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Toxicological Evaluation of Alginic Acid, a Polysaccharide Isolated from *Turbinaria conoides* (J. Agardh) Kutzing on Wistar Albino Rats

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Abstract

Introduction: Alginic acid, a polysaccharide is one of the important phytochemical ingredients of brown algae, *Turbinaria conoides* (J. Agardh) Kutzing. *T. conoides* has been studied for various pharmacological activities, yet no toxicological information found in the literature therefore, preset study aimed at extraction and isolation of alginic acid and to assess the safety profile through acute and sub acute toxicity study in both male and female rats. **Materials and Methods**: Alginic acid was characterized through Fourier transform infrared spectroscopy, thermo gravimetric and differential scanning calorimetric analysis. In acute toxicity study, female rats received 2000 mg/kg of isolated product, at a single dose on oral administration. In subacute toxicity study, both male and female rats were given with 100, 200 and 400 mg/kg of the isolated product, orally, for a period of 28 days consecutively and behavioral changes, hematological, biochemical and histopathological investigations were verified. **Results and Discussion**: In acute toxicity study, no morbidity or mortality was reported with alginic acid treated animals at a dose of 2000 mg/kg. In sub-acute toxicity study, there were no treatment related abnormalities observed in hematological and biochemical parameters except, decreased red blood cell count (400 mg/kg); increased platelets (200 mg/kg) in female rats and increased levels of liver parameters (serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, gamma glutaryl transferase); lipid parameters (total cholesterol, triglycerides and blood glucose) in both male and female rats. Histopathology studies revealed a slight infiltration of cells and congestion in blood vessels in the liver; congestion of alveolar tissue in lungs with 400 mg/kg treated animals. No behavioural changes observed. **Conclusion**: From the obtained results it is indicated that the oral administration of alginic acid (active principle of *T. conoides*) did not produce any significant adverse effects in rats of both the sex. Hence, alginic acid was considered as safe to use for further therapeutic purpose.

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1. Introduction

Marine organisms are the potential source which provides novel biologically active substances for the development of pharmaceuticals[1]. Seaweeds are extensively used as a source of food, feed, fertilizer and medicine; in India it is used for commercial production of agar, agarose, carrageenan and seaweed liquid fertilizer[2]. Marine algae promote the formation of oxidising agents and secondary metabolites which are responsible for specific biological activity[3]. There are approximately 8000 species of marine macroalgae and of those, 1500 species are identified as brown algae[4].

Turbinaria conoides (J. Agardh) Kutzing, is one of the species of brown marine algae belongs to the family Sargassaseae, order Fucales[5]. It has been traditionally used for children's fever, as a fertilizer, pesticide, insect repellant and antibactericidal[6]. The therapeutic value of *T. conoides* was further supported by its biological and pharmacological applications reported in the literature such as anti-bacterial; anti-cancer; anti-histaminic, anti-cholinergic, anti-viral; antioxidant and anti-pyretic activities[5-9].

Alginic acid $(C_6H_8O_6)_n$ represents the main structural polysaccharide of brown algae which is a polymer of D-mannuronic acid and L-glucuronic acid[10]. Alginic acid has been investigated for the activities like; antioxidant, antibacterial and anti-viral[11-14] but there is no data reported on the safety profile of the alginic acid. Therefore, the aim of this study was to assess the *in vivo* toxicity study of alginic acid, active principle of *T. conoides* on Wistar albino rats.

2. Materials and Methods

2.1 Collection and Isolation of Alginic Acid

Turbinaria conoides (J. Agardh) Kutzing, marine brown algae, belongs to the family Sargassaceae, procured from the Mandapam coastal area, Rameshwaram (District), Tamil Nadu (State), India. The algae was identified and authenticated by Prof. P. Jayaraman, and a voucher specimen (PARC/2016/3294) was deposited at Plant Anatomy Research Centre, Institute of Herbal Science, Chennai, India.

Alginic acid was isolated according to Marudhupandi *et al.*[8] with slight modifications. The algae was shade dried, powdered through a blender and the powder (1 kg) was macerated with 70% ethanol (2L), at room temperature for 12-15 hours by subjecting to mechanical stirring at a speed of 4000-5000 rpm. The solution was centrifuged at 2000 rpm for 10-20 minutes; discard the supernatant, collect the residue, add approximately 500ml of distilled water and allowed to stir continuously at 60^0 C for 1h. Repeat the procedure twice, collect and pool both the extracts, allowed for centrifugation at 10,000 rpm for 10-20 minutes. Collect the supernatant and treat with 1% $Cacl_2$ and kept at 4^0 C for 12 hours to precipitate alginic acid which was then obtained by filtration of the solution through nylon membrane of 0.45 µm size. The yield of isolated product was found to be 6.2%.

2.2 Physicochemical properties of Isolated Alginic Acid

The pH of 1% isolated alginic acid of *T. conoides* was found to be 8.0±0.6 and the molecular weight was found to be 8×10^5 g mol^{-1}. The extracted alginic acid was easily soluble in distilled water and insoluble in ethanol, methanol, chloroform, acetone and DMSO.

2.3 Characterization of Isolated Alginic Acid

Alginic acid was characterized to identify the functional groups, thermal decomposition and melting temperature through various techniques like Fourier Transform-Infrared (FT-IR) spectroscopy, Thermo Gravimetric Analysis/Derivative Thermo Gravimetry (TGA/DTG) and Differential Scanning Calorimetry (DSC).

FT-IR spectroscopy of alginic acid was analyzed by using potassium bromide (KBr) pellet, which was prepared by mixing 1 mg of alginic acid with 100 mg of anhydrous KBr. The spectrum was recorded from 40 to 4000 cm^{-1} using FT-IR 8300 Spectrophotometer, Shimadzu, Kyoto, Japan[15].

