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Effect of Apple Cider Vinegar on Expression of *SASH1* Gene in Liver Toxicity Induced by Gentamicin in Albino Rats

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Abstract

The liver plays an essential part in xenobiotic metabolism of the body. Gentamicin is effective against the life threatening gram-negative bacterial infections, while apple cider vinegar decreases the tissue damage and inflammation as proved by histopathological analysis.

This experiment was designed to study the protective role of apple cider vinegar in hepatotoxicity induced by gentamicin and using the qt - PCR technique, gene expression of *SASH1* was done in adult albino rats.

In this experiment, three groups, including 10 adult albino rats each were labelled A, B, and C. Group A was controlled and it received distilled water 4ml/kg/day via intraperitoneal. Group B received only Gentamicin 100mg/kg/day for 11 consecutive days. Groups C received Apple cider vinegar 2ml/kg/day for first 10 days, then along with Gentamicin 100mg/kg/day for next 11 days upto day 21st. Rats were sacrificed on 22nd day 24hrs after administration of the last dose. Gross and histological parameters were studied. The results were analyzed by SPSS version 22.0. A p-value ≤ 0.05 was considered as statistically significant. Expression of *SASH1* gene was analyzed by Real time reverse transcriptase–polymerase chain reaction (RT—PCR). For this purpose, ABI PRISM 7300 sequence detection system was used with FAM dye. For internal reference, *HPRT* was considered as a housekeeping gene.

Keywords: HPRT, PCR, SASHI, Ligament.

Introduction

The liver is an intra-abdominal organ that weighs approx. 1.5grams, which makes up to 2.5% of body weight. [1] Falciform ligament demarcates the division of liver into two lobes, right and left.

Histologically, the structural unit of the liver consists of hepatic lobule that is roughly hexagonal in shape. [1]At each corner of every lobule, a portal triad is present that is composed of hepatic arteriole, portal venule and bile ductule. [2]

Gentamicin has a narrow therapeutic window and is toxic at high doses.[3]Aminoglycosides have a structural unit of amino sugars. These are linked to a 4,6-di-substituted deoxystreptamine ring through glycoside bonds. This family includes gentamicin, amikacin, plazomicin and tobramycin. [4]

The FDA of the U.S approved its routine preparation to treat infective endocarditis, sepsis, meningitis, peritonitis, bacterial conjunctivitis and infections caused by gram-negative bacteria. [5.] Despite the beneficial effects, it has been reported that free oxygen radicals are generated by gentamicin that causes injury to tissues like ear and kidneys. [6]In the kidneys, gentamicin interferes with protein synthesis. It triggers necrosis in renal tubular cells. [7]LD50 of gentamicin in rats is ~ >5000mg/kg (orally), 96mg/kg (I/V), 384mg/kg (I/M), 559mg/kg (I/P) body weight. [8]

Unpasteurized or organic apple cider vinegar has a cobweb-like appearance and is present at 5% concentration. [9]LD50 of apple cider vinegar in rats is 3310mg/Kg body weight.

SASH1 (SAM and SH3 Domain Containing 1) gene is responsible for encoding proteins. Also, many studies showed significant down regulation of this gene in tumor cells of multiple human cancer cases.

Materials and Methods

An experimental study was performed on rats to observe the morphological changes in liver and *SASH1* gene expression. This study was carried out in Research Laboratory, Postgraduate Medical Institute (PGMI) and Central research lab, Lahore General Hospital. The therapeutic reagents used in this study were Gentamicin and Apple cider vinegar. The protocols for the study were approved by the Advanced Studies and Research Board of University of Health Sciences, Lahore.

For the detection of a difference of 70% - 90% in the histological findings, a sample size of 30 albino rats was needed at a significance level of 5% with a Power of the study of 90%, a sample size of 10 in each group (total groups =3) was required to make a total sample size of 30.

Procedure

30 adult male albino rats were procured from animal house of PGMI. Each animal was then weighed and evaluated thoroughly before the commencement of study.The healthy rats were about 8-10 weeks of age with a weight range of 180-220g. The environment of animal house was well ventilated and the temperature maintained at 24±2ºC, humidity 55 ± 5% and dark & light cycles, each cycle of 12 hours.[10]All the protocols, laid by the international, natural and institutional guidelines for the care and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care, were followed while the animals used in this study. [11]

Grouping and Treatment

The grouping of rats was in three groups, namely, A, B and C. 10 rats were placed in each group by using a random number generator. Rats and their cages were labeled by using waterproof markers as group A (black), group B (blue), and group C (Red). Every rat's tail was marked for identification.

