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TOXICOLOGICAL STUDIES OF AN AYURVEDIC MEDICINE "SLESMA SAILENDRA RAS (SLS)" AFTER CHRONIC ADMINISTRATION TO MALE SPRAGUE-DAWLEY RATS

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ABSTRACT

Slesma Sailendra Ras (SLS) an Ayurvedic preparation used as a traditional medicine in the treatment of cold, flu and fever in the rural population of Indian subcontinent. After 54 days chronic administration of the SLS preparation to the male rats at a dose of 400 mg/kg, the following toxicological changes were noted. In the male rats there were decrease in Total Protein, Albumin and Globulin content in the plasma. Statistically very highly significant decrease plasma Total Protein content was noted. There was a negligible increase Triglyceride level and VLDL content in the plasma whereas statistically very highly significant decrease in Total Cholesterol, HDL and LDL content. After chronic administration of SLS to the male rats' decrease of plasma Bilirubin and Creatinine level were noted in comparison to their control group. On the contrary, a very highly significant decrease in urea & Urea/Creatinine level in plasma were noted. SLS caused decrease in plasma uric acid which was not statistically significant.

KEYWORDS: Ayurvedic Medicine, Cholesterol, Slesma Sailendra Ras (SLS), Triglycerides, Uric Acid.

INTRODUCTION

Ayurvedic medicine (also called ayurveda) is one of the world's oldest medical systems, originated in India and has evolved there over thousands of years. Slesma Sailendra Ras (SLS) along with 25 medicinal plants used as an Ayurvedic medicine used as a traditional medicine in the treatment of cold, flu and fever in the rural population of Bangladesh[1].These medicinal plants having different therapeutic uses. Sunthi (Rz.) is used for the management of nausea, morning sickness & chemotherapy[2]. Extracts from Marica have been found to have antioxidant properties [3] and anti-carcinogenic effects. Pippali is used for the treatment of respiratory tract diseases like cough, bronchitis, asthma and cold, as counter-irritant and analgesic it is applied locally in muscular pain and inflammation and internally it is used as a carminative in conditions such as loss of appetite, sleeplessness and obstruction in liver and spleen[4]. Ziraka (Fr.) has antimicrobial properties against pathogenic bacteria and fungi, indicated that it is an aromatic herb and has a powerful bactericidal action [5] .The seeds of Krsnajiraka has protective antioxidant and anti-inflammatory effects, and promotes apoptosis (cell death) of the cancer cells[6]. The rhizome of Sati has been reported to possess anti-inflammatory, anti-asthmatic, hypoglycemic, vasodilator, spasmolytic & hypotensive[7]. Saindhava is high in minerals, cooling, sweet and easy to digest. As a muscle relaxant, Saindhava is applied in a warm bath[8]. Gajapipali (Fr.) enters into the composition of many medicines used as carminatives and digestives[9]. Lavanga may reduce blood sugar levels[10]. The pharmacological activity and therapeutic uses of individual ingredients are well established but no

report was found on the formulation of SLS. That is why; this study was performed to observe the effect of chronic administration of SLS to Male Sprague-Dawley rats at a high dose. The objective is to have a better understanding of the potential toxicological profile of the drug and to decide how justifiable the use of this drug is under the stated condition. The study provides directions for further research as well.

MATERIALS AND METHODS

Animal models used in the experiment: To perform the toxicological experiments and growth studies, healthy 7 weeks old rats (*Rattus novergicus*: Sprague-Dawley strain) were taken at the animal house of the Department of Pharmacy, Jahangir Nagar University, Savar, Dhaka. The weight of the rat was between 65 to 75 gm.

Experimental design: In all the experiment a total of twenty male rats were used. The rats were divided into two groups of ten animals. One group was treated with SLS and another was used as a control. The control animals were administered with distilled water only as per the same volume as the drug treated group for 54 days.

Collection of the ayurvedic formulation, chemicals & reagents:The Ayurvedic formulation of Slesma Sailendra Ras(SLS) was collected from Sri Kundeswari Aushadhalaya Ltd, Chittagong. All other reagents, assay kits and chemicals used in this work were purchased from Human GmbH Germany.

Preparation of sample: Slesma Sailendra Ras (SLS) is included in the Bangladesh National Formulary of Ayurvedic Medicine (BNFAM) 1992 (Approved by the Government of Bangladesh vide Ministry of Health and Family Welfare Memo No. Health-1/Unani-2/89/ (Part-1) 116 dated 3-6-1991).

Statistical analysis used: The results are expressed as mean ± SEM (Standard error of mean). Means were compared by independent sample t-test. The statistical program "SPSS 12.0 for Windows" was used to test the level of significance. Probability (p) value of 0.05 or less ($p<0.05$) was considered as significant.

