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Toxicity studies of crude extracts from marine *Streptomyces* sps. with potential antibacterial sensitivity against antibiotic resistant human pathogens

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ABSTRACT

Objective: To investigate the crude extract of marine actinomycetes with adverse effect locally on the adult Wister albino rats or systematically in the blood circulation. **Methods:** Acute toxicity, sub acute toxicity, biochemical and histopathological were tested. **Results:** In the results acute toxicity (LD_{50}=2 500 μg/kg bw), sub acute toxicity study (2 500 μg/kg bw) were significant at 5% level of each experimental groups compared to the control group. Biochemical and histopathological study also showed better as compared with control group **Conclusion:** This crude microbial extract from *Streptomyces* sp. RSAUT 20 and *Streptomyces scabiei* (*S. scabiei*) RSAUK 49 is potential source for novel antimicrobial compounds. The crude extract of *Streptomyces* sp. RSAUT 20 and *S. scabiei* RSAUK 49 were tested for *in vivo* toxicity study.

1. Introduction

In order to develop and to establish the safety and efficacy level of a new drug, toxicity studies are very essential and no drug is used clinically without its clinical trial as well as toxicity studies. In these studies, laboratory animals (Mice, Rats, Guineapigs, Dogs and Monkey *etc*.) help in deciding whether a new drug should be tested in humans for clinical use[1]. Depending on the duration of exposure of the animals to the drug, toxicological studies may be classified as acute, subacute or chronic[2,3]. In acute toxicity studies, the animal is given a single dose of drug to determine the immediate toxic effect. Acute toxicity studies are commonly used to determine lethal dose (LD_{50}) of a drug. Subacute toxicity studies are used to determine the effect of drug on the biochemical and hematological parameters of blood and any histopathological changes. Here, toxicological data helps to make decision whether a new drug should be adopted for clinical use or not[1].

Therefore, in connection of this objective, the present work was conducted to report whether the crude extract of marine actinomycetes have any adverse effect locally on the adult Wister albino rats or systematically in the blood circulation[4]. The study was supported by local animal ethical committee of Alagappa University, Karaikudi.

2. Materials and methods

2.1. Experimental animals

The study was carried out in Alagappa University, Karaikudi. Adult Wister albino rats weighing 150-200 g were housed in large spacious cages, maintained in controlled environment of (32±2) ℃ temperature, <30% humidity and 12 h light/dark cycles. Animals were fed with standard pellets diet obtained from Sai

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Durga Feeds and Foods (Bangalore, India) and water ad libitum.

2.2. Grouping of rats

Individual weight of the rat was taken and they randomly divided in two groups. The first groups of eight rats were used as the experimental group, while the second group of eight rats were used as control.

2.3. Acute toxicity studies

For the determination of median lethal dose (LD_{50}), 10 animals were used for each test. The animals were kept fasting for overnight providing only water, after which the extracts were administrated orally at the dose of 500 μg/kg bw. through oral gavage and observed for physical signs of toxicity for 14 d. If mortality was observed in 7 out of 10 animals, then the dose administrated was assigned as toxic dose. If the mortality was observed only in 4 out of 10 animals, then the same dose was repeated once again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose *i.e.* 1 000 μg/kg. 2 mL of 1% acacia gum is administrated through oral gavage for control. The LD_{50} was calculated by the method of Miller and Tainter[5].

2.4. Sub acute toxicity studies

Male Wister Albino rats weighing 150-200 g were divided into 2 groups of 10 animals each and were housed under controlled conditions (32±2) ℃ temperature, <30% humidity and 12 h light/dark cycles). Crude extracts from the most potential actinomycete strains *Streptomyces* sp. RSAUT 20 and *Streptomyces scabiei* (*S. scabiei*) RSAUK 49 (unpublished data) were administered daily for up to 28 d at doses of 250 μg/kg bw. respectively by oral gavage. The changes in the body weight of each rat was assessed using sensitive electronic balance (A and D Company Limited, Tokyo, Japan) during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and once on the day of sacrifice. During the fourth week of dosing period, all the animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing up to 4 h. The average food consumption was recorded weekly.