Group A: (n=10) was a control group. Rats were given 4ml distilled water intraperitoneally at the time of dose administration for 21 consecutive days**.**

Group B: (n=10) was experimental group. Rats were treated with 4ml distilled water for 1st-10 days, then gentamicin, 100mg/kg bodyweight dissolved in 1ml of distilled water, was administered intraperitoneally once daily for next 11 days up to day 21.

Group C: (n=10) was experimental group. Rats were treated first with Apple cider vinegar 2ml/kg bodyweight only by oral gavagefor 10 days, then along with Apple cider vinegar 2ml/kg bodyweight through oral gavage, gentamicin 100mg/kg body weight diluted to a volume of 1ml of distilled water was administered once daily intraperitoneally for next 11 days up to day 21.

Table 1: Experimental Groups of Animals, Mode of Intervention and Dosage of Drug

Groups	Intervention And Dosages	Number of Animals (N)	Method of Administration	Duration of Dosage	Day of Sacrifice
Group A	4ml of distilled water	10	Intraperitoneally	21 days	22nd
Group B	Initially 4ml distilled water for 10 days, then gentamicin 100mg/kg b.w/day for next 11 days upto day 21	10	Intraperitoneally	21 days	22nd
Group C	Apple cider vinegar 2ml/kg b.w/day for 1st 10 days then both Apple cider vinegar 2ml/kg b.w/day plus gentamicin 100mg/kg b.w/day for next 11 days upto day 21	10	Gentamicin intraperitoneally an Apple cider vinega Orally by gavage tube	21 days	22nd

Dissection and Tissue Sampling

At the end of the experimental period, on the 22nd day, 24 hours after the administration of the last dose of the agent, each rat was given anesthesia. For 2-3 minutes, the rat remained in the tightly closed jar. [13]Liver was divided into two halves. One half was snap-frozen at -80°C for RT-PCR. The other half was fixed using 10% NBF (Neutral Buffered Formalin).

Quantitative Real-Time PCR

Real time reverse transcriptase–polymerase chain reaction (RT—PCR) was utilized to determine the expression of *SASH1* gene. For this purpose, ABI PRISM 7300 sequence detection system was applied with FAM dye. For internal reference, expression of *HPRT* was used as the housekeeping gene.

Statistical Analyses

The analysis of gathering data was done by applying SPSS 22.

To determine the association of quantitative variables, one way ANOVA was applied. The Post Hoc Turkey test was used for multiple comparisons of quantitative variables between different experimental groups.

P-value of ≤0.05 was considered as statistically significant.

Observations and Results

Body Weight of Rat: The body weight of the rat was done at the start of the experiment and at the end of the experiment. All the rats stayed healthy during the experiment. One way ANOVA test was used for the analysis of change in weight of the rats before and after the experiment. The data obtained shows that the mean body weight of the rats was not significantly different

before the experiment (p-value=0.654) but it was different at the end of the experiment (p-value=0.044 for mean body weight, p-value<0.001 for weight gain).

Table 2: Comparison of Initial Body Weight and Final Body Weight amongst Groups

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	P-value#
Initial body weight (g)	178.9 ± 5.78	179.4 ± 5.50	181.1 ± 5.36	0.654
Final body weight (g)	183.4 ± 5.32	178.4 ± 5.72	184.8 ± 5.96	0.044*
Weight gain (g)	4.50 ± 1.18	-1.00 ± 1.83	3.70 ± 1.57	< 0.001*

P value ≤ 0.05 is regarded as significant statistically

For the multiple comparisons between groups Post hoc Turkey test was applied. The result shows that there is a significant difference in mean body weight between group B and C. There is no difference in mean final body weight of groups A and C.

The mean weight gain in group B was significantly lesser in comparison to group A and C. There is no notable difference in weight gain of group A and C.

