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SCREENING OF TERATOGENICITY OF THE MARINE ALGA *SYMPHYOCLADIA LATIUSCULA*

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Keywords:

Polyphenols, *Symphocladia latiuscula*, Sodium Valproic acid, Teratogenicity, Gross abnormalities

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ABSTRACT: Objective: To explore the Teratogenicity of isolated Polyphenols of *Symphyocladia latiuscula* (PSL) on pregnant female rats. **Experimental Design:** The marine alga *Symphyocladia latiuscula* were the preparation of methanolic extract followed by isolation of Polyphenols. The Teratogenic study was performed according to the OECD guidelines, no 415. And the test drug; PSL 100, 200 and 1000 mg/kg and positive control Valproic acid (VPA); 1000 mg/kg was administered from 6th to 17th day of gestation during the period of fetal organogenesis. All females were killed on the 20th day of pregnancy, and their uterus was dissected to examine the Gross Abnormalities. **Results and Conclusion:** VPA significantly reduced all body measurements in terms of growth retardation, body weight, height, lower and upper limb, VPA showed mortality of 2 pups in the concentration of 1000 mg/kg/bw i.v whereas in PSL no significant differences were noted concerning the positive control group. The highest difference was shown especially PSL 1000 mg/kg/bw fetal morphological abnormalities; regarding the weight and length of the fetuses, there was a reduction, visceral abnormalities were revealed in the high dose of PSL 1000 mg/kg and positive control VPA 1000 mg/kg groups compared to the normal control group. These alterations were dose-dependent and found that the use of PSL was safe at 100 and 200 mg/kg dose, did not show toxic during the gestational period, and any anatomical and structural abnormalities in all the doses studied especially PSL 100 and 200 mg/kg.

INTRODUCTION: Medicinal plants have important contributions in the healthcare system, and herbal medicine represents a long history of human interactions with the environment. The Indian subcontinent is well known for its diversity of medicinal plants, forest products, and the age-old healthcare traditions; there is an urgent need to establish these traditional values in both the national and international perspectives realizing the ongoing developmental trends in traditional knowledge and side effects caused by these medicinal plants.

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Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Toxicology "is the study of the detection, occurrence, properties, effects, and regulation of toxic substances," although more descriptive, do not resolve the difficulties. Toxicity itself can rarely, if ever, as a single molecular event but is, rather, a cascade of events starting with exposure, proceeding through distribution and metabolism, and ending with interaction with cellular macromolecules (especially DNA or protein) and the expression of a toxic endpoint.

According to the extent of exposure (dose), the number of cells affected increases to the point where the whole organ or tissue is changed, biochemically, or morphologically. The fact that the molecule is not metabolized to a less toxic form

but is regenerated, leaving it free to repeat the cycle many times, leads to depletion of NADPH with significant effects on cellular homeostasis [1]. Toxicological assessment of any herbal medicine is to identify adverse effects and to determine limits of exposure level at which such effects occur. Two important factors which are taken into consideration in evaluating the safety of any herbal drug are the nature and significance of the adverse effect and also, the exposure level where the effect is observed.

Marine algae compounds have conducted to the global search for novel medicine agents and have a significant number of novel metabolites with potent pharmacological properties have been discovered. The marine algae are a rich natural resource of many biologically active compounds such as polyunsaturated fatty acids, sterols, proteins, polysaccharides, antioxidants, and pigments. They contain more than 60 trace elements in a concentration much higher than in terrestrial plants.

They also contain protein, iodine, bromine, vitamins, and substances of stimulatory and antibiotic nature. Marine algal toxins are a major source of seafood contamination worldwide with serious adverse impacts on human health. A few species produce toxins that may be unsafe or poisonous for human life and can cause diseases by directly attacking human tissues, although the frequency is rare.

Teratology is the study of abnormal development in embryos and the causes of congenital malformations or birth defects. These anatomical or structural abnormalities are present at birth although they may not be diagnosed until later in life. They may be visible on the surface of the body or internal to the viscera. Congenital malformations account for approximately 20% of deaths in the prenatal period.

Approximately 3% of newborn infants will have major malformations, and another 3% will have malformations detected later in life [2]. Currently, Teratogenicity testes *in-vivo* or genetic toxicity tests are the methods employed to study the reproductive and developmental toxicity [3, 4]. Whole embryo cultures or micro-mass embryo cell cultures have also been used to reveal the developmental toxicity [5].

