum of 10 min at 3000 rpm at 5. The supernatant (plasma) was aspirated at room temp and pooled in another ape drop tube by using separate cleaned syringe or micro tip and following parameters are taken for the studyThe parameters were Cholesterol, Glucose, HDL, Creatinine, Alkaline phosphatase, GPT (ALT), GOT (AST), Urea and ϒ- Glutamyl Transferase.

Cholesterol esters are hydrolysed by cholesterol esterase to give free cholesterol and fatty acid. In subsequent reaction cholesterol oxidase (CHOD) oxidises the 3-OH group of free cholesterol to liberate cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidise hydrogen are coupled with 4-Amino antipyrine (4AAP) and phenol to produce red Quinoneimine dye. Absorbance of coloured dye measured by spectrophotometrically at 505 nm (Nader et al., 1994; Warnick et al., 1995).

Glucose Oxidase oxidises Glucose to gluconic acid and Hydrogen peroxidise. In presence of enzyme peroxidise, released Hydrogen Peroxide is coupled with phenol and 4-Aminoantipyrine (4-AAP) to from coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to glucose concentration in the sample (Kaplan and Lavernel,1983).

Low density lipoprotein cholesterol very low density lipoproteins cholesterol and Chylomicron fraction are precipitated by addition of Poly ethylene Glycol 6000. After centrifugation the high density lipoprotein fraction remains in the supernatant and it is determined with the CHOD-PAP method (Nader et al., 1994; Warnick et al., 1995).

Creatinine in protein free solution reacts with alkaline picrate and produces a red coloured complex which measured spectrophotometrically at 520nm (Bohses and Taussky, 1945).

Alkaline Phosphates from serum converts phenyl phosphate to inorganic Phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidizing agent. Potassium ferricyanide and forms an orange-Red coloured complex, which can be measured by spectrophotometrically at 510nm (Verley, 1975).

ALT was also determined by 2,4-DNPH (Reitman and Frankel,1957) method. Alanine amino transferase (alt) catalysed transamination of L-alanine and ά-keto glurate (ά-kg) to form pyruvate and L-Glutamate. Pyruvate so formed is coupled with 2,4-Dinitro hydrazine (2,4-DNPH) to form a corresponding hydrazones, a brown colour

complex in alkaline medium and this can be measured by spectrophotomatrically at 505nm (Toro and Ackermann,1975).
Aspatate amino transferase (AST) catalysed transamination of L-aspartate and ά-keto glutarate (ά-Kg) to form Oxaloacetate and L-Glutamate. Oxaloacetate so formed is coupled with 2,4-Dinitro hydrazine (2,4-DNPH) to form a corresponding hydrazone, a brown color complex in alkaline medium and this can be measured by spectrophotomatrically at 505nm (Reitman and Frankel,1957; Toro and Ackermann,1975).
Urea reacts with hot acidic Diacetylmonoxime in presence of thiosemicarbazide and produces a rose-purple colour complex, which is measured spectrophotometrically (Crocker, 1967; Wybenga, et al. 1971).
ϒ-Glutamyl Transferase (GGT) is an enzyme found mainly in serum from hepatic origin through the highest levels are in kidneys. Elevated levels are found in hepatobiliary and pancreatic diseases, Chronic alcoholism, myocardial infection with secondary liver damage and diabetics.GGT catalyses the transfer of amino group between L-ϒ-Glutamyl-3-carboxy-4-nitroanilide and glycylglycine to form L-ϒ-Glutamylglycylglycine and 5-amino-2-nitrobenzoate is measured as an increase absorbance which is proportional to the GGT activity in the sample.
In test sample preparation firstly dissolve 1 substrate tablet in 2.2 ml buffer reagent .0.1 ml of serum were added 1 ml of working reagent mix well & read the initial absorbance & reading are continued for every 1 min interval till 3 minutes in spectrophotometrically at 405 nm (IFCC, 1986).

2.7 Histological study in rat

Histological study was conducted only on liver and kidney to check the level of hepatotoxicity and renal toxicity after chronic feeding of this fruit .The tissue was preserved in 10% formalin, embedded in paraffin, sectioned at approximately 5mm, stained with hematoxylin and eosin, and examined with an optical microscope (Wasfietal,1994).

2.8 Gaschromatography–massspectrometry (GC–MS) analysis

The dried fruit of *Sonaratia apetala*, was dissolved in absolute ethanol (1mg/ml) and extract by the ultrasonicator. Then the extracted fruit samples (each 10µl) were separately injected for gas chromatography- mass spectrometric (GC-MS) system consisting of an Agilent 6890 gas chromatograph (column) coupled with an Agilent 5973 mass spectrometer.

GC-MS technique was used to identify the phyto-constituents present in the extract. The plant extract was analyzed using Agilent Technologies 6890 N Network GC system and interfaced to Agilent Technologies 5973 Inert Mass Selective Detector employing the following conditions: column DB-1 ms fused silica capillary column (30X0.25 I.D.X 0.10 Film, composed of 100% Dimethylpolysiloxane) chosen for improved signal to noise ratio for better sensitivity and mass spectral integrity, operating in electron impact mode; helium (5.0) was used as carrier gas at a constant flow of 1ml/min. The injector, MS Source & MS Quadrapole temperature were fixed at 250^{o}C, 230^{o}C and 150^{o}C respectively and turbo Speed of the pump was 100%. The oven temperature was programmed from 50^{o}C (isothermal for 5 minutes), with an increase of 10^{o}C/min to 100^{o}C (isothermal for 2 minutes), then 10^{o}C/min to 300^{o}C (isothermal for 5 minutes) For tuning of the MSD in EI mode Perfluorotributylamine (PFTBA) was used as tuning compound. Mass spectra were taken at 2235 EM Volts and fragments from 69 to 502.
Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST)/National Bureau of Standard (NBS) and Wiley having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST/NBS and Wiley libraries. The name, molecular weight and structure of the components of the test materials were ascertained (Merlin et al., 2009).

2.9 Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS) version15. Data are expressed as the mean ± S.D., and were analyzed using one way analysis of variance . Significant differences between the control and treatment groups were determined using the Dunnett test and $P < 0.05$ was considered to be significant.

3. Results

In this study it was observed that *Sonneratia apetala* fruit has several nutritional importance and have higher value of the different nutrients e.g. calorie, protein, fat, calcium, iron, magnesium, potassium, copper, manganese, zinc, sodium, and moisture content. The nutritive values in the fruits mainly macronutrients and micronutrients contents are showed in Table 1 and 2.

In acute toxicity study, during the 4 week treatment period, all animals were observed regularly for clinical signs and mortality patterns once before treatment, during treatment and up to 4 hrs after treatment. No mortality was found with doses of 200, 400, 1000, 2000mg/kg of *Sonneratia apetala* during 72 hrs observation period. It was also observed that the animals became quite, less response, to external stimuli and used to sit in the corner of the cage for 5 min after the administration of each fruit extract of *Sonneratia apetala*. The behavioural changes were exhibited till 20 to 40 min.

In sub acute or chronic toxicity study, during the experiment no mortality was observed with *Sonneratia apetala*, at 400mg/kg of body weight. There were no significant difference in body weight gain which was noted between the control and experimental groups when fruits (*Sonneratia apetala*) were administered at 28 days of time period.

Oral administration of the amount of dose 400mg/kg body weight of this type of fruits (*Sonneratia apetala*) shows significant changes during the estimation of Haemoglobin in each 14 day after up to 28 days . As the data were showed statistically significant ($p<0.05$) values. *Sonneratia apetala* male and female both groups were shown significantly increased the haemoglobin level as compare to the control group of animal till 28 days.

Oral administration of the amount of dose 400mg/kg body weight of the fruit (*Sonneratia apetala*) showed variable changes during the estimation of Cholesterol in each week up to 1 to 28 days (4 week study). It was also showed statistically significant value ($p<0.05$). *Sonaratia apetala* male group of rats cholesterol level become same as compared to the control group but female group of treated animal cholesterol level become increased till first 2 weeks of treatment.

Oral administration of the amount of dose 400mg/kg body weight of fruit (*Sonneratia apetala*) did not show any statistically significant changes during the estimation of Glucose level up to 1 to 28 days (4 week study).

In the case of HDL levels for *Sonneratia apetala* a significant value ($p<0.05$) was observed by oral administration of the amount of dose 400mg/kg body weight.
Sonneratia apetala were showed a very higher level of significance till 28 days of treatment of HDL as compared with the control groups of animals for either sex.

