HISTOMORPHOLOGICAL EFFECTS OF SALICYLIDENE SALICYL HYDRAZIDE ON LIVER IN SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: *This study was aim to identify histomorphological changes in the liver of Sprague dawley rats after exposure to Saliyclidene salicylhydrazide and dose dependent histomorphological changes in the liver.*

Materials and Methods: *This study was started from Mar 2019, to June 2019. In this study, acute toxicity of seven days, the sixteen Sprague dawley female rats were selected. They were divided into four groups. Two test groups (A & B). Group A was given 25mg/kg SCS, while in group B 50mg/kg of SCS was given. Positive control group (C) was given paracetamol 300mg/kg. Group D was a negative control group. Each group had four animals. After seven days of exposure to SCS through oral route all the animals were euthanized. Their livers were harvested and processed for preparation of histological slides. These slides were then analyzed by microscopy and histomorphological changes were recorded and analyzed according to Roenigk classification system.*

Results: *Necroinflammatory changes were observed in Group A, B and C compared to Group D(-control). However, difference between Group A and B was not significant. Nuclear pleomorphism was observed in hepatocytes of Group A, B and C compared to group D(control). Pleomorphic changes in the intervention group A & B were same. Steatosis was observed in group A, B and C compared to Group D. Steatosis in group A and B were same while in group C it was relatively more significant. The overall grading according to ROENIGK classification system showed significantly higher toxicity in group B, compared to other intervention groups (A, B, C) and control group D.*

Conclusion: *Significant effects of SCS were observed in liver of Sprague dawley rats, however, the toxic effects were less severe. Liver Parenchyma and architecture were preserved.*

Keywords: *salicylidene salicyl hydrazide (SCS), liver, toxicity*

INTRODUCTION

Salicylidene salicylhydrazide (SCS) is synthesized from methyl salicylate (winter green oil).Methylsalicylate is reacted with hydrazine to get salicylhydrazide. Salicylhydrazide is then treated with salicylaldehyde to get Saliyclidene salicyl hydrazide.[1] SCS is a selective antagonist of β1subunit-containing GABAa receptors. Salicylidene salicylhydrazide is unusual in selectively inhibiting GABAa receptors which contain the β1 subunit through the allosteric mechanism. Recently it has been discovered that lack of Salicylidene salicylhydrazide interaction with multiple GABAa receptor modulators predicts

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that SCS is interacting at the previously unknown location on the receptor.[2]

Salicylates are distributed throughout the volume of body water which appears to be much greater than extracellular fluid. Studies on rats has shown that the concentrations of salicylates in liver, kidney and lung are similar to its concentration in the serum. When the salicylate concentrations are calculated on the basis of water content, the liver contains about two thirds as much as serum.[3] These activities also predict its potential benefits and provide support for its application in new drug development.

Liver has a fundamental role in metabolism of the substances absorbed through gastro intestinal tract. It has its own system of drug-metabolizing enzymes which is largest system of enzymes compared to any other organ of the body and due to this drug metabolizing capacity and functions, it is therefore the most common target organ for drug toxicity.[4] Liver functions are diverse and complex including, metabolism, conjugation, detoxification, endocrine and exocrine activity, and haematopoiesis in early embryonic and foetal development.

The metabolic and morphological response to noxious stimuli depends on duration, severity and nature of stimuli. These stimuli may lead to cellular degeneration and death of animal (at high doses). While at lower doses they may lead to cellular changes which may not lead cellular degeneration or death of animal. Such adaptive changes may be adverse or not? depends upon the change which have taken place. In some cellular adaptations physiologic and morphologic changes involving changes in cellular organelles and intracellular accumulations of different endogenous and exogenous substances allow the cells and animal to survive the chemical insult and live normally. But adaptive changes or doses of chemicals that induce these adaptive changes may not result in illness or death of animals.These adaptations are chemical and dose dependent.[5]

It has been established that an acute toxicity test can give more information about the biologic properties of a chemical compound than any other single test.[6] The purpose of acute toxicity testing is to acquire information on the biologic activity of a chemical and understand its mechanism of action.

Till now SCS has only been evaluated in in-vitro experiments. In-vivo studies have not been conducted for its therapeutic effects. Toxic effect on tissues of body has not been studied. This study was designed to check the histologic effects of SCS on liver in two different doses.