Many drugs have originated from biologically active plant chemicals, and their medicinal uses are attributed to various active chemicals found in them. Synthetic drugs usually consist of a single chemical, while medicinal plants can contain a complex mixture of 400 or more chemicals. It's comparatively easy to figure out the activity and side effects of a single chemical, but there is just no way scientists can map all the complex interactions and synergies that might be taking place between all the various chemicals found in a plant, or crude plant extract containing all these chemicals which are used traditionally [6].

It is easy to understand the reason for the use of old plants for medicinal purposes. Only after the industrial revolution and the advancement of organic chemistry, did synthetic products gain supremacy in pharmacological treatments, but before that, natural products were practically the only treatment option for diseases that afflicted humanity. According to estimates by the World Health Organization (WHO), 80% of the world's population uses the resources of popular medicines to meet the needs of primary health care, which can circulate about 22 billion dollars annually.

Plant Information: *Symphyocladia latiuscula* is red alga, regarded as the most vital source due to their contents of potent biologically active substances compared to other algal species. *Symphyocladia latiuscula* is belonging to the division Rhodophyta and family Rhodomelaceae; order Ceramal's that is distributed mainly in Korea, Japan and the north part of the Chinese coast. The people of China, Korea, and Japan use this alga as an edible component in their diet. Toxicity is hard to predict in part because a single species of algae can have toxic and non-toxic strains. With this objective in view, the present study was done to demonstrate the Teratogenic effects of the marine alga *Symphyocladia latiuscula* to explore the knowledge database of potent marine algae.

MATERIALS AND METHODS:

Collection of Plant Material: The marine alga *Symphyocladia latiuscula* were brought from the coastal areas of North China and Kept in Karnataka College of Pharmacy, Bangalore, batch no: ACPL/CSH07/141101.

Extraction and Isolation of Polyphenols of the Plant Material and Sample Preparation: The plant material was powdered and used for the preparation of the methanolic extract. And methanolic extract was subjected to isolation and preliminary phytochemical screening [7]. The extract was transferred to separating funnel containing the same volume of n-Hexane and shake vigorously for a few minutes, followed by allowing the liquids to separate. This process was repeated for 3 times. The n-hexane layer was discarded, and the aqueous layer was extracted twice with ethyl acetate. The aqueous layer was collected, and the ethyl acetate layer was again extracted with acetonitrile. Finally, the aqueous and acetonitrile fractions were combined and allowed to evaporate at room temperature. The presence of phenols was determined using Folin-Ciocalteu's method.

Experimental Animals: Wistar rats (Female and Male) weighing 200-250 g were used for the experiment. All the experiments conducted on the animals were by the standards set for the use of the laboratory animal use, and the experimental protocols were duly approved by the IAEC (Institutional Animal Ethical Committee) of Karnataka College of Pharmacy, Bangalore (Ref. no. KCP/IAEC/4/18-19/4/8.9.2018).

Experimental Design:

Teratogenic Study: The Teratogenic study was performed according to the OECD guidelines no. 415 [8].

Animals: The study comprised 50 virgin healthy female rats, weighing 200-250 gm are chosen for experiments. The study also included 25 male rats used to commit intercourse with female rats to get pregnant. Intercourse was assured by getting vaginal smear and detecting sperms, and this was considered as gestation day one.

Estrous Cycles: Holding the animal ventral side up and a drop of normal saline was inserted into the vagina with Pasteur pipette. The drop of normal saline was aspirated and placed several times and then withdrawn and transferred to a microscopic slide and allowed to dry. Vaginal secretion of female rats was collected without the aid of the condenser lens. Using the 10 and 40x objectives lens, it was easier to analyze the proportion among the different cell types, which are present in the vaginal smear. And then the different phases of the oestrous cycle (Proestrous, Estrous, Metestrous, and Diestrous) were recorded. Female rats with regular estrous cycle were selected for mating.

Mating Procedure: 1:2 (one male to two females) mating was used in this study, and each morning, the females were examined for the presence of sperm or vaginal plugs. Females were checked for plugs the following morning before 9 AM. The plug forms copulation and is visible within, or slightly protruding from the vaginal opening. The plugs presence indicates a successful mating.