Table 3: Pairwise comparison of mean body weight and weight gain after experiment amongst groups

	Group	Group	Mean Difference	Std. Error	P-value
Weight after experiment	A	B	5.00	2.54	0.139
		C	-1.40	2.54	0.846
	B	C	-6.4000*	2.54	0.045
Weight gain	A	B	5.50000*	0.69	<0.001
		C	0.80	0.69	0.489
	B	C	-4.70000*	0.69	< 0.001

*p value ≤ 0.05is regarded asstatistically significant

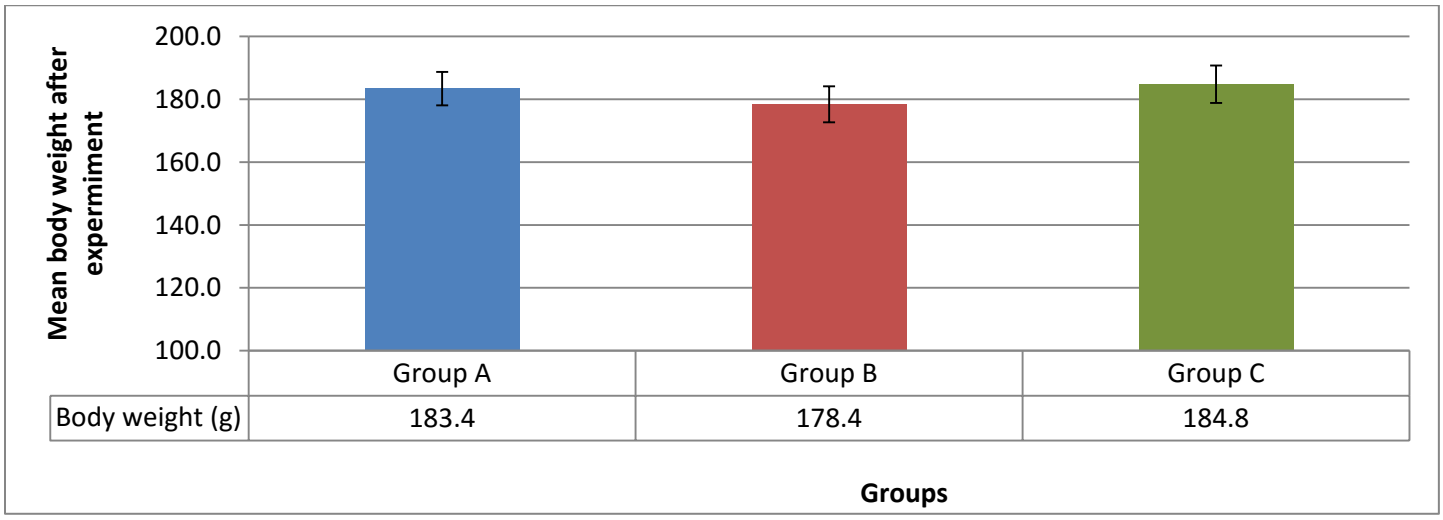

Fig. 1: Bar chart displaying a comparison of mean weight (g) after experiment among groups.

Weight of Liver and Relative TissueWeight Index (RTWI)

The mean liver weight and relative tissue weight index were observed in all groups. The results of one way

ANOYA showed a great difference in these parameters in the groups (p-value<0.001).

Table 4: Comparison of weight of liver and relative tissueweight index between groups

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	P-value#
Liver weight (g)	4.25 ± 0.55	3.87 ± 0.46	4.89 ± 0.26	< 0.001*
Relative tissue weight index	2.32 ± 0.25	2.17 ± 0.21	2.65 ± 0.12	< 0.001*

P value ≤ 0.05 is regarded as significant statistically

The Post hocTurkey test was applied for pairwise comparisons amongst groups, which indicated that liver weight and relative tissue weight index of group C was notably higher when compared between group A and B. Significantvariation was also observed in mean liver weight and relative tissue weight index among group A and B.