TGA/DTG was carried out using TGA 4000, Perkin Elmer, Waltham, MA. The sample was heated in a silica crucible from 0 to 900^0 C at a heating rate of 10^0 C/min in a nitrogen atmosphere[16].

DSC measurements were carried out in Differential scanning calorimeter- MDSC 2920 Mettler-Toledo, USA. 2 to 3 mg of powder sample was packed in aluminium lid,

placed on the top of the pan and crimped. The crimped pan was placed in the sample cell along with an empty pan as a reference. Temperature was increased from 20^0 C till the powder sample undergoes decomposition at a rate of 10^0 C/min. The thermal cell was purged with dry nitrogen at 50ml/min[17].

2.4 Biological Assay

All the animals (Wistar albino rats, 200± 20g, 6 weeks old) used for the study were obtained from the Animal house facility, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences (Reg No: 220/PO/abc/2000/CPCSEA/29.05.2015). The rats were kept under standard laboratory conditions with 12 hr/ 12 hr light/dark cycles at around 22°±1^0 C and 45-55% of relative humidity in polypropylene cages with free access to standard pellet diet and water. The animals were acclimatized to the laboratory conditions for 5 days before the experiment. The study was carried out at Faculty of Pharmacy, MSRUAS, and Bangalore after obtaining Institutional Animals Ethical Committee clearance bearing the number, IAEC No: MSRFPH/PFP-60/2015.

The acute and subacute toxicity studies of alginic acid on both male and female rats were carried out based on the OECD (Organization for Economic Co-operation and Development) guidelines 423 and 407 respectively.

2.5 Preparation and Administration of Test Dose

200 mg of isolated alginic acid dissolved in 1 ml distilled water and administered to animals at the dose levels of 100 mg/kg, 200 mg/kg and 400 mg/kg in the dose volume of 10 ml/kg. The test suspension was freshly prepared once in two days for 28 days. The control animals were administered with vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

2.6 Acute Toxicity Study

According to the OECD guidelines 423 (OECD 2001), alginic acid was administered with 2000 mg/kg at a single oral dose while the other group served as control, received distilled water. After dosing, all the animals were observed daily for 14 days of treatment period for mortality or any delayed signs of toxicity. Observations include any changes in skin, fur, eyes, mucus membrane, also respiratory, circulatory, and autonomic and central nervous systems, abnormal behavior, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma were noted during the test period[18].

2.7 Subacute Toxicity Study

According to the OECD guidelines 407, both male (five rats in each group) and female rats (five rats in each group) were used for the study. The animals were randomly divided into eight groups, each group consisting of five animals[19].

All the animals were dosed once daily for a period of 28 days consecutively. The doses were selected as 1/5th, 1/10th and 1/20th from the limit test of acute toxicity study report. Body weight of each animal was recorded on day 0, 7, 14, 21 and 28. The quantity of food and water consumed by a group of five animals for both the sexes with different doses was recorded weekly and from this data, food and water consumed per animal per day was calculated for control and treatment groups. All the animals were observed twice daily for mortality during the course of study.

On day 29, all the animals were sacrificed by excess ketamine anaesthesia after an overnight fasting (8 h). The blood samples were collected into EDTA coated tubes for estimating haematological parameters like, RBC, Haemoglobin (Hb), Leukocyte, Platelets, Mean Corpuscular Volume (MCV), Neutrophil, Lymphocyte, Monocyte, Eosinophil, Basophil, Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC)[20]. Blood collected in non-heparinized tubes were then centrifuged at 2500 rpm for 15 minutes to obtain serum for the estimation of biochemical parameters like, Total bilirubin, direct bilurubin, indirect bilurubin, Alkaline Phos-Phatase (ALP), Serum Glutamine Ortho Transferase (SGOT), Serum Glutamine Pyruvate Transferase (SGPT), Total Protein (TP), Albumin, Globulin, Gamma glutaryl Transferase (GGT), Creatinine, Urea, Uric acid. Sodium, Potassium, Chloride ions, Total Cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density

Groups	Treatment	Sex	Dosing
Group-I	Vehicle control	Male	10 ml/kg
Group-II	Vehicle control	Female	10 ml/kg
Group-III	Low dose	Male	100mg/kg
Group-IV	Low dose	Female	100 mg/kg
Group-V	Medium dose	Male	200 mg/kg
Group-VI	Medium dose	Female	200 mg/kg
Group-VII	High dose	Male	400 mg/kg
Group-VIII	High dose	Female	400 mg/kg

Lipoprotein (VLDL), Triglycerides (TGs) and Blood Glucose[21].

Necropsy of all animals was carried out and the weights of the vital organs such as liver, kidney, lungs, spleen, brain, heart, stomach, intestine, bone, pancreas, testes and ovaries were separated, weighed each organ on electronic balance and relative organ body weight of both control and treated groups were determined and compared by using a formula (organ weight/body weight) X 100[21].

The collected vital organs were excised, washed with normal saline and kept in 10% buffered neutral formalin for 48 hours and then with bovine solution for 6 hrs. Paraffin sections were made at 5 mm thickness using a microtome processed in alcohol-xylene series and stained with alum heamatoxylin and eosin, mounted on the glass slides and they were subjected for histopathological examination[22].

3. Statistical Analysis

The results were expressed as mean ± Standard Error Mean (SEM). Findings such as body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by Dunnett's test using a computer software programme -INSTAT-V3 version. A probability level of $P < 0.001$ and $P<0.005$ were accepted statistically.