The duration of gestations was recorded and has been calculated from day 0 of pregnancy by examining the presence of sperm or vaginal plugs [9].

The isolated Polyphenols of alga was subjected to explore the Teratogenic activity on pregnant female rats.

The female rats (200-250 gm) were divided into five groups, and each group consists of 10 rats:

Group 1: Normal control
Group 2: Rats were received test drug at a dose of 100 mg/kg. b.w. P.O.
Group 3: Rats were received test drug at a dose of 200 mg/kg. b.w. P.O.
Group 4: Rats were received test drug at a dose of 1000 mg/kg. b.w. P.O.
Group 5: Positive control (Sodium Valproic acid) at dose of 1000 mg/kg.b.w. I.V.

The drug was administered from 6^{th} to 17^{th} day of gestation during the period of fetal organogenesis. Throughout the test period, each animal was observed once daily. Pertinent behavioral changes, signs of difficult or prolonged parturition, and all signs of toxicity, including mortality, were recorded. During pre-mating and mating periods, food consumption was measured weekly.

Optionally, during pregnancy food consumption was measured daily. Females were weighed on the first day of dosing and weekly after that. These observations were reported individually for each animal [10]. All females were killed on the 20^{th} day of pregnancy, and their uterus was dissected to examine the gross abnormalities.

Statistical Analysis: The results are expressed as mean ± S.D from n = 10 rats in each group. Fisher's exact test was used to ascertain the significance of variations between the numbers of abnormal fetuses in different groups. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION:

Acute Toxicity Study: Acute toxicity study of isolated Polyphenols of *Symphyocladia latiuscula* was done as per the OECD 425 guidelines. Hence, had taken 3 doses; *i.e.* 100, 200, & 1000 mg/kg for Teratogenicity study.

Teratogenic Toxicity of the Polyphenols of *Symphocladia latiuscula (PSL)* in Females Rats was Evaluated by Measuring the Following Parameters: Clinical Observations: No signs of illness, gastrointestinal intolerance or abnormal behavior were observed between the control and treated groups. There were no females aborted, delivered prematurely, or died throughout the experiment. However, no significant differences were noted in food consumption and water intake of pregnant rats among all treatment groups compared with negative control groups as the same.

TABLE 1: SHOWS THE DATA COMPARISON OF NUMBER OF PREGNANT RAT, NUMBER OF DEAD RAT DURING EXPERIMENT, AND BODY WEIGHTS MEAN ± SEM OF PREGNANT RATS

Groups	Normal control	PSL (100 mg/kg)	PSL (200 mg/kg)	PSL (1000 mg/kg)	The positive control group (Sodium Valproic acid)
Number of pregnant rats	10	10	10	10	10
Number of dead rats during experiments	0	0	0	0	0
Body weight (gm) of pregnant rat (Gestation 0^{th} day)	198 ± 1.21	195 ± 1.34	190 ± 1.25	183 ± 1.24	198 ± 1.65
Body weight (gm) of pregnant rat (Gestation 20^{th} day)	263 ± 2.24	208 ± 2.35	240 ± 2.67	237 ± 2.27	240 ± 2.25

Values are expressed as mean ± SEM, n = 10 in each group. $P < 0.05$ compared with control group.

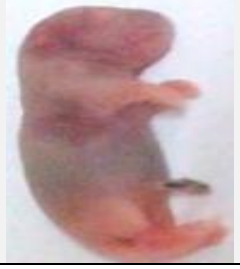
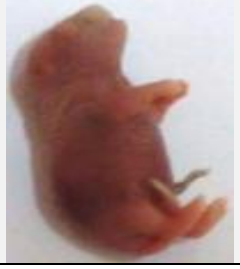
TABLE 2: SHOWS THE DATA COMPARISON (MEAN ± SEM) OF NUMBER OF PUPS, NUMBER OF DEAD PUPS, AND BODY WEIGHT, HEIGHT (cm), LOWER LIMB (mm) AND UPPER LIMB (mm) AFTER GESTATION PERIOD OF 20^{th} DAY SACRIFICED THE PREGNANT RAT