Table 5: Pairwise comparison of weight of liver and relativetissue weight index among groups

Variable	Group	Group	Mean Difference	Std. Error	P-value
Liver weight (g)	A	B	0.38	0.20	0.150
		C	-0.64000*	0.20	0.008
	B	C	-1.02000*	0.20	< 0.001
Relative tissue weight index	A	B	0.15	0.09	0.236
		C	-0.33100*	0.09	0.003
	B	C	-0.48000*	0.09	< 0.001

P-value ≤ 0.05 is regarded as significant statistically

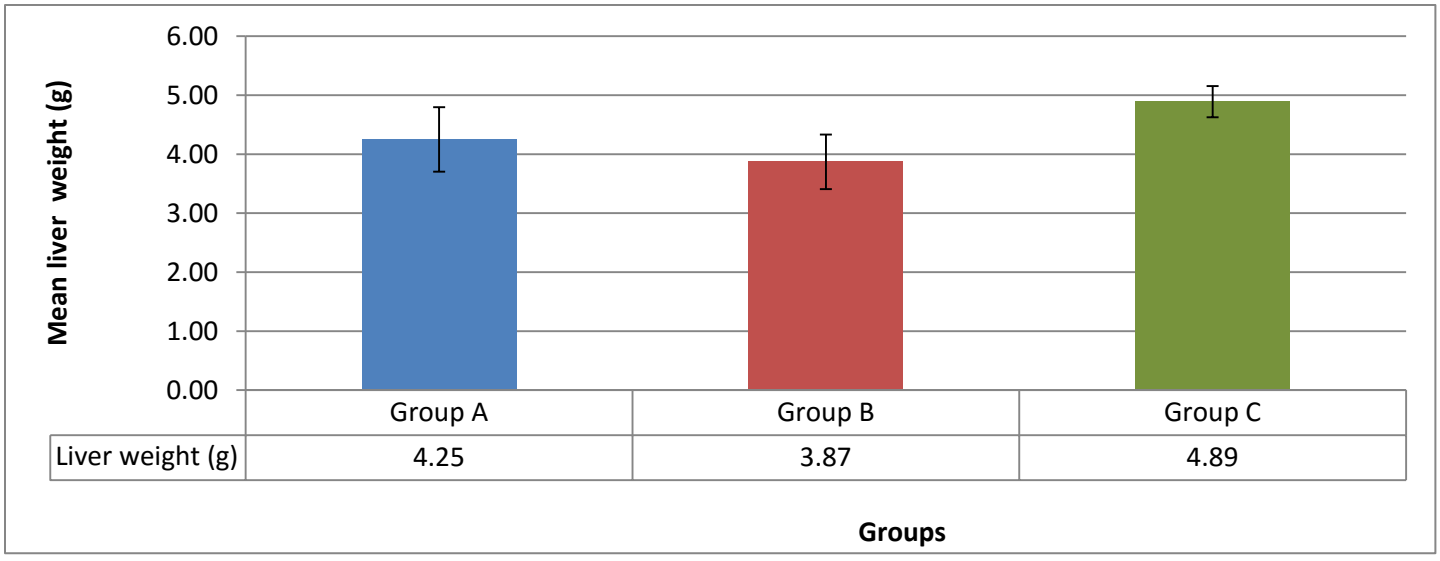

Fig. 2: Bar chart displaying a comparison of liver weight index among groups

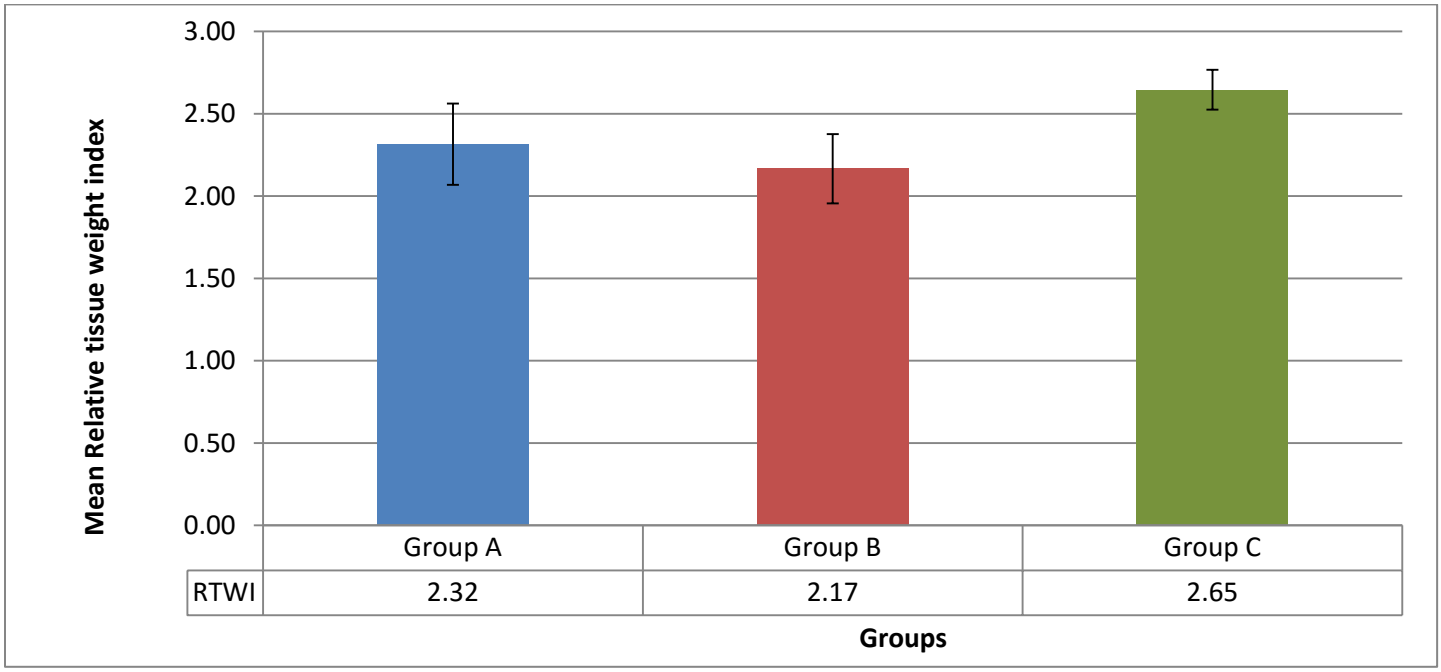

Fig. 3: Bar chart displaying a comparison of relative tissue weight index among groups

Expression of *SASH1* gene (CT *SASH1*)

The mean CT *SASH1* in all groups was determined. One way ANOVA test was used for the comparison of the CT *SASH1* among groups. It was found that the mean CT *SASH1*in all groups were significantly different(p value < 0.001) (Table 8; Fig 5).

Table 6: Comparison of CT *SASH1* among groups

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	P-value#
CT *SASH1*	20.51 ± 1.15	17.04 ± 0.63	19.75 ± 0.72	< 0.001*

One way ANOVA

p value ≤ 0.05 is regarded as significant statistically

For multiple comparisons, post hoc Tukey test was used which revealed that CT *SASH1* in group A and C was significantly higher when compared with group B. However, no significantdifference was observed in the CT *SASH1* between the group A and C.

Table 7: Pairwise comparison of CT *SASH1* among groups

Group	Group	Mean Difference	Stnd. Error	p-value
A	B	3.46800*	0.39	< 0.001
	C	0.75	0.39	0.143
B	C	-2.71300*	0.39	< 0.001