MATERIALS AND METHODS

Sixteen female Sprague Dawley rats were bought from Pharmacy department, University of Peshawar, with approximately 170-210g body weight and 8-12 weeks age. The rats were kept in plastic cages in the animal house at Pharmacy Department University of Peshawar. This study was started from Mar 2019, to June 2019. All animals were kept in a proper temperature and humidity-controlled room (21-23°C, 40%−70% relative humidity) with a 12-hour light/dark photoperiod, proper ventilation and 30-70% humidity maintenance according to OECD guidelines 243 7. Food and water were provided ad libitum.[8,9] The experimental protocol was approved by the Ethical Committee of Khyber Medical College Peshawar.

Animals were divided into four groups. Test group A and test group B 25mg/kg and 50 mg/kg body weight of SCS was given respectively for seven days. Drug was administered by oral route through oral gavage tube. Negative control group C was given only normal diet while positive control group D was administered the known hepatotoxic drug, paracetamol, in its already established hepatotoxic dose (300mg/kg/day).[10]

Animals were euthanized by cervical dislocation and immediately after death, laparotomy was done. Livers were removed, washed and weighed. Then were fixed in formalin (10% formaldehyde solution), embedded in paraffin, and sectioned at 3.0 μm thickness for routine hematoxylin–eosin (HE) staining. The histo-morphological assessment was carried out on a light microscope (Nikon Eclipse E100). Microscopic Parameters were calculated according to the pre-set variables of Roenigk classification system.[11]

Descriptive statistical analysis was conducted through SPSS version 20. The four groups were compared by Kruskal-Wallis test. P= <0.05 was considered significant for analysis. The Qualitative data of liver Roenigk Grading were compared between the four groups by Kruskal-Wallis test. Mann-Whitney U test was used for individual group comparison

with Bonferroni adjustment. P<0.05 was considered significant for analysis.

RESULTS

The study sample included 16 rats, divided into four groups. Each group had four rats. Weights of all the experimental groups' rats were more than the controls; however, the difference was non-significant (table 1). Moreover, at the end of the study weights of the control group D were lowest while weight of 25 mg/Kg SCS group A rats were the highest. However, the mean difference before and after the intervention was non-significant. Similarly, the liver weight after dissection at the end of study was almost similar between the groups (p = 0.699).

Liver injury was evaluated by Roenigk Grading. All of the parameters of grading (except for fatty change) and final grading were significantly different between the groups. Mean ranks were highest in the 50 mg/Kg SCS group and lowest in the control group. Body and liver weight were also lower in the control group but not significantly different from the other groups.

For Individual group comparison Mann-Whitney U test with Bonferroni adjustment was used. Following groups were made for individual group comparison.

Overall grading 25 and 50 mg/kg SCS were significantly different from each other (table 2). There was no difference in the 25 mg/kg SCS and paracetamol groups (table 2). The 25 mg/Kg SCS and control groups were significantly different for nuclear polymorphism, Necroinflamatory changes and overall grading (table 2). The 50 mg/kg SCS and paracetamol groups were significantly different for liver fibrosis and overall grading.The 50 mg/KG SCS and control groups were significantly different for all the liver grading variables except for fatty change (table 2). Similarly, paracetamol and control groups were significantly different from each other for nuclear polymorphism, necro-inflammatory changes and overall grading (table 3.2). No fibrosis was seen in any group. As It was acute toxicity study that's why fibrosis could not be seen. Necroinflamatory changes in liver were observed in group A, B and C compared to group D (control) fig 1. However, no difference was observed between intervention groups A and B (table 3) nuclear pleomorphism was observed in hepatocytes of group A, B and C compared to group D(control) fig 3.2. pleomorphic changes in the intervention groups (A & B) were same (table 3) overall grading according to Roenigk grading system showed significantly higher toxic effects in group B(grade IIIa), compared to low dose group A (grade I), positive control group C (grade I 75% and grade II 25%). This difference was also statistically significant p=<0.001 (table 3)

DISCUSSION

Our study aim was to observe the histomorphological changes in liver after 7 days treatment of SCS two different concentrations. Before euthanizing the rats, they were examined. Rats of SCS groups (25mg/kg and 50mg/kg) were aggressive and agitated. Same effect was observed by Adams et al.,1993; Roeling et al.,1993 by microinjection of GABA antagonist[12] and also by Haller et al.,1998[13] with concurrent treatment of a glutamate agonist facilitated the attack behavior in rodents.