Oral administration of the amount of dose 400mg/kg body weight of *Sonneratia apetala* shows variable changes during the estimation of Creatinine in each week up to 1 to 28 days (4 week study) was observed a statistically significant value ($p<0.001$).
Sonneratia apetala decreased the level of Creatinine and it was continued for end of the treatment as compared with the control groups of animal.

Oral administration of the amount of dose 400mg/kg body weight of *Sonneratia apetala* fruit was showed statistically significant data ($p<0.05$) during the estimation of Alkaline phosphatase in each week up to 1 to 28 days (4 week study). *Sonneratia apetala* fruit was showed a lower Alkaline phosphatase level of both male and female groups of animal as compared with the control group till 28 days of treatment.

Oral administration of the amount of dose 400mg/kg body weight of *Sonneratia apetala* fruit showed significant value ($p<0.05$) during the estimation of SGOT in each week up to 1 to 28 days (4 week study). A higher SGPT level by the fruit of *Sonneratia apetala* as compared with the control group in both of male and female group of animal, but after 14 days of treatment next 7 days showd higher SGPT level by the fruit. Next and last 7 days of treatment it was seen that the male and the female group of rat SGPT level were remain same as compared with the control group of animal and statistically significant ($p<0.05$) data were observed (Figure 7a and 7b).

Oral administration of the amount of dose 400mg/kg body weight of *Sonneratia apetala* showed significant value ($p<0.05$) during the estimation of SGOT in each week up to 1 to 28 days (4 week study). *Sonaratia apetala* these mangrove fruits decreased the SGOT level during first two weeks as compared with the control group, but after 2 weeks the SGOT level was increased and statistically significant data were observed with increasing trend till end of the study period.

Oral administration of (400mg/kg) of *Sonneratia apetala* showed significantly ($p<0.05$) decrease in serum urea level as compared with the control group during 28 days (4 week) study.

Oral administration of (400mg/kg b.w) *Sonneratia apetala* showed moderately significant increase in serum ϒ glutamyl transferase level till 28 days (4 week) of the study as compared to the control group.

During chronic exposure to rat no histological anomalies were observed in the liver, kidney, heart and stomach tissue (Figure 11,12) when compared to control groups.

Gas chromatography was used to know the valuable components, which are presents in the fruit *Sonneratia apetala* of and few of the components are to be beneficial for human. According to the peak height, there were eight types of components found and showed in Figures.13,14 and Table 3.

Table 1.**Macro Nutrient content in each 100gms of** *Sonneratia apetala* **fruit**

Name of the fruit	Moisture (gm)	Total calorie (Kcal)	Carbohydrate (gm)	Protein (gm)	Fat (gm)
Sonneratia apetala	**59.23**	**194.5**	**25.38**	**3.29**	**8.24**

Table **2.Micro Nutrient content in each 100gms of** *Sonneratia apetala* **fruit**

Name of the fruit	Calcium (mg)	Iron (mg)	Sodium (mg)	Potassium (mg)	Zinc (mg)	Copper (mg)	Magnesium (mg)	Manganese (mg)	Chromium (mg)	Boron (mg)
S. apetala	**1.86**	**0.15**	**3.45**	**1.51**	**0.12**	**12.17**	**2866.36**	**35.11**	**0.724**	**54.68**

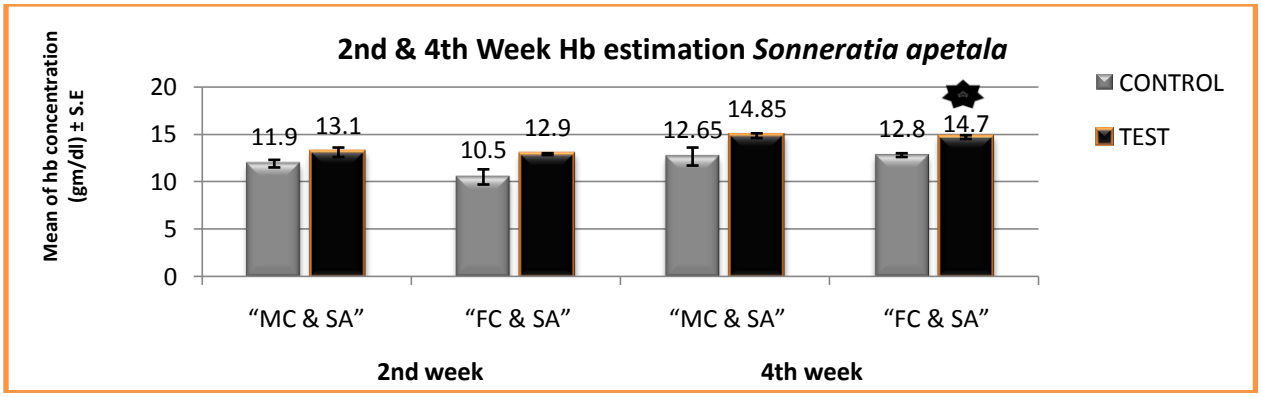


Fig.1. Effect on mean haemoglobin (gm/dl)in rats treated *Sonneratia apetala* (S.A.400mg/kg,) orally for 28 days values are expressed as mean ±S.E. (n=5). P vs Control, by student't' test, ✸= ($p<0.05$)

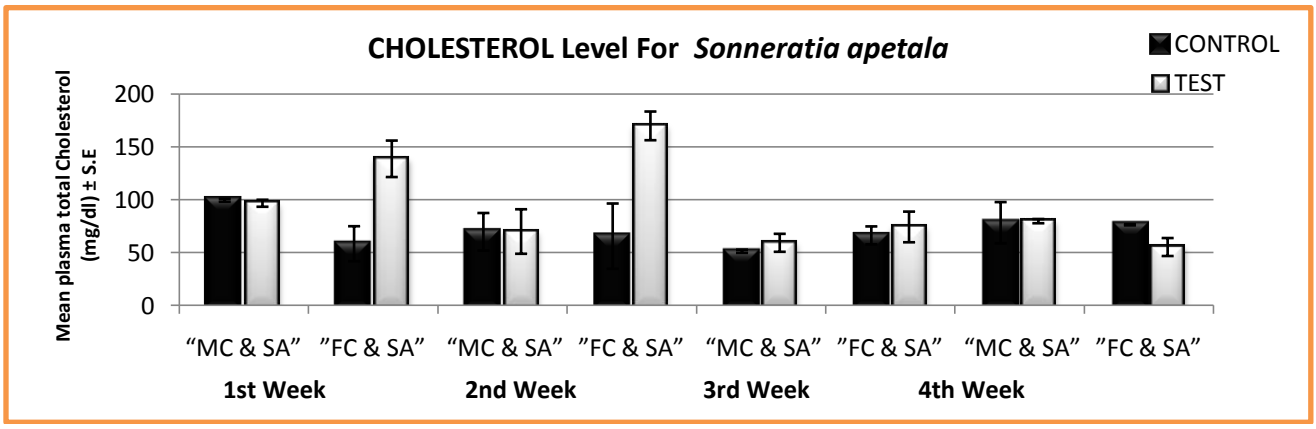


Fig. 2. Effect on mean Cholesterol level (mg/dl)in rats treated *Sonneratia apetala* (S.A.400mg/kg,) orally for 28 days values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, ✸=($p<0.05$)

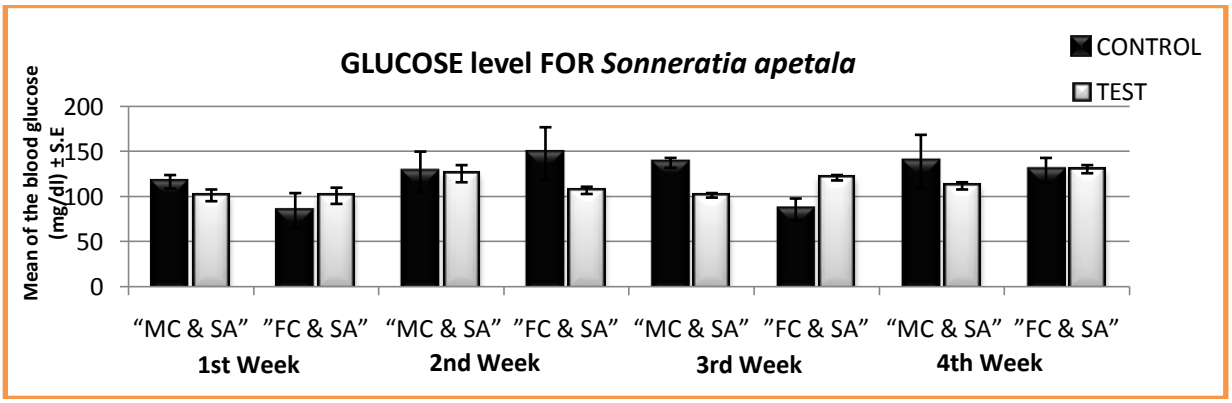


Fig. 3. Effect on mean Blood Glucose level (mg/dl)in rats treated *Sonneratia apetala* (S.A.400mg/kg,) orally for 28 days values are expressed as mean ±S.E. (n=5). P vs Control, by student't' test, ✸=($p<0.05$)