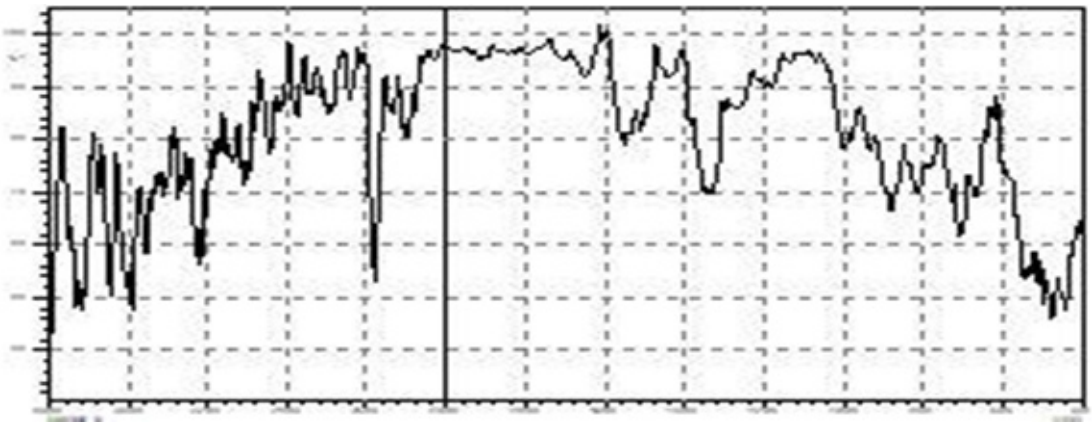


Figure 1. FT-IR spectrum of alginic acid, isolated from *T. conoides*.

4. Results

4.1 FT-IR Analysis

The FT-IR of isolated alginic acid of *T. conoides* is shown in the (Figure 1). The characteristic IR bands were observed in 3250, 1600 and 1050 cm^{-1} which shows the presence of OH stretching and C=O stretching of carboxylic acid group in glucuronic acid and mannuronic acid; C-O-C stretching which connects glucuronic acid to mannuronic acid respectively.

4.2 Thermo Gravimetric Analysis (TGA/DTG)

TGA (%) and DTG (%/^{0}C) of isolated alginic acid are shown in (Figure 2). From TGA graph, three degradation stages observed. The first zone of degradation started from 27.69 ^{0}C to 232.7 ^{0}C with a weight loss of 17.33% signifies loss of moisture. The second degradation started from 232.7 ^{0}C to 410.7 ^{0}C with a weight loss of 23.38% attribute to loss of adsorbed moisture and volatile substances. The third step of degradation from 410.7 ^{0}C to 895.93 ^{0}C with a weight loss of 26.94% signifies thermal and oxidative decomposition.

From DTG graph, three decomposition phases observed. First phase at 81.76 ^{0}C indicating the decomposition rate of 0.18%/^{0}C; second phase at 262.85 ^{0}C indicating the maximal decomposition rate of 0.28%/^{0}C; third phase at 670^{0}C with a decomposition rate of 0.064%/^{0}C.

4.3 Differential Scanning Calorimetry (DSC)

The DSC thermogram is shown in the (Figure 3). The DSC thermogram of sample is showing two endothermic transitions; first endothermic peak at 81.43^0 C with a peak area of 291.88 mJ and heat of enthalpy (DH) 115.83 Jg^{-1} indicated the loss of water and high decomposition of physical structure of the sample; second endothermic peak at 167.95 ^{0}C with a peak area of 1.18mJ and heat of enthalpy (DH) 0.47 Jg^{-1} indicated a slight decomposition of the sample.

4.4 Acute Toxicity Study

The acute toxicity study was carried out with the limit test dose of 2000 mg/kg. Acute toxicity study is required to categorize further range of doses in animal studies and also to explain the clinical signs which are likely to be induced by the test compound. Throughout the 14 days of observation period, there were no adverse effects and no morbidity or mortality was observed in 2000 mg/kg treated rats.

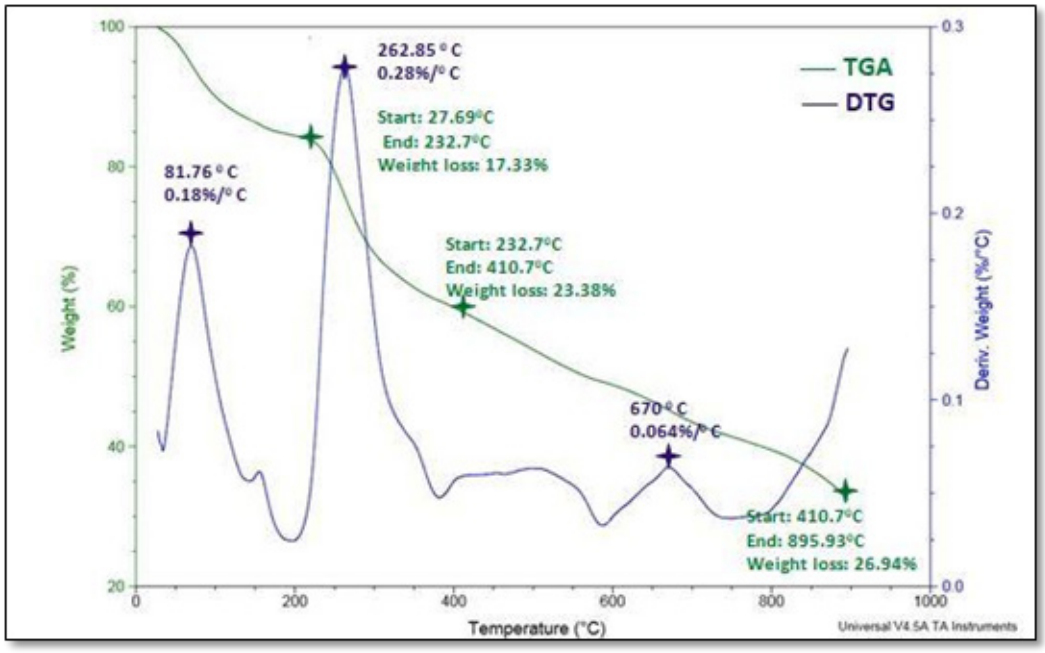


Figure 2. TGA graph of alginic acid, isolated from *T. conoides*.