2.5. Biochemical and histopathological analysis

On 30th day, all the animals were euthanized with ether and blood sample was collected through eye bleeding method and serum was separated at 4 000 rpm at 4 ℃ for 10 min, and the serum was stored at −20 ℃ for further analysis[6]. Organs *viz.*, liver, kidney, spleen, testis and heart were taken out immediately and washed in PBS solution, weighed and stored at −80 ℃ for further analysis. The biochemical parameters *viz.*, aspartate amino transferase (AST), alanine transaminase (ALT)[7], alkaline phosphatase (ALP), protein, albumin and globulin[8], bilurubin[9] were analyzed in serum samples. Haematological studies were assessed with blood sample. To determine the cross pathology and microscopic examination of the organs, tissue biopsies from the heart, liver, kidney and spleen were removed and separated from the surrounding tissues and weighed. The tissues fixed in 10% formalin were decalcified, sectioned and finally stained with haematoxylin and eosin to examine the histopathological changes in all the above groups under light microscope. All the values were statistically analysed to find not the level of significance.

3. Results

In vivo studies on the effect of ethyl acetate extract from *Streptomyces* sp. RSAUT 20 and *S. scabiei* RSAUK 49 the toxicological changes in tested animals were carried out by the present study. The LD_{50} value of the *Streptomyces* sp. RSAUT 20 is 2 500 μg/kg bw. At the level of 2 500 μg/kg bw. 6 animals were dead (Table 1). The LD_{50} value of the ethyl acetate extract of *Streptomyces scabiei* RSAUK 49 is 2 500 μg/kg bw. At the level of 2 500 μg/kg bw. 5 animals were dead (Table 1).

Table 1.
Effect of different concentrations of *Streptomyces* sp. RSAUT 20 *S. scabiei* RSAUK 49 crude extract on the percentage mortality of rats (n=10).

Treatment	Concentration (μg/kg bw.)	*Streptomyces* sp. RSAUT 20 dead animals n(%)	*S. scabiei* RSAUK 49 dead animals n(%)
1	500	0(0)	0(0)
2	1 000	0(0)	0(0)
3	1 500	0(0)	3(30)
4	2 000	4(40)	4(40)
5	2 500	6(60)	5(50)

Table 2.
Effect of *Streptomyces* sp. RSAUT 20 extract (2 500 μg/kg bw.) on the behavioural and clinical characteristics of treated animals.

	Time	0.5 h	1 h	2 h	3 h	4 h	5 h
Stimulation	Hyperactivity	0	++	0	0	0	0
	Bioerection	0	0	0	0	0	0
	Twitching	0	0	0	0	0	0
	Rigidity	0	0	0	0	+	0
	Irritability	0	+	++	0	0	0
	Jumping	+	++	++	0	0	0
	Clonic convulsion	0	0	0	0	0	0
	Tonic convulsion	0	0	0	0	0	0
Depression	Ptosis	0	0	0	0	0	0
	Sleep (Loss of RR)	0	0	0	0	0	0
	Sedation	0	0	0	0	0	0
	Loss of pinna reflex	0	0	0	0	0	0
	Loss of PI reflex	0	0	0	0	0	0
	Catatonia	0	0	0	0	0	0
	Ataxia	0	0	0	0	0	0
	Loss of muscle tone	0	0	0	0	0	0
	Analgesia	0	0	0	0	0	0
	Loss of traction	+	++	++	0	0	0
	Sturaub tail	0	0	0	0	0	0
	Labored response	0	0	0	0	0	0
Autonomic effect	Cynosis	0	0	0	0	0	0
	Blanching	0	0	0	0	0	0
	Reddening	0	0	0	0	0	0
	Abnormal secretion	0	++	++	++	++	++
	Remarks/Mortality	0	0	0	0	0	6/10

Table 3.
Effect of *S. scabiei* RSAUK 49 extract (2 500 μg.kg bw.) on the behavioural and clinical characteristics of treated animals.