RESULTS

Effect on total protein contents: After 54 days chronic administration of the SLS preparation in the male rats there were decrease in Total Protein (24.069 % decr.), Albumin (37.789 % decr.) and Globulin (67.935 % decr.) content in the plasma. A statistically very highly significant decrease in Total Protein ($p=0.001$) content in plasma was noted. The decrease in Albumin ($p=0.043$) and Globulin ($p=0.024$) content were significantly different from their corresponding control values in table 2 and figure 1.

Effect on lipid profiles: In the male rats there was a negligible increase noted in Triglyceride (TG) level (6.49 % incr.) ($p=0.816$) and VLDL (6.49 % incr.) ($p=0.816$) content in the plasma which was obviously not statistically significant. Whereas statistically very highly significant decrease in Total Cholesterol (TCHO) (88.458 % decr.) ($p=0.001$), LDL (92.433 % decr.) ($p=0.001$) and HDL (63.942 % decr.) ($p=0.001$) content in the plasma were noted.

Note: n=10, *p<0.05, **p<0.01, ***p<0.001, NS=Not Significant

Effect on liver function test: After chronic administration of SLS to the male rats a decrease of Bilirubin level (39.075% decr.) in the plasma was noted in comparison to their control group, the decrease though not statistically significant, yet it was noticeable ($p=0.110$).

Effect on kidney function test: There was a decrease in the plasma Creatinine (12.037%) in the SLS treated male rats, though this decrease was not significant ($p=0.415$). On the contrary, a very highly significant decrease in urea (20.002%) content in plasma was noted ($p=0.001$). A high blood level of Creatinine & Urea indicates that the kidneys may not be working properly**.** A highly significant decrease in Urea/Creatinine ratio (26.665%) in plasma was noted ($p=0.003$). The BUN/Creatinine ratio can be elevated due to acute kidney injury or dehydration. About 11.227% decrease in plasma Uric Acid content of SLS , when treated male rats in comparison to their control male rats which was not statistically significant ($p=0.393$).

DISCUSSION

High cholesterol levels can lead to hardening of the arteries, which may cause heart disease, stroke, and other symptoms or problems throughout the body. Increased levels of VLDL & LDL are linked to atherosclerosis, which can lead to coronary heart disease. Total cholesterol, is an extremely potent predictor of CHD which reflects vulnerability of the body to heart diseases. Similarly, low ratio means high count of HDL and hence less chances of heart attacks and other diseases (19). From the above discussion, it is clear that SLS not only safe but also beneficial for the management of heart disease, stroke or likes CHD diseases. The decreased level of Bilirubin in plasma indicated lack of toxic effect on liver even after chronic administration of Slesma Sailendra Ras (SLS). According to Naganna, 1989

(20), increase in Bilirubin indicates the abnormal liver function which may be the results of higher synthetic function of the liver. So decrease level of Bilirubin in SLS treated rat is indicating the normal liver function. A high blood level of Creatinine & Urea indicates that the kidneys may not be working properly**.** The BUN/Creatinine ratio can be elevated due to acute kidney injury or dehydration. High levels of Uric Acid can cause gout or kidney disease. SLS decreased Urea, Creatinine & Uric acid level in serum compared with control male rats. This is to ensure that the concentration of the prescribed medication is not so high that it compromises the patient's kidney function (21-22).

CONCLUSIONS

In conclusion, the present investigation has shown that Slesma Sailendra Ras (SLS) significantly reduces Total Protein, Total Cholesterol, LDL, HDL, Urea and Urea/Creatinine ratio in plasma. It ensures that the concentration of the prescribed medication is not so high that it compromises the patient's normal liver and kidney functions. Especially after considering of lipid profile test, SLS may be helpful in lowering high cholesterol level in blood and will be beneficial for the management of chronic heart diseases.

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Table 1: Name of the ingredients used in the preparation of "Slesma Sailendra Ras (SLS)"