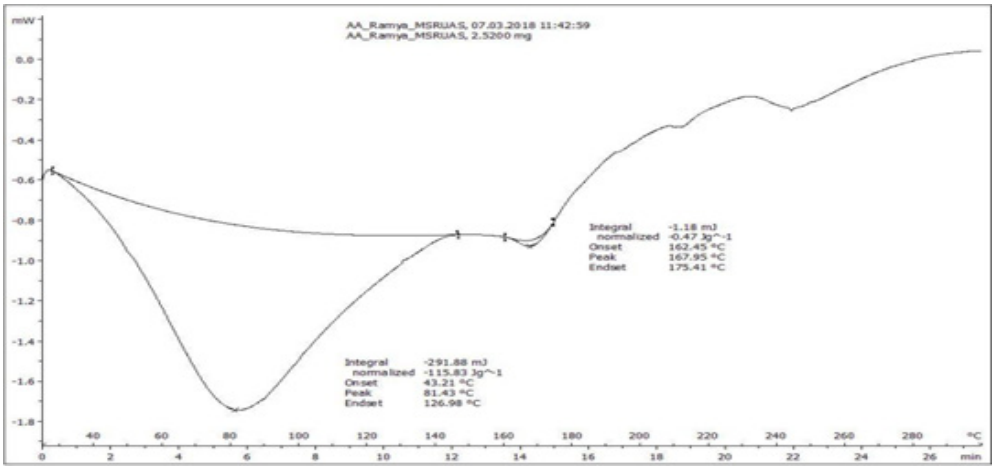


Figure 3. DSC thermogram of alginic acid, isolated from *T. conoides*.

4.5 Subacute Toxicity Study

4.5.1 Effect of Alginic Acid on the General Behavior of Rats

The oral administration of alginic acid (100, 200 and 400 mg/kg) caused no noticeable change in the general behavior of the rats. The body weight of all the animals of both the sex exhibited a structural development. There were no significant changes in the body weight compared to the control group throughout 28-days treatment period represented in Table 1.

Table 1. Effect of oral administration of alginic acid on body weight of Wistar albino rats treated for 28 days

DOSE (mg/kg/day) 1	DAYS (g/rat)				
	7	14	21	28	
Control					
Male	161±2.79	164±2.86	166.8±2.88	170.2±3.16	173±3.21
Female	154.8±2.08	157.4±2.54	160.6±2.75	163±2.88	166.4±2.99
Isolated Alginic Acid 100					
Male	134.2±4.12	148.4±5.99	161.0±9.85	177.8±15.36	187.6±17.86
Female	148.6±2.87	150.6±5.58	154.2±4.54	157.2±4.55	157.6±4.76
Isolated Alginic Acid 200					
Male	183.6±4.20	189.6±3.84	201.2±6.69	209.2±8.96	212±8.35
Female	171.6±2.66	176.2±2.61	179.6±3.41	174.6±9.07	186.4±4.36
Isolated Alginic Acid 400					
Male	193.2±5.34	204.0±4.86	212.4±7.72	216.4±9.26	223.6±10.09
Female	182.0±0.95	189.2±3.29	193.0±4.68	193.2±2.89	193.6±2.84

Values are expressed as mean ± SEM; (n=5); *P>0.05 Vs control.*

4.5.2 *Effect of Alginic acid on Hematological Parameters*

The results of hematological investigations (Table 2) conducted on day 29, revealed A significant decrease in Red Blood Cells (RBC) ($p<0.05$) was observed in female rats treated with alginic acid 400 mg/kg; a significant

Table 2. Effect of alginic acid on haematological parameters in male and female Wistar albino rats treated for 28 consecutive days.

Sex	Hematological parameters	Study group			
		Control	AA (100mg/kg)	AA (200mg/kg)	(AA 400mg/kg)
Male	RBC ($X10^5/mm^3$)	8.07±0.16	6.93±0.35	6.95±0.53	7.08±0.33
	HB (g/dL)	11.66±1.20	10.66±1.26	10.94±1.36	1050±1.18
	WBC ($X10^3/mm^3$)	9.14±0.81	9.92±0.70	8.76±0.54	9.6±0.68
	Platelets ($10^5/mm^3$)	6.46±0.55	6.02±0.38	6.17±0.41	6.19±0.46
	MCV (gl)	59.36±1.11	65.26±3.37	66.44±7.76	63.3±2.23
	Neutrophil (%)	67.0±1.70	72±2.74	71.2±2.85	66.8±4.32
	Lymphocyte (%)	18.8±1.83	20.4±3.42	20±2.03	20.2±2.38
	Monocyte (%)	3.0±0.55	2.4±0.51	2.4±0.51	2.4±0.68
	Eosinophil (%)	2.8±0.37	4±0.55	3.2±0.58	3.6±0.40
	Basophil (%)	00±00	00±00	00±00	00±00
	ESR (mm)	1±00	1±00	1±00	1±00
	PCV (%)	47.86±0.50	44.8±1.07	44.66±1.43	44.69±1.79
	MCHC (g/dl)	31.88±.40	32.23±0.76	33.38±0.70	31.29±1.49
Female	RBC ($X10^5/mm^3$)	7.87±0.13	6.89±0.34	7.22±0.32	6.83±0.29*
	HB (g/dL)	11.50±1.15	10.73±1.29	10.59±1.26	10.99±1.36
	WBC ($X10^3/mm^3$)	9.60±0.58	9.53±0.32	9.15±0.38	10.06±0.71
	Platelets ($10^5/mm^3$)	4.30±0.17	5.16±0.28	5.87±0.42*	5.25±0.40
	MCV (gl)	57.8±1.38	64.48±6.13	57.84±2.90	66.94±6.18
	Neutrophil (%)	63.8±3.48	65.2±3.61	71.4±3.27	71.6±4.16
	Lymphocyte (%)	19.2±1.86	22.8±2.22	19.8±1.59	16.8±1.46
	Monocyte (%)	3.0±0.55	2.4±0.51	2.6±0.40	2.4±0.51
	Eosinophil (%)	2.6±0.51	3.2±0.58	3.2±0.58	3±0.32
	Basophil (%)	00±00	00±00	00±00	00±00
	ESR (mm)	1±00	1±00	1±00	1±00
	PCV (%)	44.2±0.79	42.7±0.57	42.78±0.45	42.84±0.77
	MCHC (g/dl)	33.66±0.94	33.99±1.09	33.26±1.46	34.65±1.53