	Time	0.5 h	1 h	2 h	3 h	4 h	5 h
Stimulation	Hyperactivity	+	++	0	0	0	0
	Bioerection	0	0	0	0	0	0
	Twitching	0	0	0	0	0	0
	Rigidity	0	0	0	0	+	0
	Irritability	0	+	++	++	0	0
	Jumping	+	++	++	0	0	0
	Clonic convulsion	0	0	0	0	0	0
	Tonic convulsion	0	0	0	0	0	0
Depression	Ptosis	0	0	0	0	0	0
	Sleep (Loss of RR)	0	0	0	0	0	0
	Sedation	0	0	0	0	0	0
	Loss of pinna reflex	0	0	0	0	0	0
	Loss of PI reflex	0	0	0	0	0	0
	Catatonia	0	0	0	0	0	0
	Ataxia	0	0	0	0	0	0
	Loss of muscle tone	0	0	0	0	0	0
	Analgesia	0	0	0	0	0	0
	Loss of traction	+	++	++	0	0	0
	Sturaub tail	0	0	0	0	0	0
	Labored response	0	0	0	0	0	0
Autonomic effect	Cynosis	0	0	0	0	0	0
	Blanching	0	0	0	0	0	0
	Reddening	0	0	0	0	0	0
	Abnormal secretion	0	0	+	+	+	++
	Remarks/Mortality	0	0	0	0	0	5/10

Table 4.
Effect of *Streptomyces* sp. RSAUT 20 and *S. scabiei* RSAUK 49 extract. on the average weekly weight of experimental animals.

Source of Extract	Concentration (μg/kg bw.)	Average body weight (g)				
		On first day	On first week	On second week	On third week	On fourth week
TI-20	2 500	160.00±12.26	167.00±16.69	174.00±12.65	183.00±14.36	195.00±10.69
KKD-49	2 500	162.00±15.30	176.00±15.61	187.00±14.35	193.00±12.69	201.00±17.26
Control	–	162.00±10.69	172.00±12.68	185.00±14.65	191.00±13.24	196.00±17.47

Values are significant at 5% level for each experimental groups compared to the control group.

Table 5.
Effect of *Streptomyces* sp. RSAUT 20 and *S. scabiei* RSAUK 49 extract on the organs weight (g) in the control and treated rats.

Name of the organ	Control	TI-20	KKD-49
Liver	2.87±0.25	2.62±0.33	2.92±0.41
Kidney	0.73±0.09	0.76±0.15	0.77±0.16
Heart	0.39±0.09	0.38±0.21	0.43±0.18
Testis	2.32±0.21	2.21±0.24	2.42±0.17
Lungs	0.15±0.05	0.14±0.05	0.16±0.16

Values are significant at 5% level for each experimental groups compared to the control group.

Table 6 .
Effect of *Streptomyces* sp. RSAUT 20 and *S. scabiei* RSAUK 49 extracts on the average food consumption of experimental animals.

Source of the extract	Average food consumption (g)				
	Concentration(μg/kg bw.)	Ist week	IInd week	IIIrd week	IVth week
Control	2 mL of 1% Acacia gum	140.0±1.5	144.0±1.0	149.0±1.0	151.0±1.5
TI-20	250	136.0±1.5	142.0±1.0	146.0±1.5	149.0±1.0
KKD-49	250	139.0±1.0	144.0±1.5	149.0±1.0	151.0±1.5

Values are significant at 5% level for each experimental groups compared to the control group.

Table 7.
Effect of *Streptomyces* sp. RSAUT 20 and *S. scabiei* RSAUK 49 extracts on the serum parameters in treated and control rats.

Parameters	Control	TI-20	KKD-49
AST	17.90±0.70	16.07±2.06	15.03±2.14
ALT	11.33±0.94	13.26±2.15	13.60±3.12
ALP	108.00±5.65	72.30±3.58	81.33±6.45
Protein	4.03±0.45	7.23±0.22	8.70±0.25
Albumin	3.46±0.74	4.30±0.53	5.13±0.38
Globulin	1.76±0.40	2.66±0.53	3.16±0.71
Bilirubin	1.13±0.59	1.14±0.44	1.32±0.60

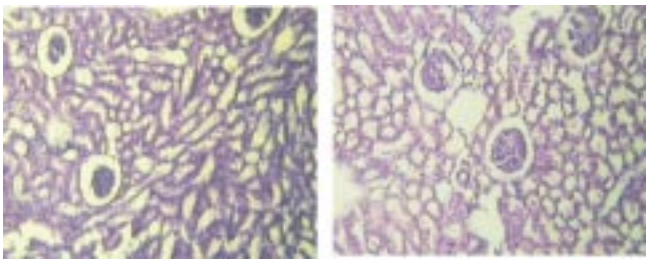
Values are significant at 5% level for each experimental groups compared to the control group.

Table 8.
Effect of *Streptomyces* sp. RSAUT 20 and *S. scabiei* RSAUK 49 extracts on the haematological and biochemical parameters in treated and control rats.