On microscopic examination the results of our study showed a significant damage in liver histological structure when SCS was used in higher concentration (50mg/kg). However, when used in lower concentration the liver damage was less severe compared to the damage caused by paracetamol in 300mg/kg dose.

Histological damage was mostly observed as necrosis in the liver tissue. Necrosis was spotty necrosis at multiple site with little mild or no inflammatory changes at necrosis site. This is in accordance with the mechanism of drug induced liver injury (DILI)

Table 1: Body and liver weight of rats at baseline and after intervention

	Group A. 25 mg/kg SCS n=4	Group B. 50 mg/kg SCS n=4	Group C. Paracetamol 300mg/kg n=4	Group D. Control. n=4	P Value
Weight Baseline	209.50 ± 19.416	196.25 ± 10.046	200.00 ± 15.232	182.25 ± 8.655	0.158
Weight After	202.50 ± 12.069	196.25 ± 16.153	200.75 ± 7.805	183.75 ± 7.500	0.169
Liver Weight	8.25 ± .957	7.75 ± 1.500	8.25 ± 1.258	7.50 ± 0.577	0.699

Kruskal-Wallis test

Table 2: Group comparison of mean ranks for body and liver weight and liver Roenigk Grading

	Group A. 25 mg/ kg SCS n=4	Group B. 50 mg/ kg SCS n=4	Group C. Paracetamol 300mg/ kg n=4	Group D. Control. n=4	P Value
Fatty Change	8.38	10.75	8.38	6.50	0.424
Nuclear Polymorphism	10.50	10.50	10.50	2.50	0.002
Necroinflamatory Changes	9.50	11.00	11.00	2.50	0.008
Fibrosis	8.50	8.50	8.50	8.50	1.000
Grading	8.00	14.50	9.00	2.50	0.002
Weight Baseline	11.75	8.75	9.25	4.25	0.158
Weight After	10.50	9.00	10.50	4.00	0.169
Liver Weight	10.00	8.00	9.50	6.50	0.699

Kruskal-Wallis test

Table 3: Comparison of Roenigk classification in different groups

Roenigk Grade	Group A (25 mg/ kg) SCS	Group B (50 mg/ kg) SCS	Group C. Paracetamol 300mg/kg	Group D. Control	P Value
0	0 (0%)	0 (0%)	0 (0%)	4 (100%)	<0.001
1	4 (100%)	0 (0%)	3 (75%)	0 (0%)	
2	0 (0%)	0 (0%)	1 (25%)	0 (0%)	
3	0 (0%)	4 (100%)	0 (0%)	0 (0%)	

Chi square test

described by Ramachandran et al 2009.[14] Inflammation was mostly at periportal sites, with infiltrates of acute inflammatory cells. In some areas hydropic changes in hepatocytes were observed. Necroinflamatory changes were more in group B and group C.

Macro vesicular steatosis was present in group A(25mg/kg), group B(50mg/kg) and group C (paracetamol 300mg/kg). In group A macro vesicular steatosis was seen in acinar zone 2 of hepatic acini (fig 3.2). While in group B macro vesicular steatosis was extensive but limited to zone 1 of hepatic acini. In group C steatosis was less as compared to group B and it was seen mainly in zone 1 of hepatic acini with little overlap in zone 2 also. In humans such macro vesicular pattern is common finding in general population, alcoholics, obese people and diabetics.[15]

Nuclei were hyperchromatic which showed cellular toxicity of the SCS. While in the areas of spotty necrosis pyknotic nuclei were seen along with hydropic changes in the hepatocytes. Binucleate cells were also observed at multiple sites which shows cells in the recovery phase.

As it as acute toxicity study so fibrosis was not observed in all groups. After all these histomorphological changes hepatic lobular architecture was still intact.

Scores derived from these types of observations, no matter how precisely defined, invariably contain a subjective element related to the observer, and thus there is always some degree of observer variability.

CONCLUSIONS

Significant effects of SCS were observed in liver of Sprague dawley rats, however, the toxic effects were less severe. Liver Parenchyma and architecture were preserved.

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