Groups	Normal control	Positive control group (Sodium Valproic acid)	PSL (100 mg/kg)	PSL (200 mg/kg)	PSL (1000 mg/kg)
Total number of pups	11	6	9	8	7
Number of dead pups	0	2	0	0	0
Body weight (gm) of pups	5.6 ± 1.11	$2.7 \pm 1.98^{*}$	$4.8 \pm 1.54^{\#\#}$	$3.9 \pm 1.35^{\#\#}$	$3.2 \pm 1.28^{\#\#}$
Pups Height (cm)	4 ± 1.26	$2.8 \pm 1.67^{*}$	$4 \pm 1.28^{\#\#\#}$	$4 \pm 1.64^{\#\#\#}$	$3.2 \pm 1.86^{\#\#}$
Pups Lower limb (mm)	10 ± 1.45	$8 \pm 1.87^{*}$	$10 \pm 1.27^{\#\#\#}$	$10 \pm 1.29^{\#\#\#}$	$10 \pm 1.64^{\#\#\#}$
Pups Upper limb (mm)	10 ± 1.23	$7 \pm 1.34^{*}$	$10 \pm 1.21^{\#\#\#}$	$9 \pm 1.22^{\#\#\#}$	$7 \pm 1.25^{\#}$

Values are expressed as mean ± SEM, n = 10; *P < 0.05 when compared to normal control, $^{\#}P<0.01$, $^{\#\#}P<0.001$ & $^{\#\#\#}$ P<0.0001 when compared to the positive control.

Positive control VPA significantly reduced all body measurements in terms of body weight, height, lower and upper limb, and while VPA showed mortality of 2 pups in the concentration of 1000 mg/kg. b.w. i.v. whereas in test drug, no significant differences were noted concerning the positive control group.

TABLE 3: PICTOGRAM ILLUSTRATES TERATOGENIC EFFECTS ON PSL (100, 200 AND 1000 mg/kg), VPA (1000 mg/kg) TREATED GROUPS WERE COMPARED WITH NORMAL CONTROL GROUP

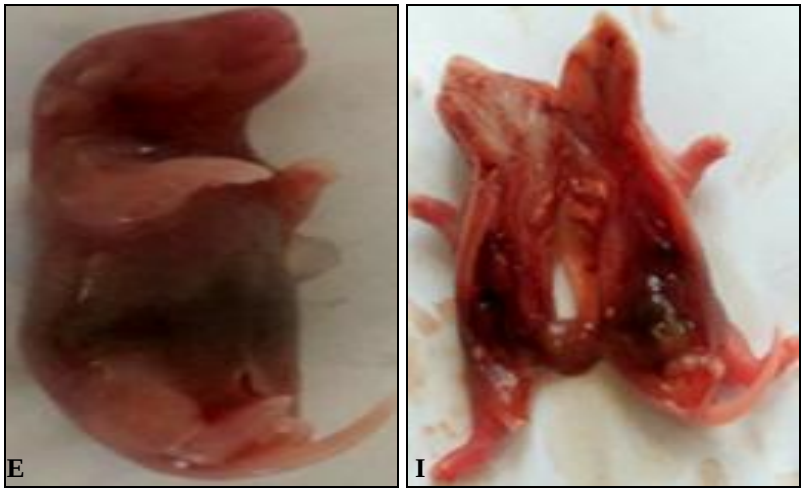
Normal Control	PSL 100 mg/kg.b.w.po	PSL 200 mg/kg. b.w.po	PSL 1000 mg/kg. b.w. po	VPA 1000 mg/kg. b.w.iv
				

Even though results revealed that the toxic effects of the PSL were found dependent on dose among the 3 groups. The highest difference was shown, especially 1000 mg/kg. b.w. of PSL in terms of several pups, body weight, and height when compared with their respective control groups.

On day 20th of gestation, the rats were sacrificed; the fetuses were examined, the normal control group showed no structural malformation. But in the other groups; the decreased pregnancy rate in a dose-dependent manner, the total number of pups also decreased. No structural extremity anomalies, facial anomalies or differences of eye openness were observed in any pups except in positive control group.