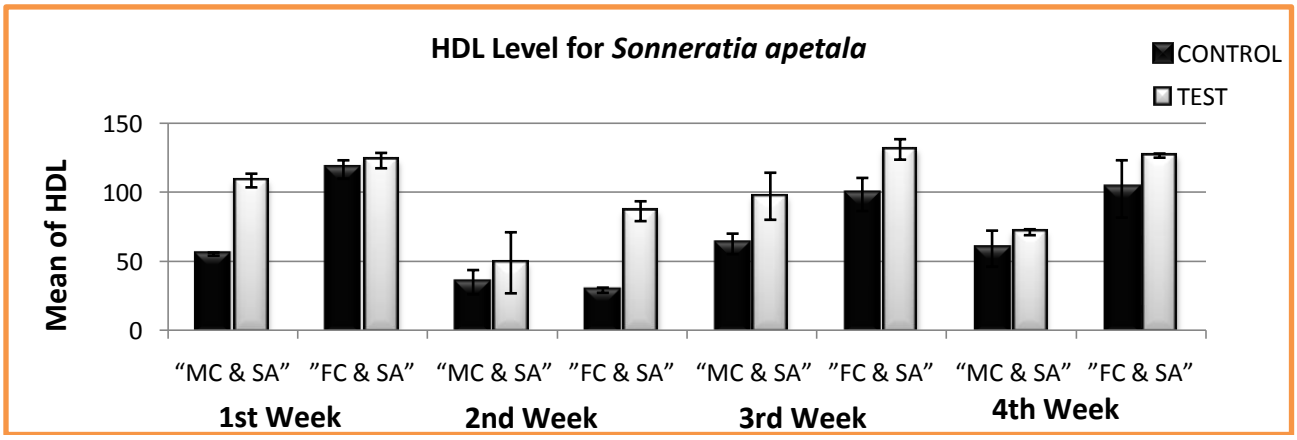


Fig. 4. Effect on mean HDL level (mg/dl)in rats treated *Sonneratia apetala* (400mg/kg,) orally for 28 days values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, ✸=($p<0.05$)

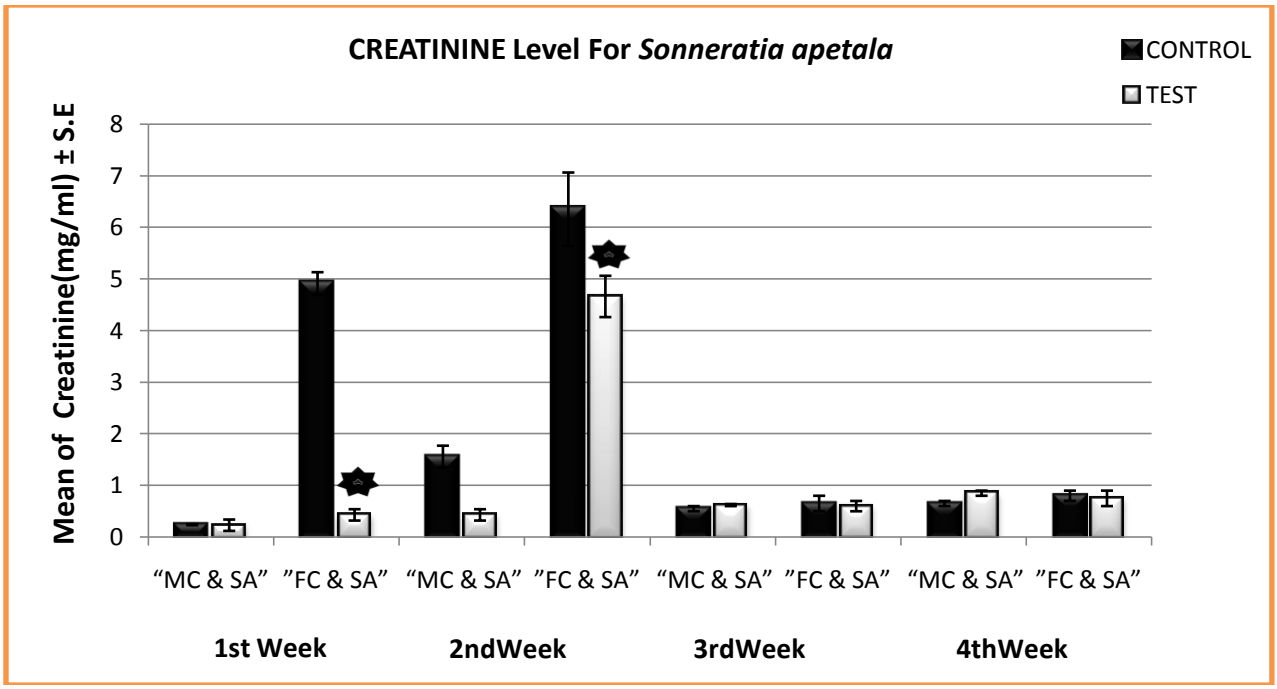


Fig. 5. Effect on mean plasma Creatinine level (mg/ml)in rats treated *Sonneratia apetala*, (400mg/kg,) orally for 28 days values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, ✸=($p<0.001$)

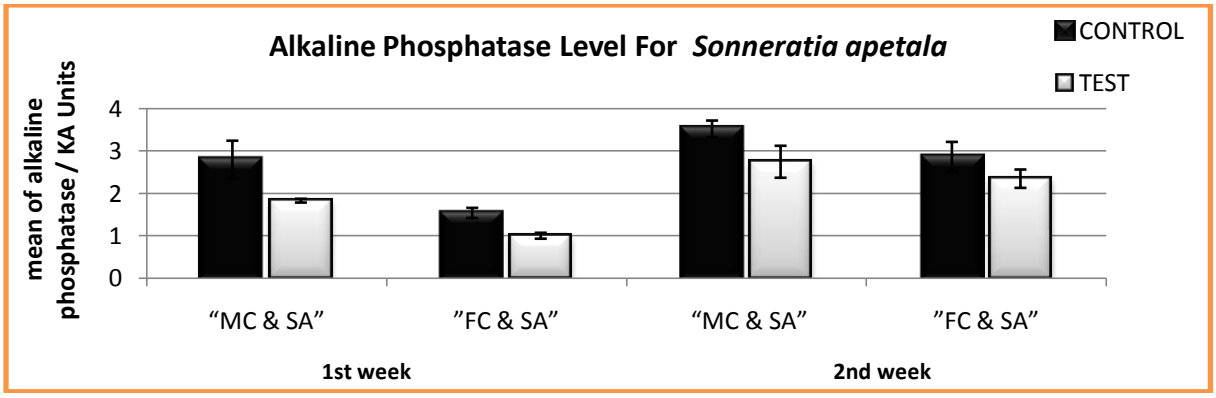


Fig. 6 (a). Effect on mean ALP level (mg/dl)in rats treated *Sonneratia apetala* (400mg/kg,) orally for first two weeks values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, ✸=($p<0.05$)

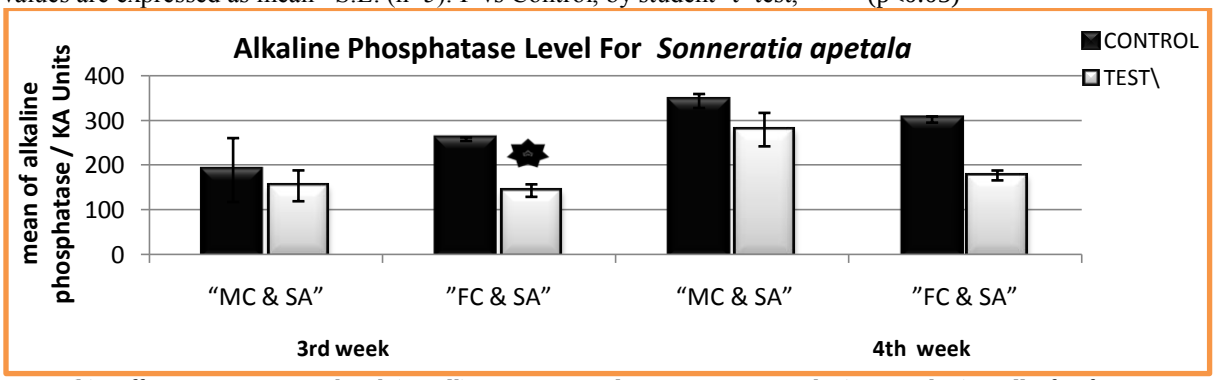


Fig. 6(b). Effect on mean ALP level (mg/dl)in rats treated Sonneratia apetala (400mg/kg,) orally for first two weeks values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, ✸=($p<0.001$)

