ORIGINAL ARTICLE

EFFECTS OF AVOCADO (*PERSEA AMERICANA*) AQUEOUS SEED EXTRACT ON GROSS FEATURES OF LIVER AND HEPATOCYTE SIZE IN ISONIAZID AFFECTED ALBINO RATS

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Background: Isoniazid (INH) has been associated with severe hepatotoxicity and fatal liver injury. This study aimed to observe the hepatoprotective effects of concomitant use of avocado aqueous seed extract on the gross features of liver and size of hepatocytes in INH induced hepatotoxicity in albino rats. **Methods:** This experimental study was conducted from Jan–Jun 2019. Thirty-six male albino rats were divided into 4 groups. Groups were administered treatment orally for 30 days. Group 1 received distilled water 1 ml/Kg/day. Group 2 received INH 100 mg/Kg/day dissolved in 1 ml distilled water. Group 3 was given aqueous avocado seed extract 250 mg/Kg/day in 2 ml distilled water and Group 4 received aqueous avocado seed extract 500 mg/Kg/day in 4 ml distilled water. **Results:** Highest mean liver weight was seen in Group 4 and lowest in Group 2. The weight of liver was significantly different among all groups but Relative Tissue Weight Index among all groups was insignificant. Colour of liver in four groups varied from reddish to pale red and reddish brown. The texture of all livers was smooth. Hepatocyte size was found to be largest in Group 2 and smallest in Group 1. Comparison among groups showed significant difference in sizes of hepatocytes. **Conclusion:** Concomitant use of aqueous avocado seed extract prevents INH-induced hepatotoxicity by maintaining body and liver weights, gross features of liver, and size of hepatocytes in a dose-dependent manner.

Keywords: Avocado, Isoniazid, Liver, Hepatocytes, Albino rats

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INTRODUCTION

Isoniazid (INH) has been used as a first line drug for the treatment of TB since 1952.[1,2] Structurally, it has a pyridine ring and hydrazine group and has potent bactericidal effects on rapidly growing TB bacilli.[1] INH has been associated with severe hepatotoxicity and fatal liver injury by causing necrosis and steatosis of hepatocytes.[3]

The incidence of liver toxicity with isoniazid is 1.6%. Metabolites of INH mainly produce oxidative stress resulting in hepatic injury.[4,5] Oxidative stress is caused by depletion of both bound and free glutathione (GSH), increase in the activity of superoxide dismutase which readily converts O_2 into hydrogen peroxide (H_2O_2), decrease in the catalases, and glutathione peroxidases that readily remove H_2O_2 resulting in buildup and accumulation of H_2O_2.[5,6] Hydrogen peroxide is a very potent oxidizing agent causing oxidative stress either directly or indirectly through the generation of reactive oxygen species (ROS).[6]

Oxidative stress is the main reason of INH induced hepatotoxicity and substances that have anti-oxidant properties can prevent it. Avocado is one of such possible fruits that can decrease the INH induced hepatotoxicity. Avocados are large berries containing a single seed weighing about 12–16% of the total weight of the fruit.[7] Avocado is a rich source of glutathione. Each 100 grams contains 27.7 mg of glutathione, making it the richest source, secondary to asparagus.[8] Glutathione is referred to as body's master antioxidant. The anti-oxidant effects of avocado are also because of the fact that it increases the serum glutathione levels as reported by Mahmoed *et al*.[9]

Ultrasonic extractions of avocado seed and peel demonstrated high contents of phenolic compounds in both ethanolic and aqueous seed and peel extracts than that of pulp.[10–12] Avocado seeds contain 64% of total phenolic compounds present in it and are responsible for 57% of total antioxidant capacity.[13]

The objective of the present study was to observe the hepatotoxic effects of concomitant use of Avocado (*Persea americana*) aqueous seed extract on the gross features of liver and size of hepatocytes in Isoniazid (INH) induced hepatotoxicity in albino rats.

METHODOLOGY

It was an Experimental study conducted on 36 adult male albino rats after taking approval from the IRB (letter# 205/RC/KEMU) and ASRB (letter# 10220/ KEMU/2018), KEMU. The study was carried out from Jan to Jun 2019 at Experimental Research Laboratory (Animal House) of Postgraduate Medical Institute, Lahore in collaboration with Anatomy Department and Histopathology Laboratory of KEMU.