increase in platelets ($p<0.05$) was observed in female rats treated with alginic acid 200 mg/kg. Even though some significant changes observed in the hematological parameters like RBC and platelets, the values of other parameters of residual test groups were found to be non-significant when compared with control group. These observed significant changes are completely treatment related changes.

Data were expressed as mean± SEM. One way ANOVA followed by Dunnett's test (n=5). Isolated Alginic acid (AA), Red blood cells (RBC), hemoglobin (HB), white blood cells (WBC), Mean Corpuscular Volume (MCV), Erythrocyte sedimentation rate (ESR), packed cell volume (PCV), mean cell corpuscular hemoglobin concentration (MCHC). The values were considered to be significantly different when *$P<0.05$ compared to control group.

4.5.3 *Effect of Alginic acid on Liver Parameters*

In this study, we have observed a significant increase in liver parameters like SGOT ($p<0.01$) in both male

Table 3. Effect of alginic acid on biochemical parameters (Liver Function Tests) in male and female wistar albino rats treated for 28 consecutive days

Sex	Biochemical parameters	Study group			
		Control	AA (100mg/kg)	AA (200mg/kg)	AA (400mg/kg)
Male	Total Bilirubin (mg/dL)	0.91±0.12	0.72±0.10	0.79±0.10	0.76±0.09
	Bilirubin direct (mg/dL)	0.046±0.01	0.05±0.012	0.046±0.012	0.056±0.014
	Bilirubin indirect (mg/dL)	0.18±0.02	0.21±0.01	0.25±0.04	0.24±0.03
	ALP (U/L)	158.5±7.08	154.80±5.66	145.5±2.97	144.30±2.04
	SGOT (U/L)	60.56±2.35	166.62±24.24**	219.1±13.90**	221.28±8.45**
	SGPT (U/L)	40.6±2.00	60.02±4.83**	62.04±2.21**	77.48±3.75**
	Total Protein (g/dL)	7.6±0.15	7.34±0.35	7.3±0.36	7.36±0.47
	Albumin (g/dL)	3.84±0.31	3.7±0.44	3.9±0.36	3.62±0.22
	Globulin (g/dL)	3.24±0.27	3.42±0.22	3.56±0.28	3.5±0.31
	GGT (U/L)	7.2±0.34	18.06±1.55**	21.0±0.72**	19.44±1.09**
Female	Total Bilirubin (mg/dL)	0.95±0.15	0.79±0.13	0.79±0.13	0.83±0.12
	Bilirubin direct (mg/dL)	0.046±0.011	0.046±0.010	0.048±0.008	0.056±0.010
	Bilirubin indirect (mg/dL)	0.18±0.01	0.25±0.02	0.21±0.04	0.20±0.03
	ALP (U/L)	160.88±6.77	149.04±2.31	145.94±3.77	142.34±3.53
	SGOT (U/L)	57±2.06	198.4±6.55**	217.2±18.73**	227.2±6.18**
	SGPT (U/L)	42.44±3.71	64.76±4.53**	66.36±3.98**	73.42±1.99**
	Total Protein (g/dL)	7.52±0.22	7.56±0.23	7.18±0.36	7.54±0.40
	Albumin (g/dL)	3.62±0.19	3.86±0.34	3.82±0.31	3.96±0.39
	Globulin (g/dL)	3.2±0.19	3.44±0.29	3.52±0.31	3.34±0.22
	GGT (U/L)	7.18±0.33	20.56±0.53**	21.4±1.78**	21.82±1.93**

and female rats treated with 100, 200 and 400 mg/kg alginic acid; SGPT ($p<0.01$) in female rats treated with alginic acid 100, 200 and 400 mg/kg alginic acid; GGT ($p<0.01$) in both male and female rats treated with 100, 200 and 400 mg/kg alginic acid whereas total proteins, albumin and globulin levels were found non-significant in comparison with the control group (Table 3). These observed significant changes are completely treatment related changes.

Data were expressed as mean± SEM. One way ANOVA followed by Dunnett's test (n=5). Isolated Alginic acid (AA), Alkaline phosphatase (ALP), serum glutamine ortho transferase (SGOT), serum glutamine pyruvate transferase (SGPT), gamma glutaryl transferase (GGT). The values were considered to be significantly different when **P<0.01 compared to control group.

4.5.4 *Effect of Alginic Acid on Kidney Parameters*

Despite of significant differences, kidney parameters which include urea, creatinine, uric acid, sodium, potassium and chloride values remained within the acceptable limits thus considered as non-significant beside control group (Table 4).