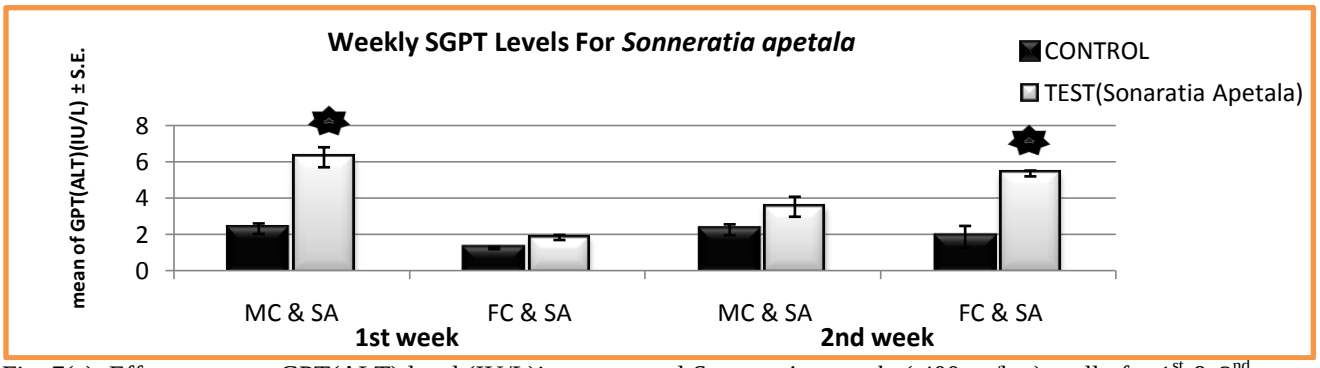


Fig. 7(a). Effect on mean GPT(ALT) level (IU/L)in rats treated Sonneratia apetala (400mg/kg,) orally for 1st & 2nd week, values are expressed as mean ±S.E. (n=5). P vs Control, by student't' test, = ($p<0.05$)

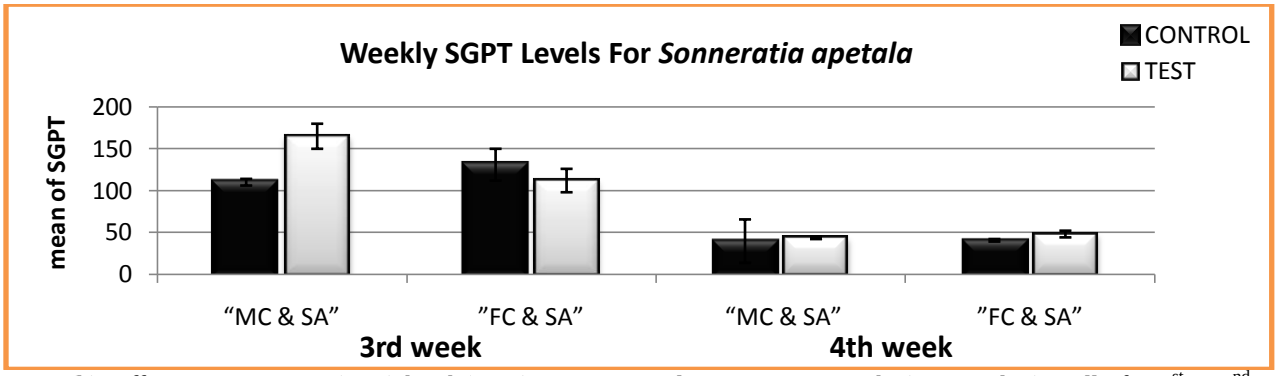


Fig. 7(b). Effect on mean GPT(ALT) level (IU/L)in rats treated Sonneratia apetala (400mg/kg,) orally for 1st & 2nd week, values are expressed as mean ±S.E. (n=5). P vs Control, by student't' test, = ($p<0.05$)

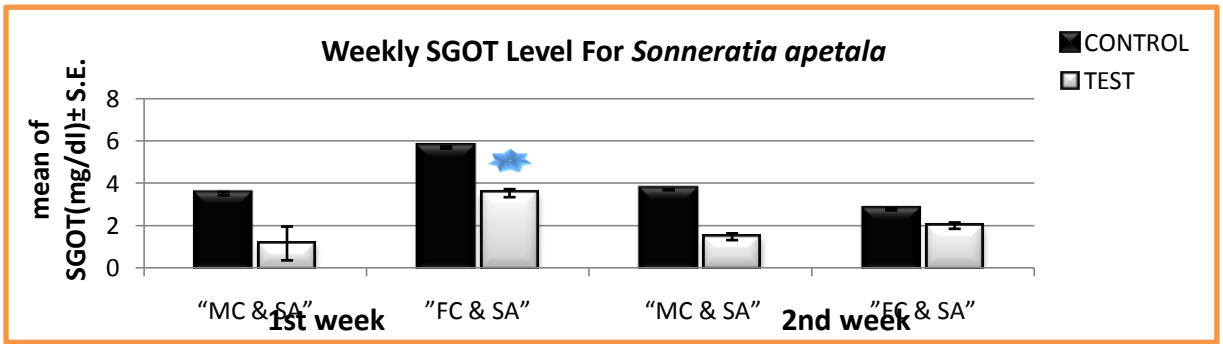


Fig.8(a). Effect on mean GOT (AST) (mg/dl)in rats treated Sonneratia apetala (400mg/kg,) orally for orally for 1st and 2nd week values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, =($p<0.05$)

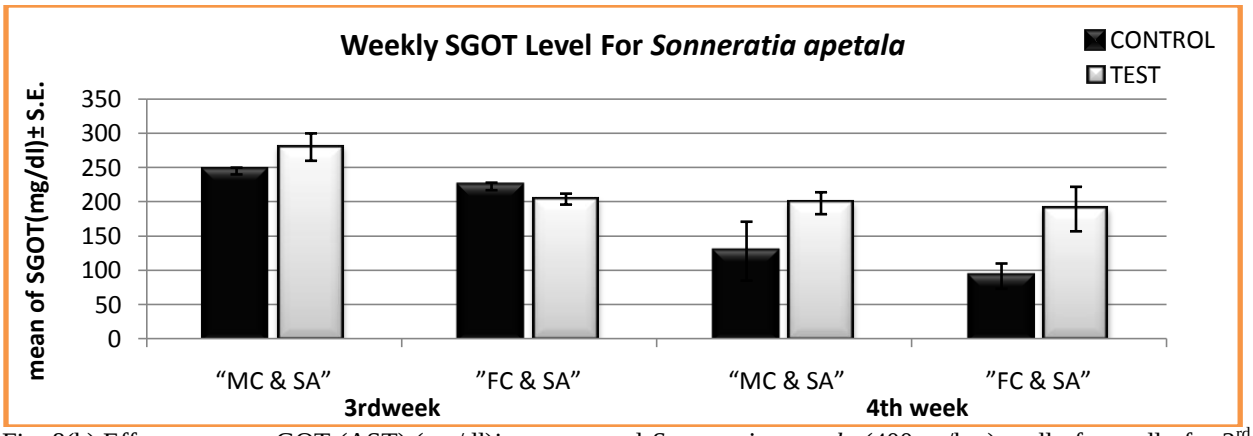


Fig. 8(b).Effect on mean GOT (AST) (mg/dl)in rats treated *Sonneratia apetala* (400mg/kg,) orally for orally for 3rd and 4th week values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, ✸=(p<0.05)