Parameters	Control	TI-20	KKD-49
WBC (Cells/mm^3)	1600.00±163.29	1600.00±294.34	1700.00±81.25
Poly morpho nulear leucocytes	31.33±6.35	27.00±0.16	22.00±0.68
Lympocytes	56.00±12.95	65.76±2.19	63.00±0.16
Eosinophils	6.33±0.92	8.43±1.49	8.16±0.49
Haemoglobin	9.20±0.81	10.76±0.18	10.96±0.30
RBC (millions/mm^3)	2.53±0.44	2.66±0.47	2.96±1.32
PCV (%)	13.33±3.09	13.23±1.24	15.33±13.71
Platelet	3.46±0.44	4.16±1.23	5.93±0.67
Cholesterol	84.00±9.09	68.44±0.49	61.43±1.45
TGL	28.33±1.69	32.67±6.28	36.22±6.32
HDL	126.66±12.28	97.66±11.89	86.12±1.41
LDL	41.00±2.94	36.67±0.24	31.45±0.22
VLDL	29.66±4.78	31.40±1.24	32.00±0.27
SGOT	17.90±0.69	18.89±1.10	19.76±0.40
SGPT	12.00±0.94	17.07±0.99	15.90±0.90
Sugar	80.66±26.10	70.87±2.43	71.11±0.24
Urea	8.00±0.81	7.11±0.64	6.13±0.49

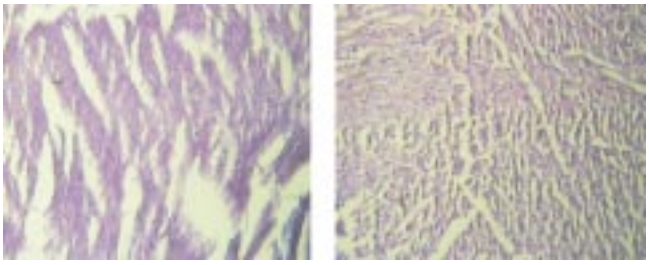
Values are significant at 5% level for each experiemental groups compared to the control group.

At the dose level of 2500 μg/kg bw. of *Streptomyces* sp. RSAUT 20 treatment, the animals were showed jumping, hyperactivity, loss of traction, irritability, abnormal secretion. No adverse effect of diarrhoea, haematuria, bioeraction, twisting, clonic convulsion and impaired movement were observed in the



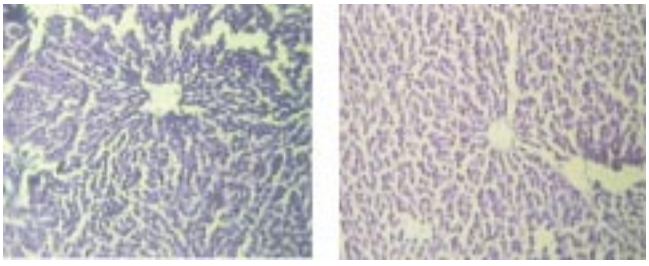
Control Treated

Figure 1. Histological observation in kidney tissue without and with administration of *Streptomyces* sp. RSAUT 20 extract.



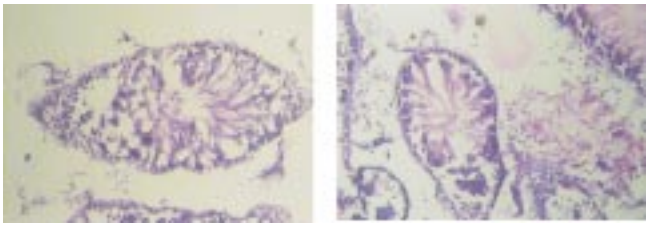
Control Treated

Figure 2. Histological observation in heart tissue without and with administration *Streptomyces* sp. RSAUT 20 extract.



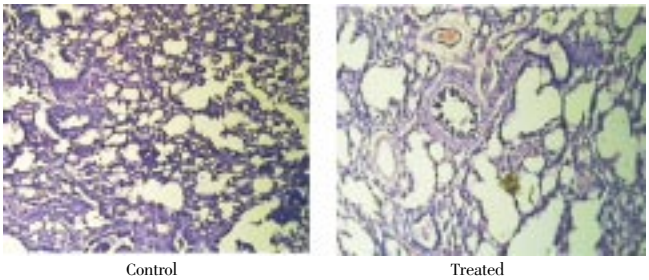
Control Treated

Figure 3. Histological observation in liver tissue without and with administration of *Streptomyces* sp. RSAUT 20 extract.