P value ≤ 0.05 is regarded as significant

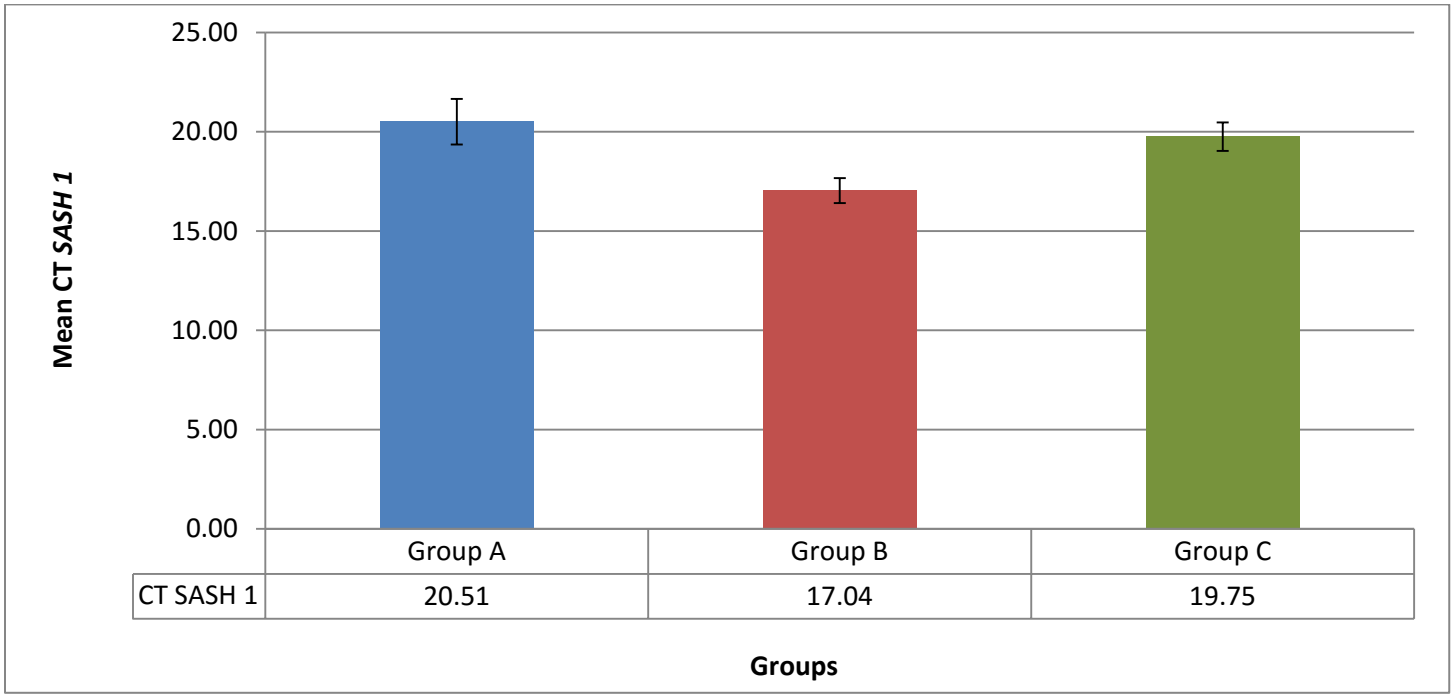

Fig.4: Bar chart displaying a comparison of CT *SASH1* among groups.

Expression of *HPRT* (CT *HPRT)*

The mean CT *HPRT* in all groups was also determined. One way ANOVA test was used for the comparison of the CT *HPRT* among groups. It was found that there was no significant difference in mean CT *HPRT*among groups(p value < 0.001).

Table 8: Comparison of CT *HPRT* among groups

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	P-value#
CT *HPRT*	30.38 ± 0.88	30.49 ± 0.48	30.72 ± 0.63	0.536