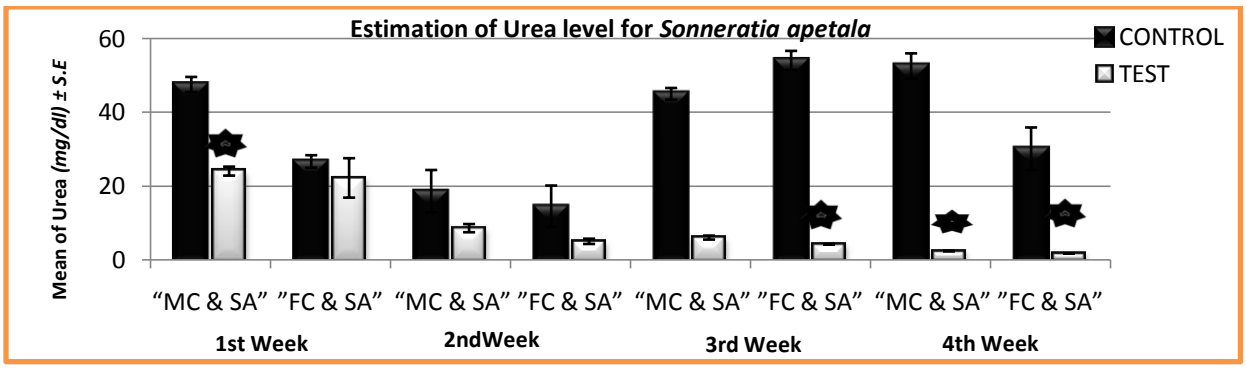


Fig. 9. Effect on mean Urea level (mg/dl)in rats treated *Sonneratia apetala* (400mg/kg,) orally for 28 days values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, ✸=(p<0.05)

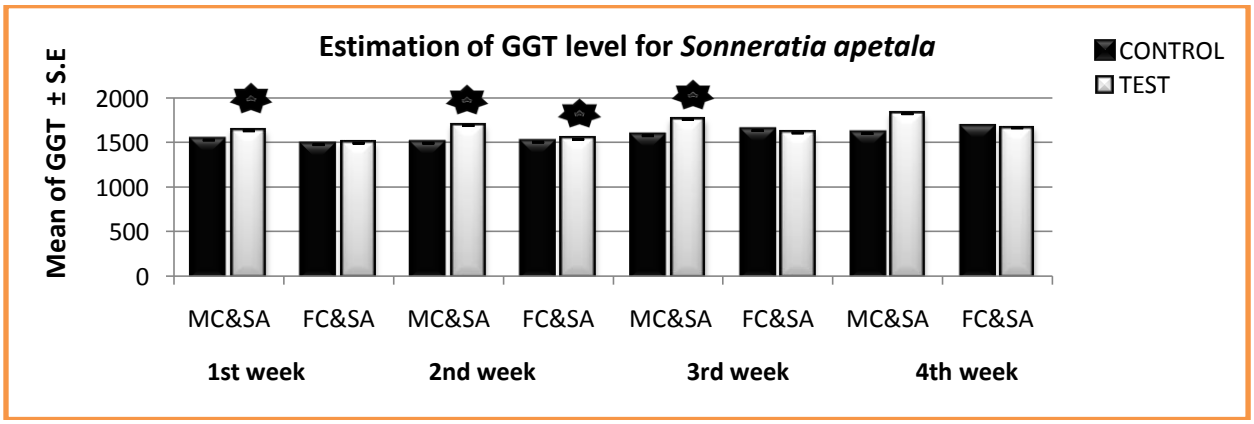
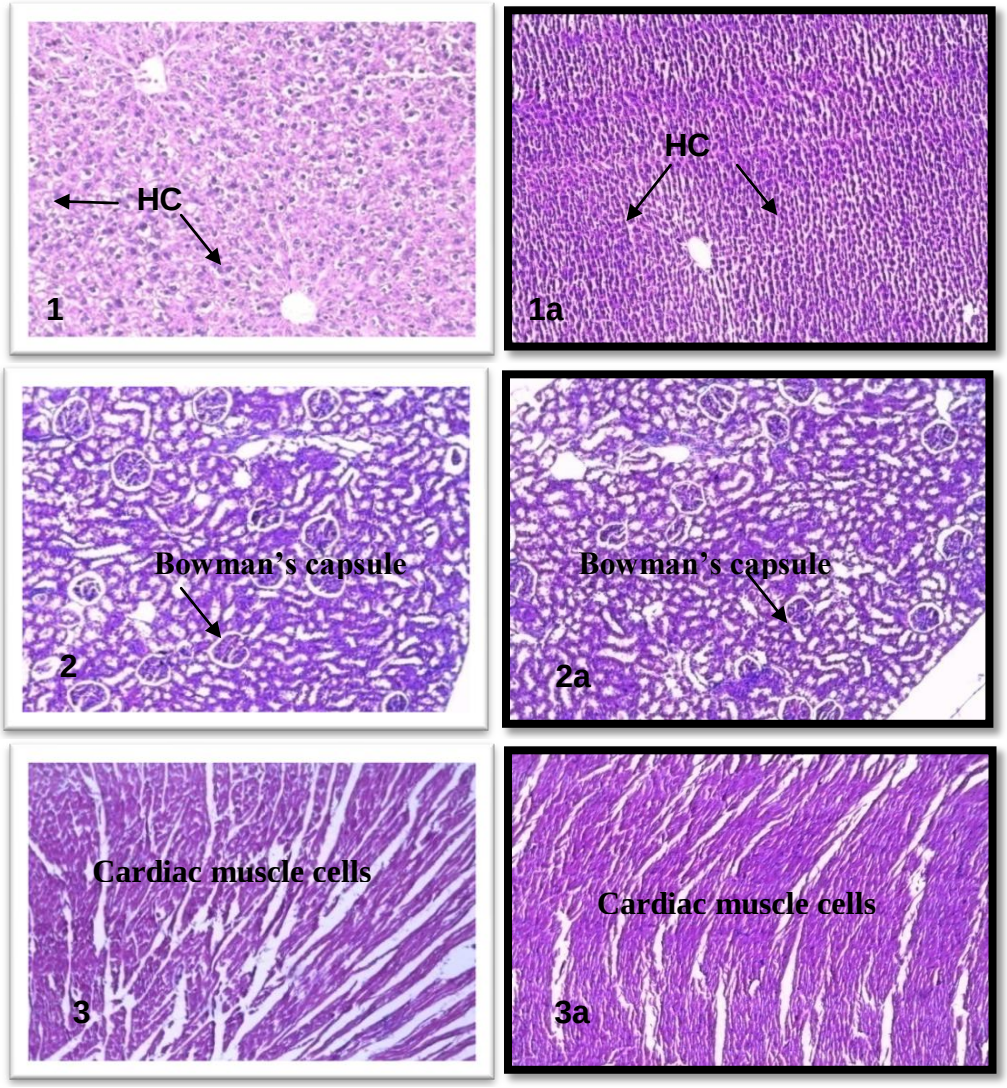


Fig. 10. Effect on mean serum ϒ glutamyl transferase level in rats treated *Sonneratia apetala* (400mg/kg,) orally for 28 days values are expressed as mean ±S.E. (n=5). P vs Control, by student 't' test, ✸=(p<0.001

HISTOPATHOLOGICAL RESULTS:

FEMALE RAT



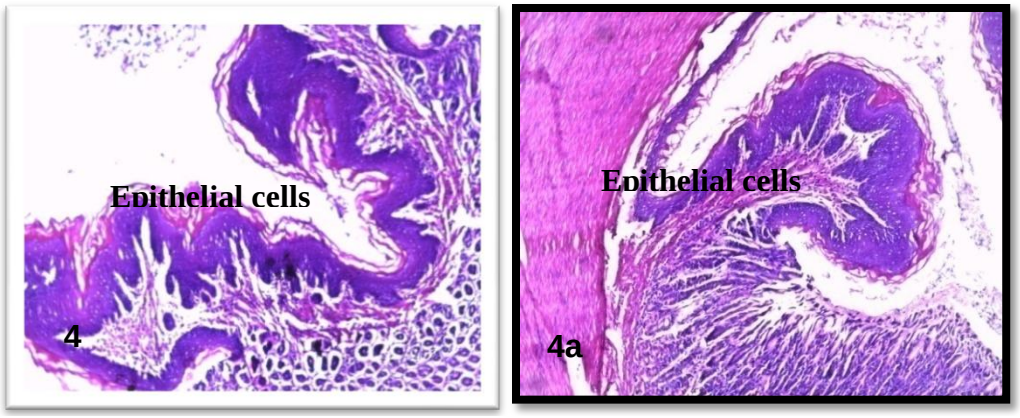
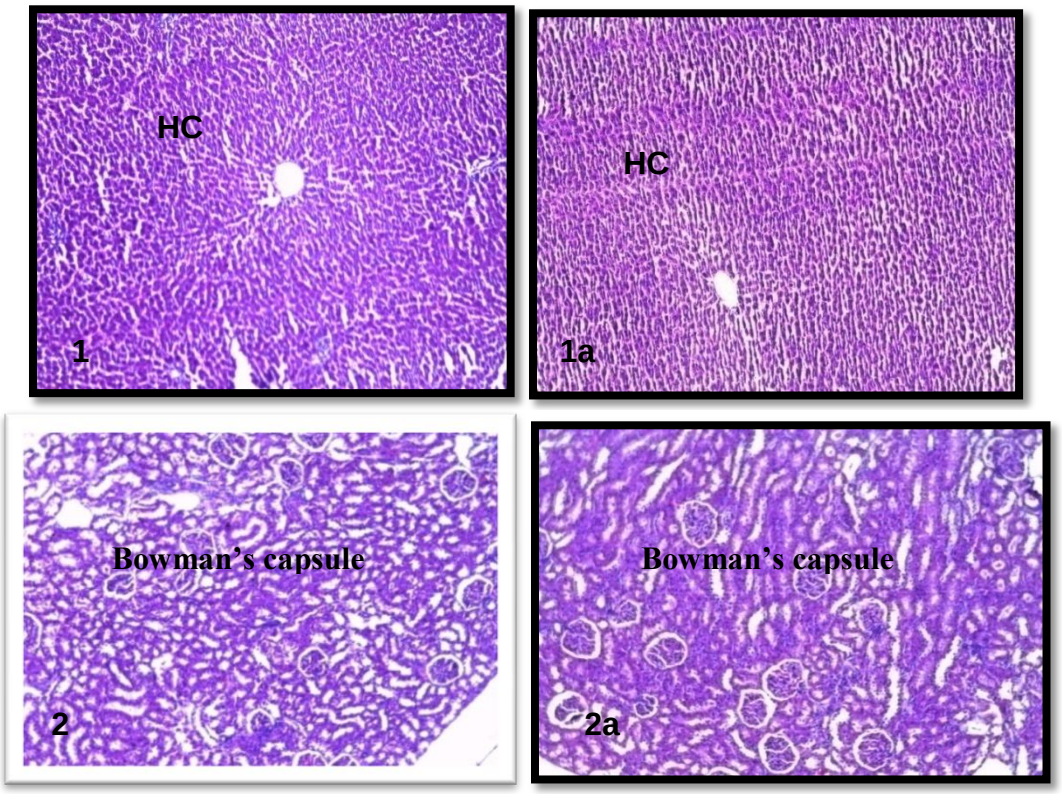


Figure11. Histological sections of female rat 1 & 1a Liver (HC = Hepatic cells), 2 & 2a Kidney (BC = Bowman's capsule), 3 & 3a Heart (CMC = Cardiac muscle cells) and 4 (normal view) & 4a (enlarged view) Stomach (EC = Epithelial cells).

1,2,3,4= female control rat, 1a,2a, 3a,4a=female treated rat

MALE RAT



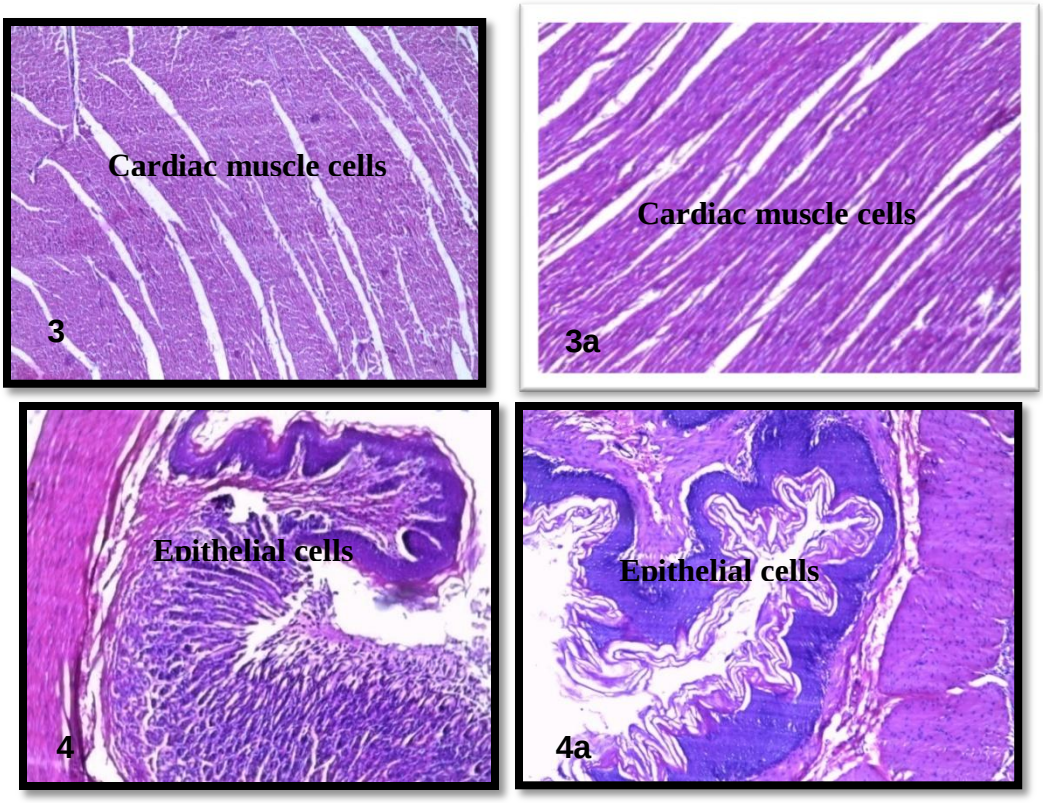


Figure12. Histological sections of male rat 1 & 1a Liver (HC = Hepatic cells), 2 & 2a Kidney (BC = Bowman's capsule), 3 & 3a Heart (CMC = Cardiac muscle cells) and 4 (normal view) & 4a (enlarged view) Stomach (EC = Epithelial cells)
1,2,3,4= male control rat, 1a,2a, 3a,4a= male treated rat

GC-MS ANALYSIS RESULTS:

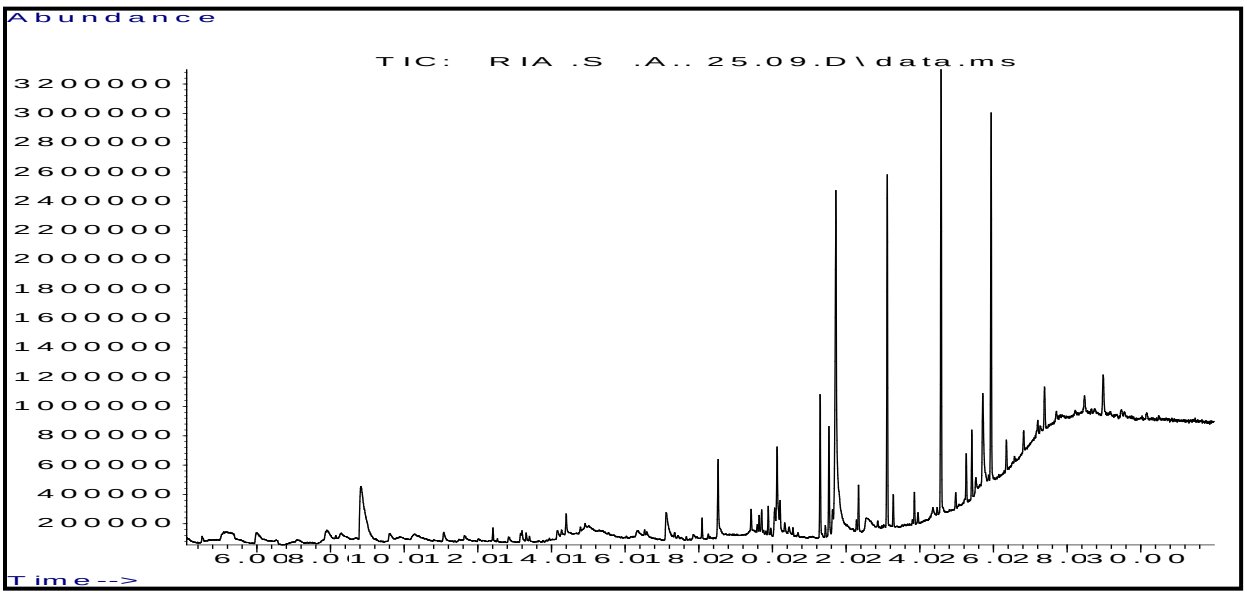


Fig. 13. GC/MS chromatogram (MRM) for 10µl methanol extract fruit sample of *Sonneratia apetala* using Agilent Inert Flow Path