Data were expressed as mean± SEM. One way ANOVA followed by Dunnett's test (n=5). Isolated Alginic acid (AA), Sodium ions (Na), potassium ions (K), chloride ions (Cl). The values were not statistically significant when compared to control group.

4.5.5 *Effect of Alginic Acid on Lipid Parameters*

In this study, there is a significant increase in lipid parameters like total cholesterol ($p<0.01$) in both the sex with 100, 200 and 400 mg/kg alginic acid treatment; High Density Lipoprotein (HDL) ($p<0.01$) in male rats treated with 200 mg/kg alginic acid, ($p<0.05$) in female rats treated with 200 mg/kg alginic acid and male rats treated with 400 mg/kg alginic acid; Low Density Lipoprotein (LDL) ($p<0.01$) in male rats treated with 100 mg/kg, female rats treated with 400 mg/kg alginic acid, ($p<0.05$) in rest of the groups of both the sex treated with alginic acid; Very Low Density Lipoprotein (VLDL) ($p<0.05$) in male rats treated with 200 mg/kg alginic acid; triglycerides ($p<0.01$) in all

Table 4. Effect of alginic acid on biochemical parameters (Renal Function Tests) in male and female wistar albino rats treated for 28 consecutive days

Sex	Biochemical parameters	Study group			
		Control	AA (100mg/kg)	AA (200mg/kg)	AA (400mg/kg)
Male	Urea (mg/dL)	35.46±1.75	40.42±4.93	42.24±5.70	41.42±4.94
	Creatinine (mg/dL)	0.68±0.07	0.70±0.06	0.73±0.04	0.69±0.06
	Uric acid (mg/dL)	2.04±0.19	2.26±0.13	2.22±0.20	2.32±0.17
	Na (m.mol)	134.4±1.72	134.8±1.46	134.8±1.24	133.6±4.96
	K (m.mol)	3.54±0.24	3.8±0.28	3.94±0.38	4.14±0.52
	Cl (m.mol)	96.44±2.44	96.06±2.59	96.92±2.79	98.34±3.07
Female	Urea (mg/dL)	36.78±1.86	40.08±6.55	40.98±5.24	41.22±6.51
	Creatinine (mg/dL)	0.71±0.07	0.74±0.06	0.70±0.07	0.68±0.08
	Uric acid (mg/dL)	2.06±0.17	2.30±0.24	2.3±0.24	2.32±0.21
	Na (m.mol)	134.4±1.72	134.2±1.77	136.2±2.82	134.4±1.50
	K (m.mol)	3.62±0.28	3.8±0.39	4.1±0.39	4.02±0.35
	Cl (m.mol)	96.44±2.74	96.82±2.14	97.88±2.61	97.58±3.15

Table 5. Effect of alginic acid on Lipid profile in male and female Wistar albino rats treated for 28 consecutive days

Sex	Lipid parameters	Study group			
		Control	AA (100mg/kg)	AA (200mg/kg)	AA (400mg/kg)
Male	Total cholesterol (mg/dL)	87.93±7.12	129.8±3.12**	141.8±3.77**	141.2±11.36**
	HDL (mg/dL)	35.0±2.92	43.4±3.57	51.0±4.53**	46.0±2.10*
	LDL (mg/dL)	29.8±2.91	60.0±0.55**	49.6±1.29*	48.8±1.02*
	VLDL (mg/dL)	20.4±1.50	24.4±1.12	27.0±3.19*	23.6±1.37
	Triglycerides (mg/dL)	52.2±4.77	123.8±2.46**	125.0±5.69**	124.6±4.95**
	Blood glucose (mg/dL)	85.46±3.41	118.6±2.84**	119.2±4.86**	123.0±1.73**
Female	Total cholesterol (mg/dL)	94.44±9.50	138.8±2.89**	132.6±5.71**	131.8±3.98**
	HDL (mg/dL)	34.8±2.33	45.8±4.50	47.0±4.27*	45.4±2.25
	LDL (mg/dL)	30.0±2.21	47.8±3.40*	44.8±4.63*	42.4±2.77**
	VLDL (mg/dL)	20.6±1.57	23.2±0.86	23.2±0.66	23.8±1.46
	Triglycerides (mg/dL)	52.0±4.67	125.6±2.99**	125.0±3.54**	129.2±6.73**
	Blood glucose (mg/dL)	84.2±3.93	119.2±3.25**	117.4±3.41**	118.8±2.35**

the groups of both the sex treated with alginic acid; blood glucose ($p<0.01$) in both male and female rats treated with 100, 200 and 400 mg/kg alginic acid when compared to control group (Table 5).

Data were expressed as mean± SEM. One way ANOVA followed by Dunnett's test (n=5). Isolated Alginic Acid (AA), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL). The values were considered to be significantly different when **P<0.01 and *P<0.05 compared to control group.

4.5.6 *Effect of Alginic Acid on Organ Weights*

Organ-body weight ratio may indicate organ swelling, atrophy, or hypertrophy[23]. In this study, the non-significant changes in the weights of all the vital organs suggest that these organs showed insignificant adverse effects or treatment-related signs of toxicity throughout the treatment period with the isolated product in the both sexes (Table 6).

Data were expressed as mean± SEM. One way ANOVA followed by Dunnett's test (n=5). Isolated Alginic acid (AA), Absolute organ weight (A), Relative organ weight (R). The values were not statistically significant when compared to control group.

4.5.7 *Effect of Alginic Acid on Histopathology of Vital Organs*

Histopathological investigations (Figures 4-7) revealed infiltrations in kidney and liver; congestion of alveolar tissue and septal edema in lungs; congestion of blood vessels in liver in alginic acid treated animals of both the sex with high (400 mg/kg) dose.