Name of plants/Ingredients	Used parts	Botanical/Scientific name	Family	Amount Used
1. Kajjali	------	Mercury	------	40 g
2. Sunthi	Rhizome	*Zingiber officinale*	Zingiberacea	20g
3. Marica	Fruit	*Piper nigrum*	Piperaceae	20 g
4. Pippali	Fruit	*Piper longum*	Piperaceae	20 g
5. Abhraka (bhasma)	-----	*Siliceous encrustation*		20 g
6. Jiraka	Fruit	*Cuminium cyminium*	Umbelliferae	20 g
7. Krsnajiraka	Fruit	*Carum bulbocastanum*		20 g
8. Sati	Rhizome	*Hedychium spicatum*	Zingiberaceae	20 g
9. Karkata (G1.)				20 g
10.Yamani	Fruit			20 g
11. Pauskara (puskaramula)	Rhizome	*Inula racemosa*	Asteraceae	20 g
12. Hingu (Exd.)		*Ferula foetida*	Apiaceae	20 g
13. Saindhava	NaCl			20 g
14. Yava ksara				20 g
15. Tankana				20 g
16. Gajapippali	Fruit	*Scindapsus officinalis*	Araceae	20 g
17. Jatikosa (Ar)				20 g
18. Ajamoda	Fruit	*Carum roxburghianum*	Apiaceae	20 g
19. Loha (lauha bhasma)	Iron			20 g
20. Duralabha (Pl.)		*Fagonia arabica*	Zygophyllaceae	20g
21. Lavanga	Flower	*Syzygium aromaticum*	Myrtaceae	20 g
22. Dhattura bija	Seed			20 g
23. Jaipal		*Croton tiglium*	Euphorbiaceae	20 g
24. Katphala	Fruit	*Myrica nagi*	Myricaceae	20 g
25. Citraka	Root	*Plumbago zeylanica*	Plumbaginaceae	20 g
are to be rubbed together, and subjected to bhavana with the juice or decoction of the roots of the following, in succession :-				
(Q.S. = drugs 2639 for bhavana)				
26. Bilva (mula) kasaya Root				Q.S.(for bhavana)

27. Arka				Q.S.(for bhavana)
28. Citraka Root		*Plumbago zeylanica*	Plumbaginaceae	Q.S.(for bhavana)
29. Danti	Root	*Baliospermun montanum*	Euphorbiaceae	Q.S.(for bhavana)
30. Apamarga	Root	*Achyranthus aspera*	Amaranthaceae	Q.S.(for bhavana)
31. Jivanti	Root	*Leptadenia reticulate*	Asclpiadaceae	Q.S.(for bhavana)
32. Vasa	Root	*Adhatoda vasica*	Acanthaceae	Q.S.(for bhavana)
33. Nirgundika svarasa	Leaves			Q.S.(for bhavana)
34. Agnimantha	Root	*Premna serratifolia*	Verbenacea	Q.S.(for bhavana)
35. Dhattura svarasa	Leaves			Q.S.(for bhavana)
36.Krsnajiraka	Fruit	*Carum bulbocastanum*	Umbelliferae	Q.S.(for bhavana)
37. Paribhadra	Root	*Erythrina variegata*	Fabaceae	Q.S.(for bhavana)
38. Kantakari	Root	*Solanum xanthocarpum*	Solanacea	Q.S.(for bhavana)
39. Sunthi	Rhizome	*Zingiber officinale*	Zingiberaceae	Q.S.(for bhavana)

Table 2: Effect of SLS on total protein content in male rats

Parameter	Control (Mean± SEM)	Test (Mean±SEM)	t/p	95%CI	Overall output
Total protein	5.9844±0.28955	4.5440±0.24997	3.785 /0.001***	0.63752 to 2.24318	↓ 24.069%
Albumin	5.1736±0.72772	3.2185±0.12052	2.650 /0.043*	0.08836 to 3.82179	↓ 37.789%
Globulin	4.5796±0.98631	1.4684±0.25490	3.054/ 0.024*	0.58333 to 5.63890	↓ 67.935%
Albumin/Globulin	1.1747±0.72676	3.0203±0 .70705	-1.679/ 0.119^{NS}	-4.2226 to 0.1398	↑ 157.110%

Table 3: Effect of SLS on lipid profiles in male rats

Parameter	Control (Mean± SEM)	Test (Mean±SEM)	t/p	95%CI	Overall output
Triglycerides	22.3684±5.2396	23.8202±3.0779	-0.239 /0.816^{NS}	-14.98131 to12.0777	↑6.490%
Total Cholesterol	132.658±1.534	15.311±1.39851	56.074±0.001***	112.931to 121.762	↓ 88.458%
VLDL	4.4737±1.0479	4.7640±0.61559	0.239 /0.816^{NS}	2.99626to2.41554	↑6.490%
HDL	24.9642±1.1184	9.0015±0.80468	11.586 /0.001***	13.0418to18.88349	↓63.942%
LDL	101.813±3.4542	7.7042±1.41567	25.209 /0.001***	85.6785to102.5401	↓92.433%
TCHO/ HDL	5.238±0.2893	2.042±0.3510	7.093/0.001***	2.2407 to 4.1512	↓61.011
LDL/HDL	4.0905± 0.38237	2.6506± 1.46109	1.174/ 0.265^{NS}	-1.25881 to 4.13854	↓ 35.2%