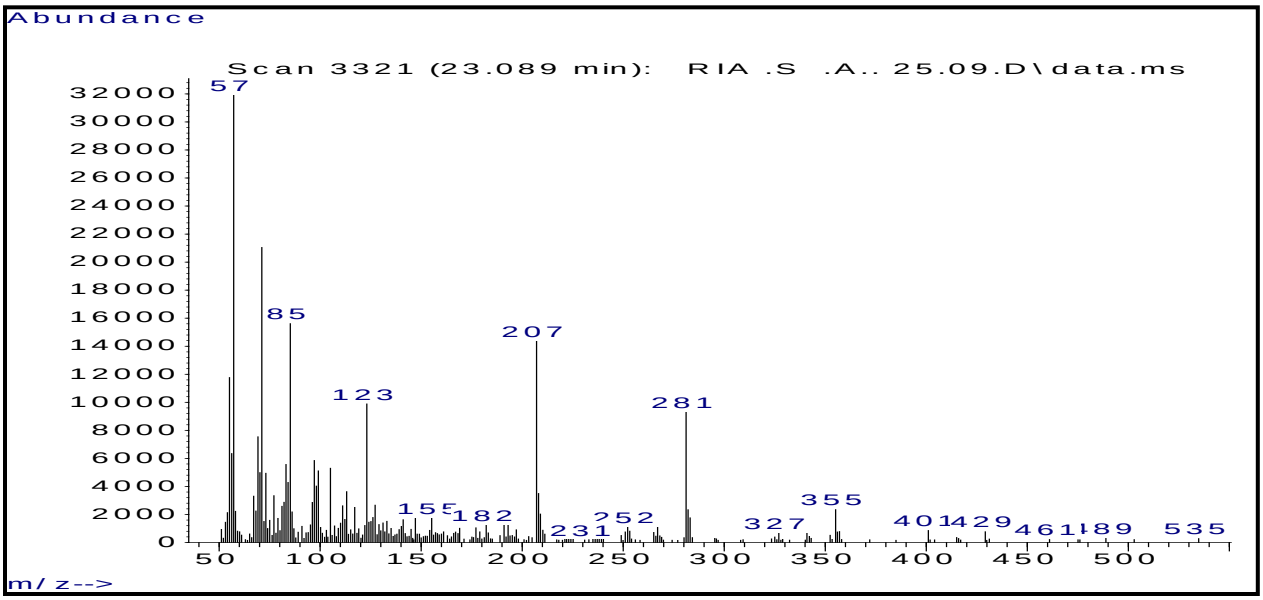


Fig. 14. GC/MS chromatogram (MRM) for 10µl methanol extract fruit sample of *Sonneratia apetala* using Agilent Inert Flow Path

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Table. 3. Analysis of compounds in the fruit of *Sonneratia apetala* through GC-MS

Sl.No	Retention Time	Peak Area (%)	Compound	Pharmacological Activity
1	17.127	1.62	Caffeine	Decreased fatigue, lower risk of diabetes, increased metabolic rate
2	18.088	0.37	Hexadecanoic acid, methyl ester	Antioxidant, hypocholesterolemic, nematicide, pesticide, anti androgenic flavor
3	18.523	2.24	Tetradecanoic acid	Antioxidant, cancer preventive, nematicide, lubricant, hypocholesterolemic
4	20.131	4.75	Oleic Acid	Anti cancer activity
5	20.337	0.29	Octadecanoic acid	5-alpha reductase inhibitor, hypocholesterolemic, suppository.
6	25.418	1.87	Squalene	Anticancer, antimicrobial, antioxidant, chemopreventive pesticide, anti-tumor, sunscreen

7	26.357	0.66	Vitamin E	Anti-ageing, analgesic, anti-diabatic, anti-inflammatory, antioxidant, anti-dermatitic, anti-leukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, anti-ulcerogenic, vasodilator
8	28.983	1.85	Beta-sitosterol	Anti-bacterial, anticancer (breast, cervix and lung), anti-inflammatory, antioxidant, hepatoprotective, hypocholesterolemic, hypoglycemic, hypolipidemic

4. Discussion

In the present results, it was observed in the fruit of *Sonneratia apetala* high content of total calorific value and fat content, which seems that it is good for the underweight patient and also for malnourished group. It contains high amount of magnesium which has a great role in our body e.g. act as a co- enzyme and helps to maintain different metabolic reactions, it is required to activate the enzymes involved in the oxidative phosphorylation of ADP to ATP and also for the return of ATP to cyclic AMP, which turn regulates parathormone secretion etc. Magnesium is essential for the functioning of heart-beat and maintaining the blood pressure and also helps to maintain the muscular contraction. Manganese content was also found in valuable level. Manganese is an essential trace nutrient in all forms of life. The classes of enzymes that have manganese cofactors are very broad, and include oxidoreductases, transferase, hydrolyses, lyases, isomerases, ligases, lectins, and integrins. The reverse transcriptases of many retroviruses (though not lentiviruses such as HIV) contain manganese. The best-known manganese-containing polypeptides may be arginase, the diphtheria toxin, and Mn-containing superoxide dismutase (Mn-SOD).

Sonneratia apetala fruit contain some amount of potassium, which helps to prevent hypokalemia, Addison's disease etc. It also contain good amount of copper which helps to formation of haemoglobin in the form of ceruloplasmin that plays an important role in the transport of iron in transferring for haemoglobin synthesis and absorption of iron in our body and also acts as a co-enzyme in various biochemical reactions and the other nutrients also present in little amount which also have various role in our body.

The fruit of *S. apetala* was non-toxic to the rodents during acute and chronic toxicity studies, since the acute dose did not show any mortality, toxic symptoms. All animals treated with the fruit extract survived beyond the 28-day observation period. Moreover, the body weights of the treated and control rats were similar. Therefore, it is safe to state that its oral LD50 exceeds 2000mg/kg. According to Loomis and Hayes (1996), substances with LD50 of between 5000 and 15,000 mg/kg are regarded as being non-toxic. In other way, Hor et al. (2012) have documented the safety assessment of methanol extract of red dragon fruit (Hylocereus polyrhizus) acute and subchronic toxicity studies revealed that the lethal oral dose of the fruit extract is more than 5000 mg/kg and the no-observed-adverse-effect level (NOAEL) of the extract for both male and female rats is considered to be 5000 mg/kg per day for 28 days.

Evaluation of oral toxicity via a repeated dose 28-day experiment has been advocated as a fundamental requirement for assessing safety and has been applied in several safety assessment studies (Hor et al., 2011; Mohamed et al., 2011; Rosidah et al., 2009). The repeated dose toxicity test conducted on rats for 28 days using the methanol extract of *S. apetala* fruit provided data regarding the cumulative toxic effects on target organs. In general, an increase or decrease in the body weight of an animal has been used as an indicator of an adverse effect of drugs and chemicals (Teo et al., 2002). Moreover, the relative organ weight and histological section indicates whether the organ has been exposed to injury or otherwise. Impaired organs often have abnormal atrophy (Wang et al., 2007). In the present result, the relative organ weights of all treated male and female rats did not show any changes when compared to

control groups. Furthermore, no related histopathological changes were observed. Gross and microscopic examinations at necropsy revealed no changes attributable to the administration of *S. apetala* fruit extract (Fig.11,12) when compared to control groups.

From medicinal point of view, it was documented that the antimicrobial activity of the mangrove plant extract of *Sonneratia apetala* on the various test microorganisms, including clinical multiple antibiotic resistant bacteria and phytopathogens. Antimicrobial activities of the extracts were determined by the well diffusion method. In vitro screening of *S. apetala* mangrove plant extracts showed species specific activity in inhibiting the growth of bacteria and fungi. Hexane, chloroform and methanol extract showed good activity against all the pathogens, where as only methanolic extracts were active against most of the pathogens. According to Bobbarala et al., 2009, few ingredients are common in the fruit of S. apetala with other fruits and plant extracts and might have exhibited the similar medicinal properties (Bellec et al., 2006; Jaafar et al., 2009; Zainoldin and Baba, 2009; Hor et al., 2012; Halder et al., 2013).