A total of 36 male Sprague Dawley Albino rats of 8–12 weeks age, weighing between 200–250 grams were randomly divided into 4 equal groups by lottery method. Animals were allowed to acclimatize for one week before start of experiment. Any rats that became inactive or stopped eating were excluded. They were kept under controlled conditions of temperature (27–30 °C) and 12 hours of light and dark cycle were maintained. The animals were fed on standard diet and tap water *ad libitum*.

Group 1, (Control group, CG) received only distilled water 1 ml/Kg/day in morning.[14] Group 2, (Isoniazid group, INHG) received only isoniazid 100 mg/Kg/day dissolved in 1 ml distilled water as a single dose in morning.[4] Group 3, (Isoniazid-Avocado (low dose) group, $INHAV_{low}$) was given Isoniazid 100 mg/Kg/day dissolved in 1 ml distilled water as a single dose in morning[4], and Avocado seed extract (aqueous) 250 mg/Kg/day dissolved in 2 ml distilled water as a single dose in morning, one hour after INH.[14] Group 4, (Isoniazid-Avocado (high dose) group, $INHAV_{high}$) received Isoniazid 100 mg/Kg/day dissolved in 1 ml distilled water as a single dose in morning[4], and Avocado seed extract (aqueous) 500 mg/Kg/day dissolved in 4 ml distilled water in two divided doses of 2 ml each. 1st dose was given in morning, one hour after INH and 2nd dose in the afternoon. All doses were given orally by gavage method for 30 days.

The body weights of experimental animals were measured at day zero, on weekly basis for dose adjustment, and at the end of experiment on 31st day. After 24 hours of the last dose of drugs animals were euthanized with morphine 0.3 to 0.5 mg/Kg intraperitoneally[15] and liver was dissected out. It was washed with normal saline and weighed. Gross examination of liver was done and photographed.

The liver specimens were fixed in 10% formalin solution for processing. The slides were stained with Haematoxylin and Eosin (H and E) stains for microscopy. The slides were examined for hepatocytes at 20× magnification using Nikon Eclipse Ci-L LED microscope. In each slide, 3 fields were studied and 5 hepatocytes with clearly demarcated cell membranes were selected. Average of hepatocyte dimensions were taken as size of that hepatocyte. Mean size of 15 hepatocytes was taken as size of hepatocyte for that particular slide. Mean size of hepatocytes of 3 slides was taken as hepatocyte size for that particular specimen.

The data from the 4 groups was entered and analysed using SPSS-26. Mean±SD were calculated for quantitative features. One-way ANOVA was used to construct the comparison among all groups. Qualitative or categorical data were presented as frequency and percentage. Pair-wise comparison was done using Least Square Difference (LSD) Test. Chi-square test was used to construct a comparison among groups on the basis of qualitative/categorical characteristics, and $p<0.05$ was taken as significant.

RESULTS

The Mean±SD of initial body weights of rats of four groups were 200.4±9.71 g (CG), 203.89±29.41 g (INHG), 200.44±14.98 g ($INHAV_{low}$) and 210.56±24.58 g ($INHAV_{high}$). The final body weights were 223.56±8.09 g (CG), 185.11±20.55 g (INHG), 197.56±22.86 g ($INHAV_{low}$), and 248.33±23.85 g ($INHAV_{high}$). An increase in final body weight in CG and $INHAV_{high}$, but a decrease in final body weight in INHG and $INHAV_{low}$ was observed. Comparison among groups for initial and final body weights by One-way ANOVA showed that the difference of final body weight among all groups was significant ($p=0.000$). However, pair-wise comparison among groups for final body weight showed significant difference in all possible groups ($p<0.05$) except between groups INHG and $INHAV_{low}$.