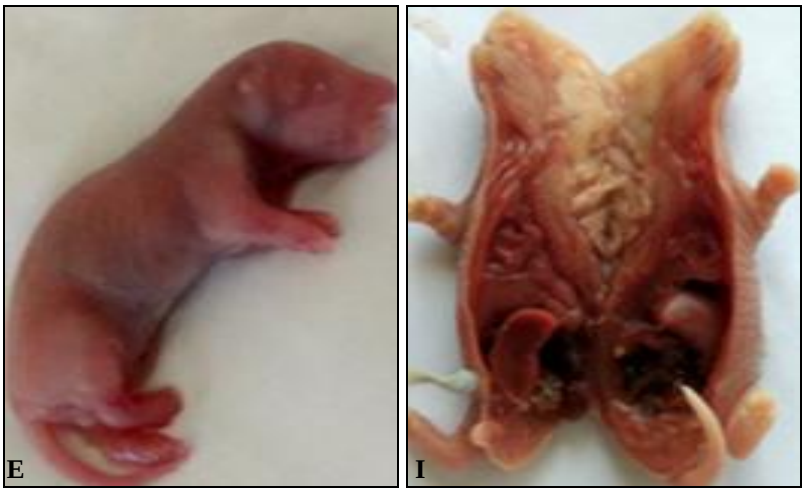
Fetal morphological abnormalities; regarding the weight and length of the fetuses, there was a reduction, visceral abnormalities were revealed in the high dose of PSL 1000 mg/kg and positive control VPA 1000 mg/kg groups compared to the normal control group. These alterations were dose-dependent.

Effects of Test drug (PSL 100, 200, 1000 mg/kg. b.w. po) and Positive Control (VPA 1000 mg/kg. b.w. IV) in External (E) and Internal (I) Features of Pups:



Normal control group

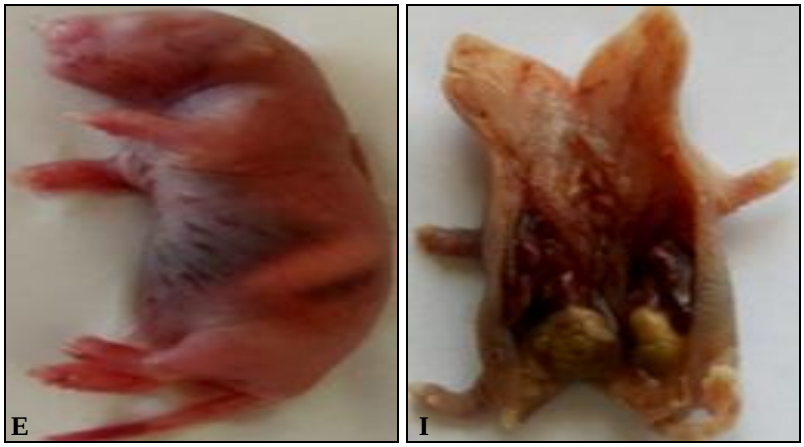
On day 20th of gestation, the rats were sacrificed; the fetuses were examined and showed no structural malformation.



POLYPHENOLS OF *SYMPHOCLADIA LATIUSCULA* (PSL) 100 mg/kg

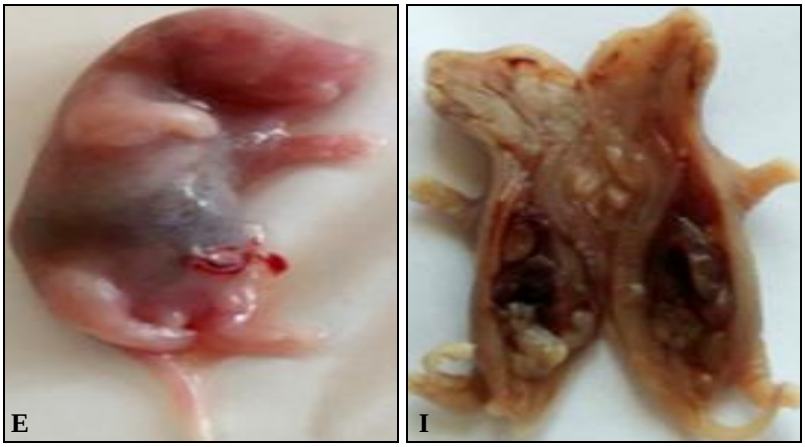
On day 20th of gestation, the rats were sacrificed; the fetuses were examined, compared with normal control group showed no structural malformation.

The size of the fetuses was measured and the result of the clinical examination showed that a low dose of PSL was not embryotoxic.