There are several studies have already been carried out on the nutritive values and presence of potent micronutrient in the fruits of different plant species (Bellec et al., 2006; Jaafar et al., 2009; Zainoldin and Baba, 2009; Hor et al., 2012; Halder et al., 2013;) as well as safety evaluation by acute and chronic toxicity studies after in-vivo exposure in animals (mice & rat) but less studies have been documented with fruits of mangrove plants (Halder et al., 2013; a; b). Recently, the fruit of *S. apetala* has increased in popularity by local people in and around Sundarban, not only because of its green coloration and economic value as a food product, but also because of its health benefits. The fruit of *S. caseolaris* has already been reported a potent anti-diabetic and anti-nephric as medicinal benefits (Halder et al., 2013). In Malaysia, the fruit of *Hylocereus polyrhizus* is rich in ascorbic acid (vitamin C) and lycopene. Lycopene is associated with a reduction in cancer risk and heart disease, and a lowering of blood pressure (Bellec et al., 2006; Jaafar et al., 2009; Zainoldin and Baba, 2009). Furthermore, H. polyrhizus and H. undatus seeds contain high levels of essential fatty acids, namely linoleic and linolenic acids (Ariffin et al., 2009).

Potent nutritional values and toxicological data is only available on the fruit of other species *Heritiera fomes*, locally called Sundari (Halder et al., 2013), but no one has attempted earlier regarding the assessment of nutritional contents and safety evaluation by acute and sub acute exposure to *Sonneratia apetala* fruit. The analysis of blood parameters is relevant to risk evaluation as changes in the hematological system have a higher predictive value for human toxicity when the data are translated from animal studies (Olson et al., 2000). In terms of hematological parameters, neither those of male nor female treated rats appeared to be toxicologically significant, being slightly higher or lower than those of the control group. These results indicate that *Sonneratia apetala* fruit extract does not interfere in the formation of erythrocytes and leukocytes nor does it cause microcytosis or macrocytosis in rats.There were no significant alterations in the hematological parameters.

Alkaline phosphatase (ALP), ALT and AST are important serum enzymes in the human liver, and monitoring their concentrations usually help to detect chronic liver diseases (Burger et al., 2005; Wang et al., 2007; Witthawaskul et al., 2003). ALP and ALT is a cytoplasmic enzyme that is found at a very high concentration in the liver, and an increase in the level of this specific enzyme suggests hepatocellular damage (Tennekoon et al., 1991). AST is also an enzyme that is present in high quantity in the cytoplasm and mitochondria in different tissues, including the liver, heart, skeletal muscle, kidney and brain (Evan, 2009). Tate and Meister, (1985) have reported GGT catalyzes the transfer of the gamma-glutamyl moiety of glutathione to an acceptor that may be an amino acid, a peptide or water (forming glutamate). GGT plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification (Siest et al., 1992). Other lines of evidence indicate that GGT can also exert a prooxidant role, with regulatory effects at various levels in cellular signal transduction and cellular pathophysiology (Dominici et al., 2005). Furthermore, it has been reported that an increase in the level of serum proteins is indicative of tissue injury (Solomon et al.,1993). Serum proteins such as albumin can act as a criterion for assessing the synthetic capacity of the liver, since early all are synthesized in hepatocytes. A reduction in serum proteins therefore tends to reflect chronic damage (Rasekh et al., 2008). In the present study, there were found statistically significant differences in ALP, AST, ALT and GGT levels between control and treated animals at any dose. Hence, *Sonneratia apetala* fruit extract does not cause hepatotoxicity. This was further confirmed by histopathological examination of the liver of treated and control rats, showing normal lesions. However, the total protein and globulin levels were significantly lower in male rats treated with 400mg/kg of the extract than in the control. In addition, the albumin/globulin ratio was significantly higher in the group of male rats treat with 400mg/kg of fruit extract due to the increase in plasma albumin at this dose. The changes were regarded as

toxicologically irrelevant because these adverse results did not appear in both sexes and were not dose-related. Additionally, these changes were within the normal laboratory range (Evan, 2009). Moreover, no concurrent changes in histopathology of the liver were observed.

Kidney function was evaluated by means of serum urea, creatinine, potassium, sodium and chloride. Serum creatinine, which results from the catabolism of creatine phosphate in skeletal muscle, increases when renal function is poor and decreases with the loss of skeletal muscle (Tortora and Derrickson, 2009). Hence, elevated blood creatinine is liable indicator of a negative impact on kidney function or impaired glomerular filtration (Evan, 2009; Hassan et al., 2007; Rhiouani et al., 2008). The creatinine level significantly decreased in a dose-dependent manner at 400mg/kg in male rats compared to the control, and thus had no negative impact on the kidneys. Serum urea increases as a result of toxic effects on the renal tubules, renal parenchyma, cardiac injury, and blockage of the urinary outflow tract by crystalluria, calculi, or
Other obstructions (Evan, 2009). In the present study, serum urea showed a non dose dependent decrease in female rats. Hence, the *Sonneratia apetala* fruit extract did not cause nephro toxicity. Other parameters like potassium, sodium, chloride and uric acid did not differ between the control and treatment group. This was further confirmed by the histopathological examination of kidneys from treated and control animals, showing normal architecture (data not shown). Subchronic or an administration of *Sonneratia apetala* fruit extract did not have any adverse effect on organ morphology.

The GC–MS analysis of the fruit extract revealed the presence of 8 compounds. These 8 compounds were caffeine, hexadecanoic acid, methyl ester, tetradecanoic acid, oleic Acid, octadecanoic acid, squalene, vitamin E and beta-Sitosterol, mostly fatty acids or fatty acid esters (Duke, 1998). Fatty acid methylesters in the range of C8 to C18 are considered non-toxic. Oral administration (by gavage) studies demonstrated that hexadecanoic acid, methylester (palmitic acid, methylester) is non-toxic to rats with a LD50 exceeding 2000 mg/kg (Pearson,1997). The acute toxicity of 2-furancarboxal-dehyde, 5-(hydroxymethyl)-(5-HMF) is very low. An acute oral LD50 of 3100mg/kg was observed in a study on rats (Ulbricht et al.,1984).There was no significant difference in terms of body weight increase, feed consumption or final weight between the rats administered 250mg5-HMF/kg/day and the control animals over a 40-week period. Histological examination of various organs found no differences between the two groups (Abraham et al., 2011). According to Fiege et al. (2000), the LD50 of phenol,2,4-bis (1,1-dimethylethyl) is 9200mg/kg.9,12-Octadecadienoicacid (Z,Z), also known as conjugated linoleic acid (CLA) has been demonstrated to have in vitro anticancer activity. In addition to its anti cancer effects, CLA was also reported to inhibit a therosclerotic lesions, to increase immune function, to decrease body fat, and to increase lean body mass in several animal models (Aydin, 2005). Rat toxicity data indicate that CLA intake as1.5% of the diet for 36weeks results in no histopathological damage to organs and no hematological abnormalities (Scimeca,1998). 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl-,(all-E),known as squalene, is a fatty compound that is found in vegetable oils including olive oil but is usually extracted from shark's liver, where it is found at high concentrations. Squalene has been reported to possess antioxidant properties. In vitro experimental evidence indicates that squalene is a highly effective oxygen-scavenging agent (Huang et al., 2009). In humans, squalene might potentiate the effect of some cholesterol lowering drugs. Experimental studies have shown that squalene can effectively inhibit the growth of tumor cells, partially preventing the development of chemically-induced cancer (Smith, 2000). No appreciable side effects nor notable toxic signs were observed in serum biochemical tests and hepatic functional tests in squalene-treated animals over a 3-month interval (Kamimura et al.,1989). Thus, the bioactive compounds in the fruit extract of *Sonneratia apetala* are not toxic.

5.Conclusion

In conclusion, *Sonneratia apetala* fruit extract is relatively safe when given orally. Oral administration of a methanol extract of *Sonneratia apetala* fruit at 400,800,1000,2000mg/kg per day to both male and female rats for 28days did not result in mortality and was not associated with adverse effects on the general condition, growth, organ weights, or hematological and biochemical parameters, nor did it's how abnormalities in macroscopic or histopathological findings. Thus, it's lethal or a dose for male and female rats exceeds 2000mg/kg. According to Faustman and Omenn (2001), the no-observed-adverse-effect level (NOAEL) is the highest exposure level at which there is no statistically or biologically significant increase in the frequency or severity of adverse effects between exposed and control groups. Some effects may be produced but they are not considered adverse or precursors to adverse effects. The NOAEL for rats in the present study was 400mg/kg per day. This study provides valuable preliminary data on the

toxicity profile of *Sonneratia apetala* fruit. Further investigations involving preclinical and clinical studies of the extract will be necessary to determine the safe dose before it is prescribed as a food and/or drug, in order to protect the population from the possible toxic effects of this fruit. The results also shown that the nutritive values of *Sonneratia apetala* also so high. However, local population has already been used as edible fruit from its traditional knowledge.

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