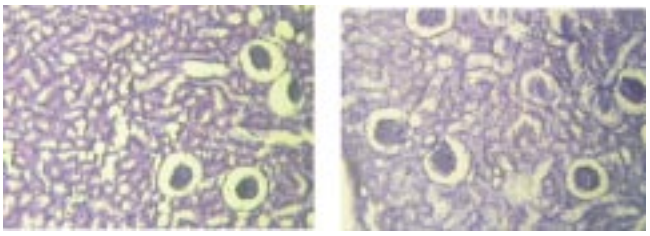
Control Treated

Figure 4. Histological observation in testis tissue without and with administration of *Streptomyces* sp. RSAUT 20 extract.



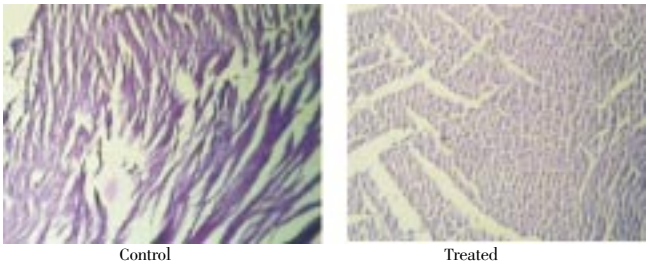
Control Treated

Figure 5. Histological observation in lungs tissue without and with administration of *Streptomyces* sp. RSAUT 20 extract.



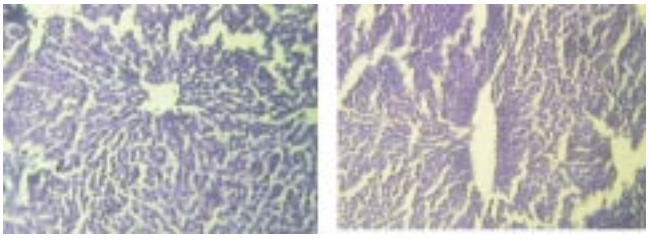
Control Treated

Figure 6. Histological observation in kidney tissue without and with administration of *S. scabiei* RSAUK 49 extract.



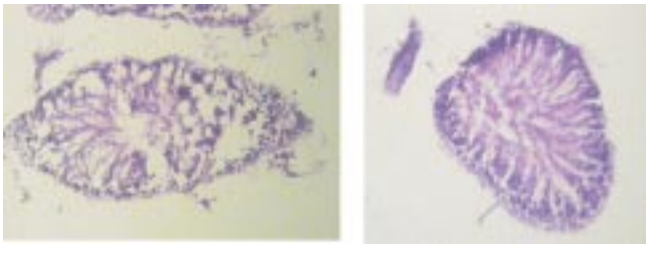
Control Treated

Figure 7. Histological observation in heart tissue without and with administration of *S. scabiei* RSAUK 49 extract.



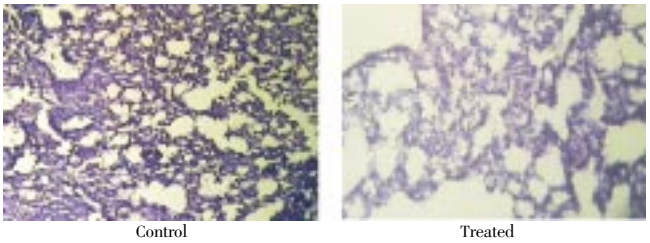
Control Treated

Figure 8. Histological observation in liver tissue without and with administration of *S. scabiei* RSAUK 49 extract.



Control Treated

Figure 9. Histological observation in testis tissue without and with administration of *S. scabiei* RSAUK 49 extract.



Control Treated

Figure 10. Histological observation in lungs tissue without and with administration of *S. scabiei* RSAUK 49 extract.

treatment days (Table 2). The dose level of 2 500 μg/kg bw. of *S. scabiei* RSAUK 49 treated animals initially showed hyperactivity, irritability, jumping, loss of traction and abnormal secretion. But not showing biorection, twisting, clonic convulsion, sedation, loss of tractions and loss of PI reflex, blanching, reddening, analagesia during the treatment dosing period (Table 3).