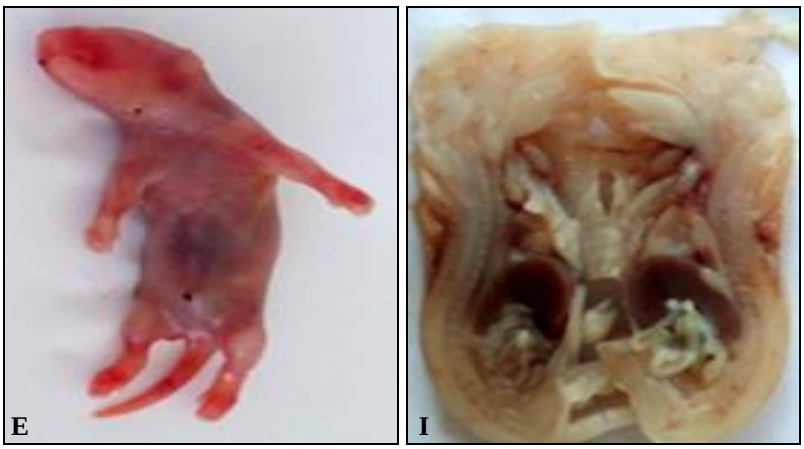
POLYPHENOLS OF *SYMPHOCLADIA LATIUSCULA* (PSL) 200 mg/kg

On day 20th of gestation, the rats were sacrificed; the fetuses were examined, compared with the normal control group showed no structural malformation. And also found no effect on the duration of gestation or offspring body weight alteration. No evidence of Teratogenic effect of the given dose of the drug was observed.



POLYPHENOLS OF *SYMPHOCLADIA LATIUSCULA* (PSL) 1000 mg/kg

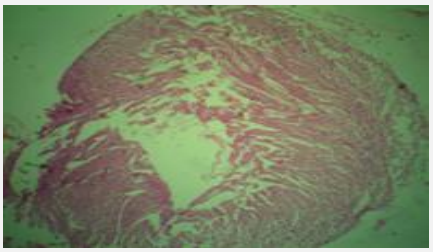
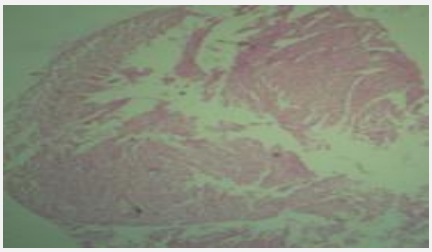
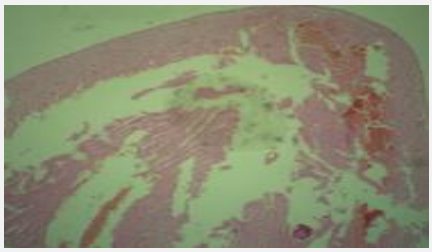
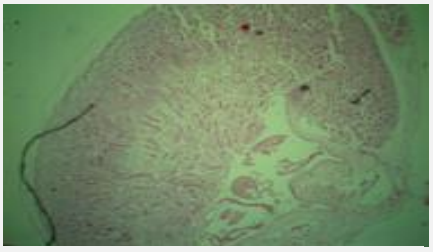
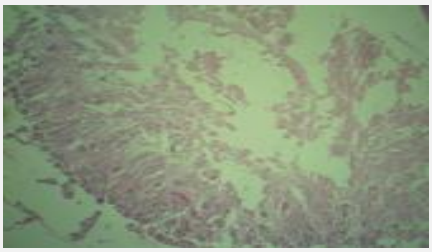
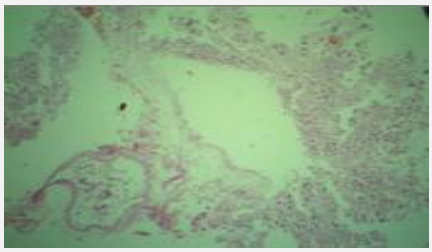
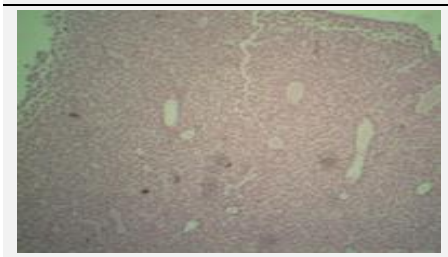
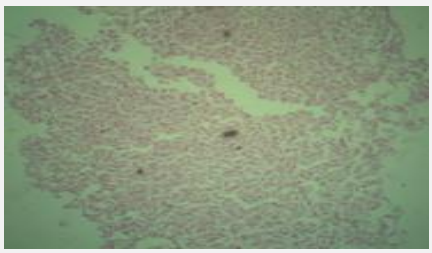
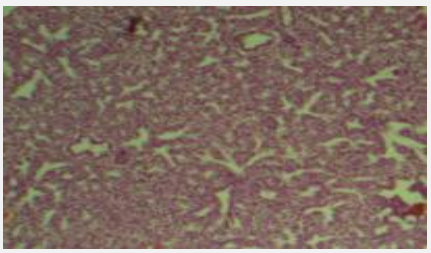
On day 20th of gestation, the rats were sacrificed; the fetuses were examined, compared with the normal control group showed structural malformation in a given dose of the drug. Reduction in body weight and shortness of upper limbs compared to lower limbs it was short. These variations occurred mostly in the higher dose of PSL. However, No macroscopic difference was determined compared with their respective control groups.