One way ANOVA

P value ≤ 0.05 is regarded as significant statistically

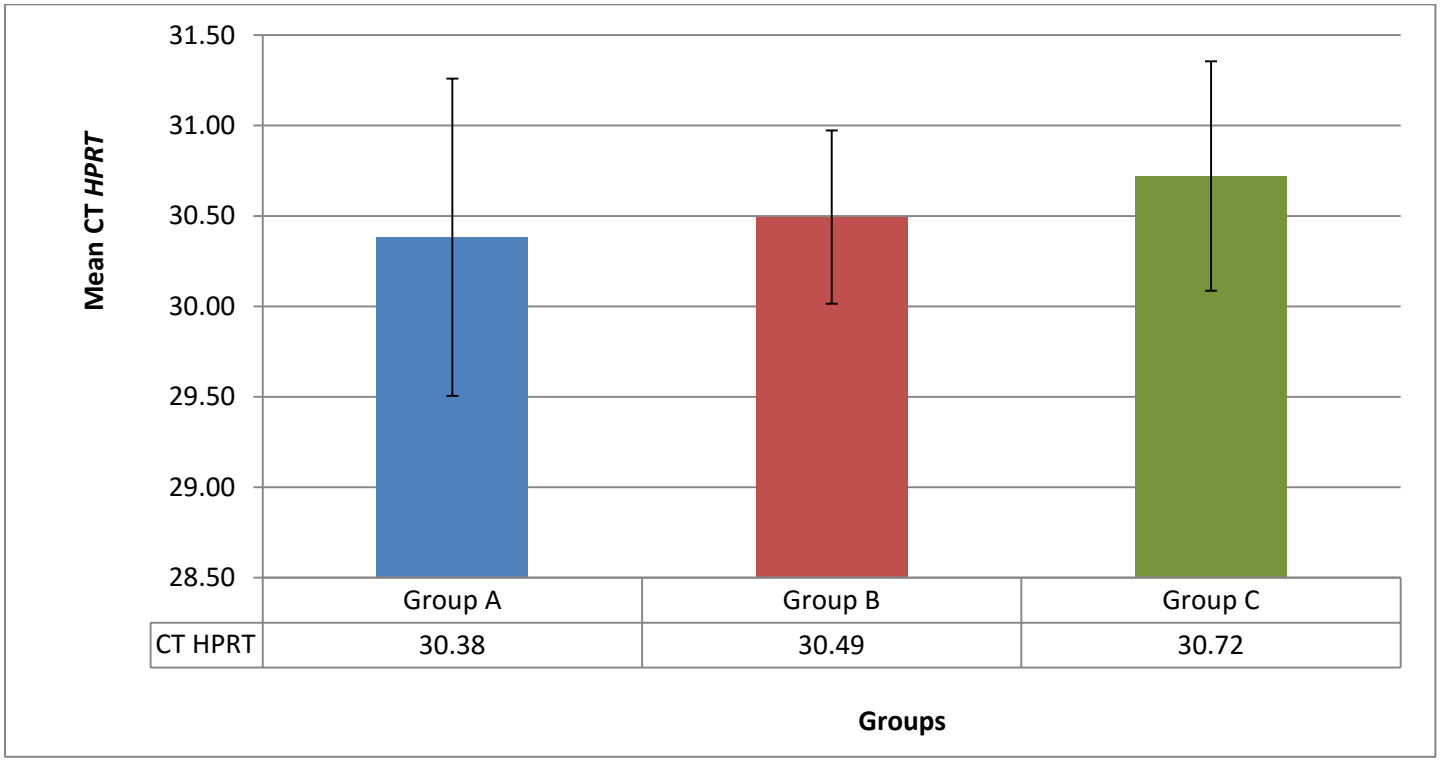

Figure 5: Bar chart displaying a comparison of CT *HPRT* among groups.

Delta CT (CT *HPRT*-CT *SASH1*):

The mean ΔCT in all groups was determined. One way ANOVA test was used for the comparison of the ΔCT among groups. It was found that the mean ΔCT in all groups were significantly different(p value < 0.001).

Table 9:Comparison of ΔCT among groups

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	P-value#
ΔCT (CT *HPRT*-CT *SASH1*)	9.88 ± 1.18	13.46 ± 0.90	10.97 ± 0.91	< 0.001*

One way ANOVA

P value ≤ 0.05 is regarded as significant statistically

For multiplecomparisons, Post hoc Turkeytest was used which revealed that ΔCT in group B was significantly higher when compared with group A and C. However, no significantdifference was observed in the ΔCT (CT *HPRT*-CT *SASH1*) between the group A and C.

Table 10: Pairwise comparison of ΔCT among groups

Group	Group	Mean Difference	Stnd. Error	P-value
A	B	-3.58000*	0.45	0.00
	C	-1.09	0.45	0.05
B	C	2.48600*	0.45	0.00