The sub acute toxicity studies revealed that, no distinct clinical changes were observed in the *Streptomyces* sp. RSAUT 20 and *S. scabiei* RSAUK 49 treated animals. There were changes in the properties of stool, urine and eye colour of all animals. No mortality was observed in any of the treatment groups. There were no significant differences were observed in the body weight of animals treated with extracts and control group (Table 4). However, there were no significant changes in weight of the various organs such as liver, kidney, heart, testis and lungs (Table 5).

Moreover, no lethality was recorded in any dose up to the maximum of 250 μg/kg bw. (TI-20 and KKD-49) during the 28 d period of the treatment. The food intake ratio with the body weight is in coincidence with both the control and treated animals (Table 6). Daily food intake was normal and there was no diarrhoea or excessive salivation.

The haematological and biochemical parameters of Streptomyces sp. RSAUT 20 and *S. scabiei* RSAUK 49 extract treated animals were also carried out in present study. Biochemical components *viz*., AST, ALP, ALT, protein, albumin, globulin and bilirubin in serum were determined after treatment and compared to that of control; there were slight changes, but all values remained within the normal range. There were no significant variations were noticed in the serum parameters (Table 7).

The hematological profiles of the experimental and control group rats were summarized in Table 8. No significant change in the values of RBCs, WBCs, haemoglobin, lymphocytes, eosinophils and packed cell volume (PCV) of experimental animals treated with the extract from Streptomyces sp. RSAUT 20 and *S. scabiei* RSAUK 49 when compared to that of control group. All the other parameters like sugar, urea, platelet, cholesterol, TGL, SGOT, SGPT *etc*. are remained within the normal limits.

It is interesting to notice that, histopathological examination of selected organs like heart, liver, kidney, testis and lungs from both control and extract treated rats showed normal architecture suggesting no detrimental changes and no morphological changes (Figure 1-10).

4. Discussion

Several studies have been carried out to find out the LD_{50} dose fix toxicity effect in the animal model experiment[10,11]. The present study investigated the acute and sub acute toxicity of crude extract of actinomycetes, in experimental animal models. In the acute toxicity study, up to the dose level of 1 000 μg/kg of body weight did not exhibit any lethality or toxic symptoms. According to Organisation for Economic Cooperation and Development (OECD) guidelines for acute oral toxicity, an LD_{50} dose of 2 000 mg/kg and above is categorized as unclassified and here in the present study the LD_{50} dose is 2 500 μg/kg bw. by this reason the marine actinomycetes crude bioactive compounds from marine actinomycetes found to be safe.

In the subacute toxicity study, the treated animals with crude actinomycetes extract did not show any significant changes in the animal behaviour study. But slightly changes in body weight increment at weekly intervals compared to the control group that means it did not have any major adverse effects on body weight. Which is used to assess the response to therapy of drugs and to indicate the adverse effects of a drug it is reported by Winder and Teo *et al*[12]. According to the Joshi *et al*[13] the weight of the liver, kidney and heart were unaltered in the experimental groups compared with the control group. The haematological and biochemical parameters did not show any significant changes in the treated groups when compared to the control group. Also the histopathological section of various organs such as the liver, kidney, heart revealed normal architecture on comparison with the control group.

Furthermore, there were no significant changes in any liver function parameters, such as SGPT, SGOT, ALP, ALT and protein, bilurubin compared to the control group here this results significant with Ravikumar *et al*[14] and Joshi *et al*[13]. The levels of SGOT and SGPT in liver tissues are found in significant in higher concentrations in cytoplasm and SGOT in particular also exists in mitochondria[15].

In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane[16], thereby causing an increased enzyme level in serum. If injury involves organelles such as mitochondria, soluble enzymes like SGOT normally located there, will also be similarly released. The elevated activities of SGOT and SGPT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver[17].

Joshi *et al*[13] reported that there were no significant changes in various haematological parameters (Hb, RBC, WBC, ESR) and differential count compared to the control group. Here the present study also showing there were no significant changes in various haematological parameters such as RBC, WBC count compared to the control group, which indicates that this crude extract of potential actinomycetes may not be toxic and does not affect circulating red cells, hematopoiesis or leukopoiesis.

The present findings suggest that this crude extract is nontoxic since no marked changes in haematological, biochemical and histopathological parameters were observed. Thus, at normal therapeutic doses, crude extract is considered to be safe for long-term treatment.

Conflict of interest

We declare that we have no conflict of interest.

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