Table 6. Effect of alginic acid on relative organ weight in male and female wistar albino rats treated for 28 consecutive days

Isolated organs		Control	AA (100 mg/kg)	AA (200 mg/kg)	AA (400 mg/kg)
Brain (g)	A	1.42±0.07	1.71±0.11	1.72±0.03	1.69±0.12
	R	0.65±0.13	0.62±0.08	0.8±0.14	0.74±0.08
Lungs (g)	A	1.13±0.06	1.38±0.12	1.30±0.17	1.34±0.10
	R	0.6±0.26	0.72±0.05	0.75±0.24	0.65±0.31
Heart (g)	A	0.95±0.13	0.75±0.06	0.70±0.06	0.73±0.03
	R	0.55±0.28	0.4±0.08	0.42±0.12	0.35±0.84
Stomach (g)	A	1.25±0.08	1.26±0.09	1.40±0.16	1.28±0.06
	R	0.6±0.05	0.65±0.14	0.72±0.48	0.7±0.03
Liver (g)	A	8.00±0.36	7.14±0.41	6.83±0.34	6.91±0.38
	R	3.5±0.20	3.5±0.62	3.2±0.52	3.5±0.32
Spleen (g)	A	0.7±0.07	0.76±0.08	0.79±0.09	0.81±0.10
	R	0.3±0.05	0.35±0.09	0.35±0.02	0.38±0.06
Pancreas (g)	A	0.89±0.02	0.85±0.07	0.90±0.05	0.91±0.03
	R	0.4±0.04	0.45±0.02	0.42±0.05	0.4±0.07
Kidney (g)	A	0.76±0.06	0.69±0.04	0.63±0.02	0.70±0.02
	R	0.4±0.13	0.52±0.24	0.35±0.18	0.45±0.09
Ovary (g)	A	0.05±0.002	0.04±0.002	0.04±0.002	0.04±0.002
	R	0.026±0.001	0.024±0.002	0.025±0.001	0.025±0.001
Testes (g)	A	1.28±0.10	1.19±0.11	1.10±0.07	1.21±0.18
	R	0.65±0.15	0.68±0.09	0.6±0.12	0.66±0.10

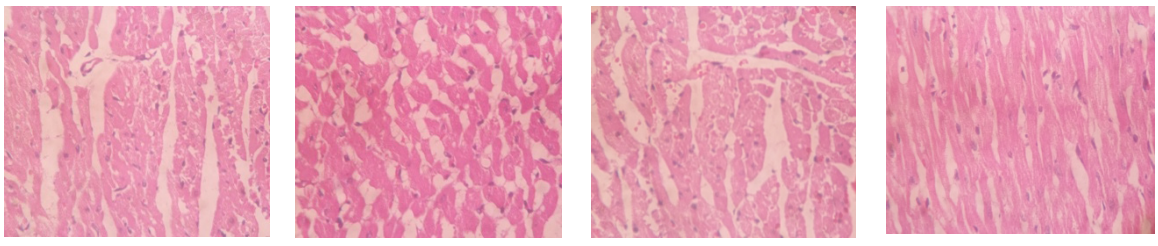


Figure 4. Histopathology (100X) of heart section from sub-acute toxicity study - **(a)** and **(b)** are male and female rats of experimental control, **(c)** and **(d)** are male and female rats treated with 400 mg/kg alginic acid- showing normal cardiac muscle bundles with myocytes.

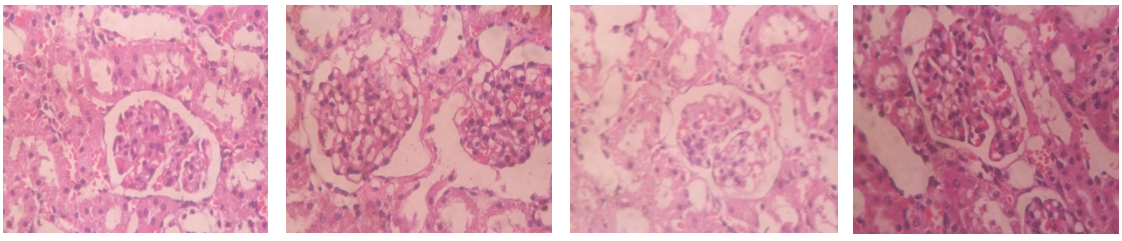


Figure 5. Histopathology (100X) of kidney section from sub-acute toxicity study - **(a)** and **(b)** are male and female rats of experimental control showing normal renal cells with glomerulus; **(c)** and **(d)** are male and female rats treated with 400 mg/kg showing partial epithelial cell damage and infiltration (arrow) of renal cells.

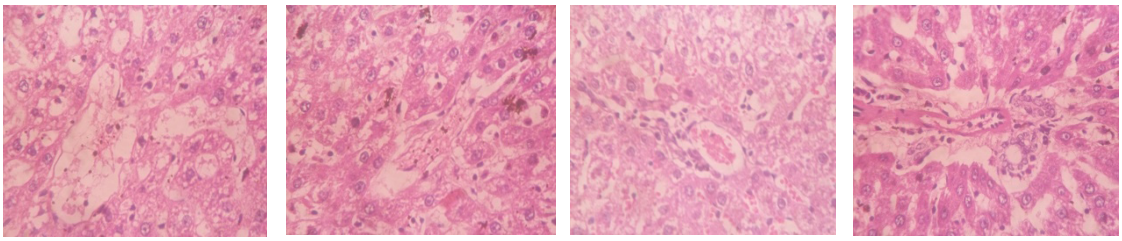


Figure 6. Histopathology (100X) of liver section from sub-acute toxicity study – **(a)** and **(b)** are male and female rats of experimental control showing normal hepatocytes with sinsoid capillaries; (c) and (d) are male and female rats treated with 400 mg/kg revealing inflammatory infiltrate and fat deposits (arrow) in hepatocyte cytosol.