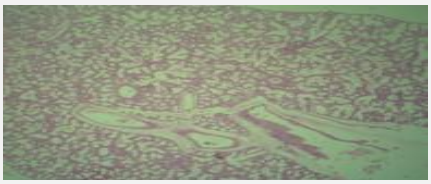
POSITIVE CONTROL GROUP (SODIUM VALPROIC ACID) 1000 mg/kg

On day 20th of gestation, the rats were sacrificed, the fetuses were examined, anatomical or structural abnormalities were observed in terms of their shortness of the upper limbs, lower limbs meant irregular shape, low birth weight, growth retardation and Internal to the viscera, and internal organ defects, focal necrosis was detected, vacuolar degeneration that were visible on the surface of the body compared to the normal control group. There are a variety of causes of malformations etiologies, including drugs.

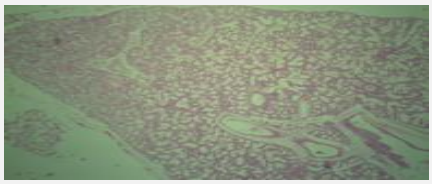
Histopathological Study: No anatomical and structural extremity anomalies were seen in dose of 100 and 200 mg/kg of Polyphenols of *Symphocladia latiuscula*. However, structural and visceral abnormalities were revealed in the high dose of Polyphenols of *Symphocladia latiuscula* 1000 mg/kg and positive control VPA 1000 mg/kg groups hence, selected for the further histological studies to find the details of malformation etiologies.

Normal control	**Polyphenols of *Symphocladia latiuscula* (PSL) 1000 mg/kg**	**Positive control group (Sodium Valproic acid) 1000 mg/kg**
HEART		
		
Normal auricles and ventricles, no observable histological changes. Hematoxylin and Eosin stain, scale bar = 100µm	Normal auricles and ventricles, no observable histological changes. Hematoxylin and Eosin stain, scale bar = 100µm	Normal auricles and ventricles, no observable histological changes. Hematoxylin and Eosin stain, scale bar = 100µm
KIDNEY		
		
Cortex and medulla are normal with normal glomeruli and tubules. Hematoxylin and Eosin stain, scale bar = 100µm	Glomeruli normal, tubules are necrotic and loss of tubular architecture, which also evident by accumulation in the center of the tubules**.** Hematoxylin and Eosin stain, scale bar = 100µm	Cortex and medulla are necrotic, degeneration with loss of capillaries surrounded by Bowman's capsule. Hematoxylin and Eosin stain, scale bar = 100µm
LIVER		
		
Normal architecture is seen with extramedullary hematopoiesis. Normal hepatocytes with pink staining cytoplasm and hepatocytes arranged in the cord like fashion surrounding the central vein. Hematoxylin and Eosin stain, scale bar = 100µm	Liver showing normal hepatocytes, mild cell necrosis, and degenerative changes were seen in the parenchyma. Hematoxylin and Eosin stain, scale bar = 100µm	Degenerative changes seen in hepatocytes with condensed nucleus and distortion in the architecture in the hepatocytes was observed. Hematoxylin and Eosin stain, scale bar = 100µm

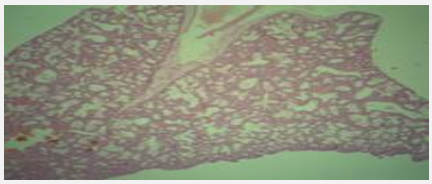
LUNG



Normal alveoli, bronchioles, and vasculature. Hematoxylin and Eosin stain, scale bar = 100µm.