P value ≤ 0.05 is regarded as significant

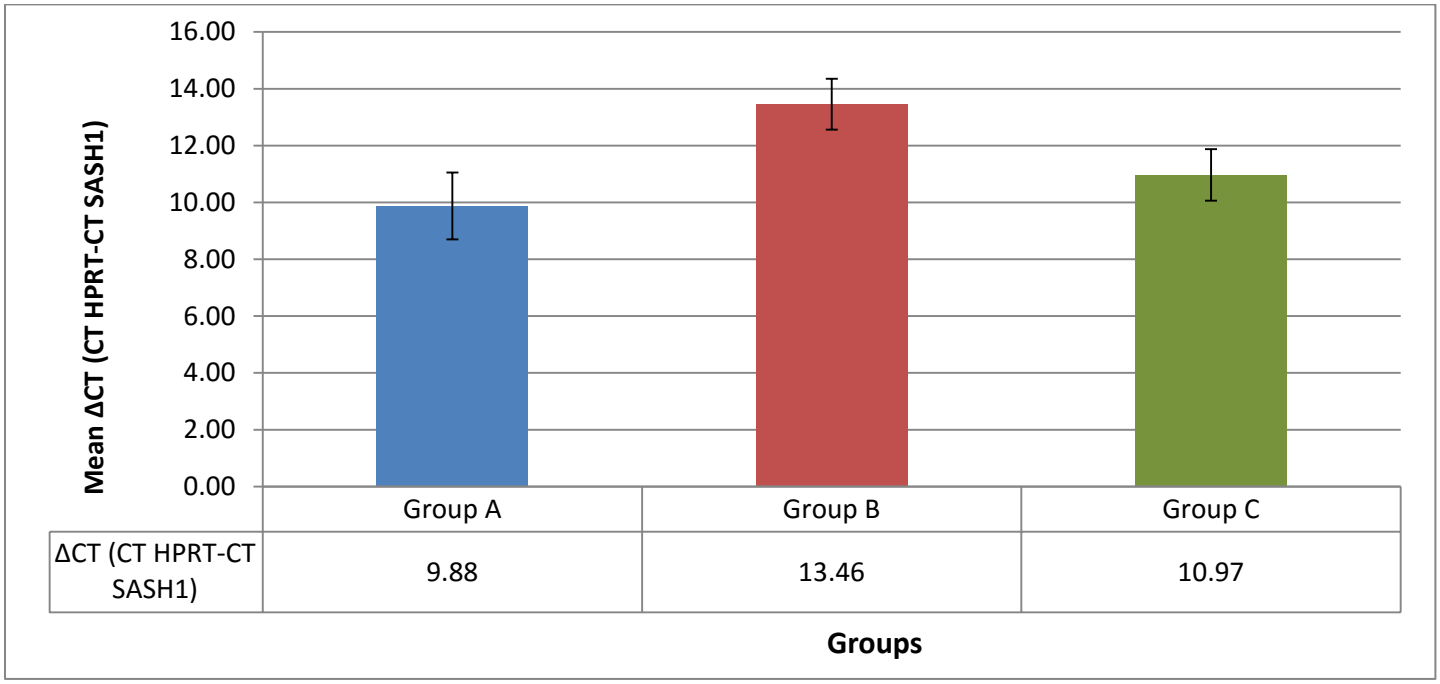

Fig.6: Bar chart displaying comparison of ΔCT (CT *HPRT*-CT *SASH1*) among groups.

Discussion

Gentamicin is responsible for cell necrosis by enhancing oxidative stress. [15]Apple cider vinegar reduces peroxidase and catalase activity and up-regulates the superoxide dismutase activity in rats. [16]*SASHI* is an anti-tumor gene.[17] The weight gain of rats of experimental group B was observed in this experiment. However, there was no considerable difference in weight of liver in apple cider vinegar treated group C with respect to the control group A. [20]

Mean relative tissue weight index was significantly less in gentamicin treated group B as compared to control group A while the mean value of relative liver weight in apple cider vinegar treated group C was near the control group A. [18]

Weight of liver declined in group B due to necrotic changes in hepatocytes. This result is same as the study carried out by Jannat. [18]However, this is in contrast with the result of study by Sharef and Aljamali[19] that showed an increase in liver weight due to inflammation and edema of hepatocytes while giving gentamicin 80 mg/kg for 15 days.

The RT-PCR results showed statistically significant changes among *SASH1* gene expression in all three group p-value. Expression of *SASH1* gene was down regulated in group B as compared to Group A and tere was an upregulation in the expression ofApple cider vinegar treated group C. Thereby showing the protective effect of apple cider vinegar against gentamicin on molecular and genetic level.

Conclusion

From the results, it is concluded that the effect of gentamicin is extended as far as genetic level shown by the down regulation of *SASH1* gene. It also shows that apple cider vinegar protect against at the genetic level by protecting the mechanisms of protein synthesis in cells that is clear from the result of *SASH1* gene expression which is near to normal level in this group. More research is required to explore the effect of these agents on liver on a genetic level.

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