Table 4: Effect of SLS on liver function test in male rats:

Parameter	Control (Mean ± SEM)	Test (Mean±SEM)	t/p	95%CI	Overall output
Bilirubin	0.04598 ± 0.3891	0.2675±0.08419	1.736/0.110	-0.04598 ± 0.3891	↓39.075%

Table 5: Effect of SLS on kidney function test in male rats:

Parameter	Control (Mean ± SEM)	Test (Mean±SEM)	t/p	95%CI	Overall output
Urea	69.7342±2.064	55.7860±1.12301	6.505/0.001***	9.3496±18.5466	↓20.002%
Urea/Creatinine	3.1078±0.18156	2.2791±0.14173	3.531/0.003**	0.32853± 1.3288	↓26.665%
Uric acid	42.9110±2.79524	38.0932±4.88610	0.884/0.393^{NS}	-6.95085 ±16.586	↓11.227%

↑= increase, ↓= decrease

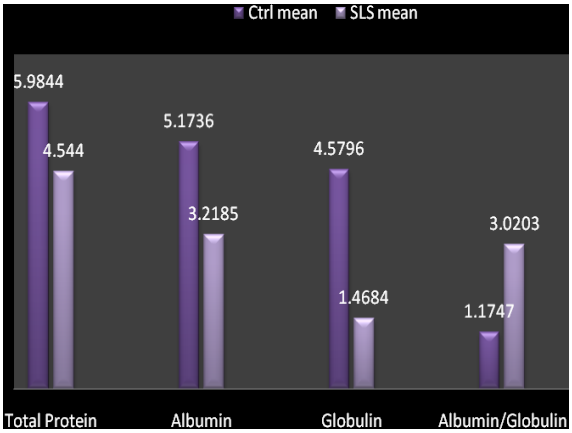

Figure 1: Graphical presentation total protein profile test test

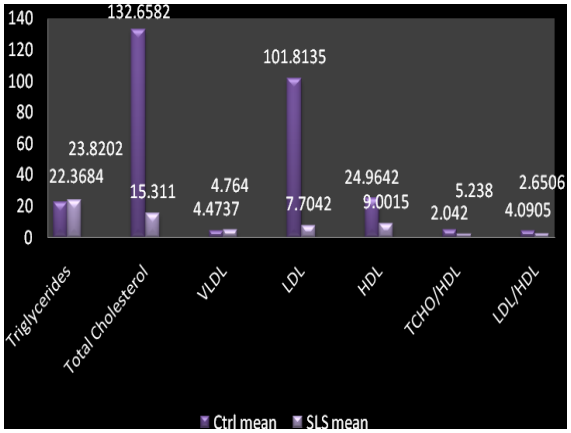

Figure 2: Graphical presentation of lipid profile

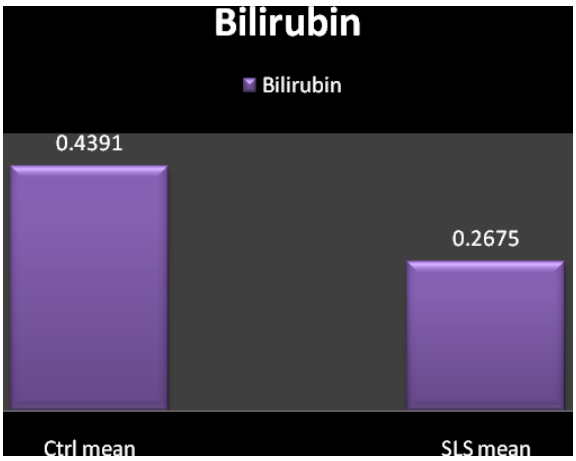

Figure 3: Graphical presentation of liver function test function test

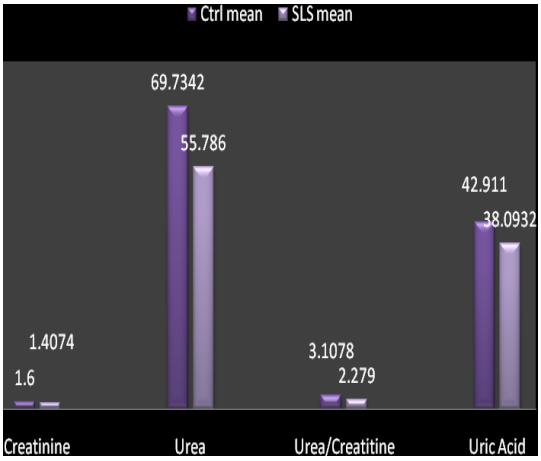

Figure 4: Graphical presentation of kidney

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