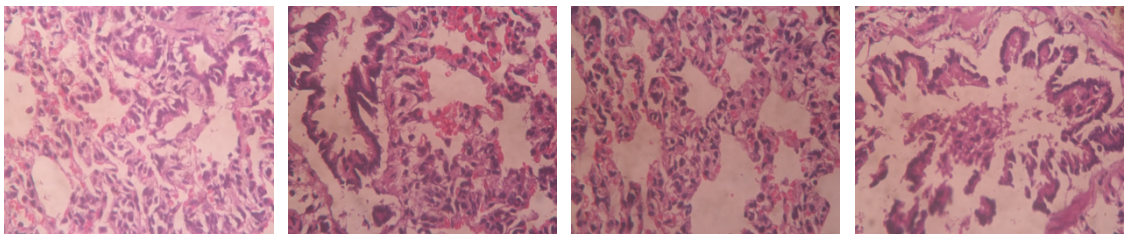


Figure 7. Histopathology (100X) of lungs section from sub-acute toxicity study – **(a)** and **(b)** are male and female rats of experimental control showing normal hepatocytes with sinsoids capillaries; **(c)** and **(d)** are male and female rats treated with 400 mg/kg showing inflammatory nodules with fibrous thickening of alveolar septae with interalveolar spaces (arrow).

5. Discussion

Natural products and their formulations have been the basis for the treatment of various diseases as they found to be safe to use due their negligible side effects[24]. Marine algae are the abundant source of natural bioactive compounds with various pharmacological effects as they contain potential nutritional values[25]. A marine alga has a

key role in the drug development area in pharmaceutical industry[26]. Nutrition-based products are being developed from algae like, *Spirulina,* which has high demand in India[27]. Use of marine algae was predominant in the field of food and drugs which are mainly used in dairy and food industries, pharmaceuticals and cosmetics[28].

In pharmacological screening of natural products, extraction, isolation and evaluation of toxicity studies are the preliminary steps involved. Though many beneficial effects of *T. conoides* and its isolated product alginic acid were reported, lack of toxicological profile suggested the present study to evaluate the acute and sub-acute toxicity study of alginic acid isolated from *T. conoides*.

The acute toxicity study is conducted to check toxic effects of any drug to an organism at a single dose or short –term exposure[29]. It is also an important effective parameter for calculating the therapeutic index of drugs and chemicals. There was no mortality reported throughout the acute toxicity study at a dose of 2000 mg/kg.

The sub-acute study or repeated dose toxicity study provides information on toxic effects, effects on animal physiology, hematology, biochemical parameters and histopathology[19]. In this course of study all the animals were active and responded to stimuli. No deaths and no clinical signs of toxicity were noticed.

The hematological parameters are capable of determining the blood relating functions of any substance. The hemopoietic system is one of the important systems of the body where the physiological and pathological changes of any toxic substances first attack in both humans and animals. Negligible or no significant changes ($p<0.05$) were noticed in RBC, WBC, platelet count, neutrophils, lymphocytes and monocytes. From this we can suggest that the isolated product neither affect erythropoiesis of RBC nor defensive mechanism of WBC[30].

The liver and the kidneys are target organs for toxic chemicals due to their essential functions in detoxification and excretion processes in the body. Thus, they are considered highly useful in toxicity studies because of their sensitivity to toxic substances and their potential to predict toxicity[31]. In our investigation there was a significant increase ($p<0.01$) in the liver parameters in both the sex of alginic acid treated animals compared to control group. Liver is the target organ for metabolism and also major site for cholesterol synthesis. Many studies have confirmed that the increased levels of liver enzymes, transaminases like SGOT and SGPT are not directly linked for liver injury but may be due to inflammation, cellular leakage and damage to cell membrane of liver cells[32].

Alteration in the concentration of major lipids such as total cholesterol, HDL, LDL, and triglycerides can give useful information on lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases[33]. In this study there was a significant increase ($p<0.01$) in TC, LDL, TGs and blood glucose were noticed. Beside these other lipid parameters found to be within the limit. Therefore, the increased levels of liver parameters and lipid parameters may be due to higher dose for prolonged period.

In histological investigation of liver shows fat deposits in hepatocytes with mild infitrate and lungs shows thickening of alveolar tissue with inflammation and kidney shows partial epithelial cell damage in renal tissue in high dose treated animals of both the sex. Pathological features of heart looks normal in architecture.

The present study was the first one to evaluate the acute and subacute toxicity study of alginic acid isolated from the marine brown algae, *T. conoides.* We aimed to assess the possible adverse effects on hematological, biochemical parameters and histopathology of major vital organs such as heart, kidney, liver and lungs. The results obtained may suggest alginic acid as drug of choice to carry out any pharmacological activities as it does not show any adverse effects at organ level. Further chronic toxicity studies may yield different results which found useful for further studies.

6. Conclusion

From the acute toxicity study results, 2000 mg/kg was found to be the safe dose. In sub-acute toxicity study, no noticeable signs of toxicity such as weakness, tremors, refusal to food, and loss of weight, hair-loss, coma and death were seen in any of the animals. Based on these findings, no adverse effects were observed up to 400 mg/kg of alginic acid treated animals via oral route for a period of consecutive 28 days treatment. Finally, it was concluded that the use of alginic acid was safe to use in further pharmacological studies as it did not show any adverse effects. Therefore, could be well considered for

further investigation for its medicinal and therapeutic efficacy.

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