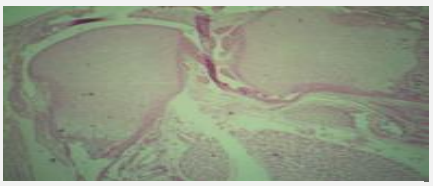


Normal alveoli, bronchioles, and vasculature. Hematoxylin and Eosin stain, scale bar = 100µm.

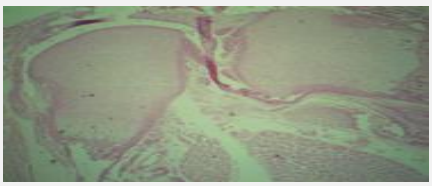


Normal alveoli, bronchioles, and vasculature. Hematoxylin and Eosin stain, scale bar = 100µm.

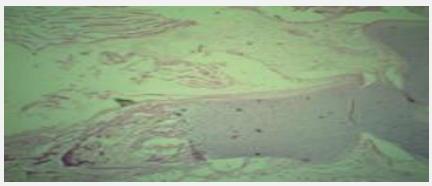
FEMUR BONE



Normal cortex, marrow with hematopoietic elements and normal articular cartilage seen



Normal cortex, marrow with hematopoietic elements and normal articular cartilage is seen. Hematoxylin and Eosin stain, scale bar = 100µm.



Histological observations of the fetal femur bones showed numerous osteoblast and osteoclast, hypertrophy, and hyperplasia of bone cells compared with the control. Hematoxylin and Eosin stain, scale bar = 100µm.

CONCLUSION: In light of these findings, we may conclude that Polyphenols of *Symphocladia* is not toxic during the gestational period in all the doses studied, especially 100 and 200 mg/kg. Herein and did not produce any anatomical and structural abnormalities. It has been found that the use of Polyphenols of *Symphocladia latiuscula* was safe in low dose. However, in high dose, *i.e.* 1000 mg/kg b.w p.o. of Polyphenols of *Symphocladia latiuscula* seen risk in pregnant rats, with growth retardation which was manifested by low body weight, length reduction, and malformations. These alterations were dose-dependent. Further studies to determine the effects of Polyphenols of *Symphocladia* on reproductive capacity and molecular mechanism are needed to complete the safety profile of this alga.

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REFERENCES:

1. Woolley A: A guide to Practical toxicology, 2nd ed. London: July 2008.
2. Chung W: Teratogens and their effects in 2004.
3. Goel RK, Prabha T, Kumar MM, Dorababu M, Prakash and Singh G: Teratogenicity of *A. racemosus* Willd. root, herbal medicine. Ind J Ex Bio 2006; 44: 570-73.
4. Moallem SA, Ahmadi A, Moshafi M and Taghavi MM: Teratogenic effects of HESA A, a natural anticancer product from Iran, in mice. Hum Ex Tox 2011; 30: 851-59.
5. Chen CC and Chan WH: Impact effects of puerarin on mouse embryonic development. Reprod Toxicol 2009; 28: 530-35.
6. George P: Concerns regarding the safety and toxicity of medicinal plants - An overview. Journal of Applied Pharmaceutical Science 2011; 01(06): 40-44.
7. Wang L, Wang X, Wu H and Liu R: Overview of biological activities and molecular characteristics of sulfated polysaccharides from marine green algae in recent years. Mar Drugs 2014; 12(9): 4984-20.
8. OEC: OECD Guidelines for the Testing of Chemicals, No. 415: One-generation Reproduction Toxicity Study, 8pp. Paris, France: Organisation for Economic Cooperation and Development 1983.
9. Cooper ERA, Jones AR and Jackson H: Effects of α-chlorohydrin and related compounds on the reproductive organs and fertility of the male rat. J Reprod Fertil 1974; 38: 379-86.
10. Ekambaram P and Paul V: Calcium preventing locomotor behavioral and dental toxicities of fluoride by decreasing serum fluoride level in rats. Environ Toxicol Parmacol 2001; 9: 141-46.

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