Mean liver weight of the rats of four groups were 7.28±0.96 g (CG), 6.69±0.88 g (INHG), 6.78±0.76 g ($INHAV_{low}$) and 8.32±1.09 g ($INHAV_{high}$). Highest mean liver weight was seen in $INHAV_{high}$ and lowest in INHG. Relative Tissue Weight Index (RTWI) was 3.26±0.43 (CG), 3.61±0.29 (INHG), 3.44±0.18 ($INHAV_{low}$) and 3.35±0.28 ($INHAV_{high}$). One-way ANOVA showed that the weight of liver was significantly different among all groups ($p<0.05$) but RTWI among all groups was insignificant (Table-1). When pair-wise comparison was done using LSD test, it showed that weight of liver in $INHAV_{high}$ is significantly more than that of other groups ($p<0.05$). RTWI was significantly higher in INHG as compared to CG but in all other possible pairs between groups, it was insignificant. (Table-1).

Table-1: Comparison of final body weights, weight of liver, and RTWI (One-way ANOVA) among groups

	Sum of Squares	df	Mean Square	F	*p*
Body Weight Final (g)					
Between Groups	21370.97	3	7123.66	18.04	0.000*
Within Groups	12639.33	32	394.98		
Total	34010.31	35			
Weight of Liver (gram)					
Between Groups	15.184	3	5.061	5.831	0.003
Within Groups	27.776	32	0.868		
Total	42.960	35			
Relative Tissue Weight Index					
Between Groups	0.617	3	0.206	2.166	0.111
Within Groups	3.039	32	0.095		
Total	3.656	35			

Colour of liver in 4 groups varied from reddish to pale red and reddish brown. In CG, it was reddish in

all animals (Figure-1). However, in INHG, 33.3% had reddish and 66.7% had pale red colour (Figure-2) and in $INHAV_{low}$ group, 66.7% had reddish, 11.1% had pale red and 22.2% had reddish brown colour. Reddish colour of the livers was seen in 77.8% $INHAV_{high}$ group animals, and 22.2% had reddish brown colour. Colour change was statistically significant in all experimental groups (p=0.002).

The texture of all livers was smooth. No other gross abnormality except haemorrhagic spots was observed. In INHG, 33.3% livers, and in $INHAV_{low}$ group 22.2% livers had haemorrhagic spots.

The slides showed hexagonal shaped hepatic lobules, each having central vein lined by squamous epithelium, one or two cell thick radiating cords of hepatocytes and peripherally arranged portal triads. Hepatocytes were polygonal in shape in all groups. Mean sizes of hepatocytes for four groups were 18.93±1.07 µm (CG), 28.69±1.45 µm (INHG), 26.71±1.52 µm ($INHAV_{low}$) and 20.52±1.57 µm ($INHAV_{High}$). Hepatocyte size was found to be largest in INHG and smallest in CG. Comparison among groups by One Way ANOVA showed significant difference in sizes of hepatocytes (p=0.000). LSD test showed a significant increase in sizes of hepatocytes in INHG, $INHAV_{low}$ and $INHAV_{High}$ in comparison to CG (p<0.05). When $INHAV_{low}$ group was compared with INHG, a statistically significant decrease in sizes of hepatocytes was observed (p=0.006). However, the test also showed that the hepatocytes of $INHAV_{High}$ group were significantly smaller in size as compared to INHG and $INHAV_{low}$ (p=0.000). (Table-2).

Table-2: Comparison among groups for size of Hepatocytes (µm) (One Way ANOVA)

Size of Hepatocytes (µm)	Sum of Squares	df	Mean Square	F	*p*
Between Groups	601.375	3	200.458	99.948	0.000*
Within Groups	64.180	32	2.006		
Total	665.556	35			

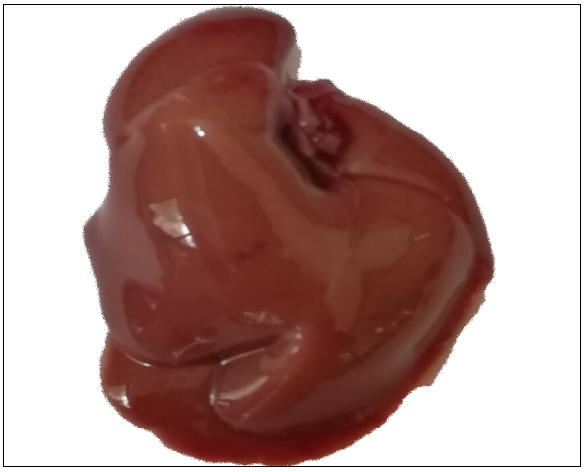

Figure-1: Reddish liver of control group

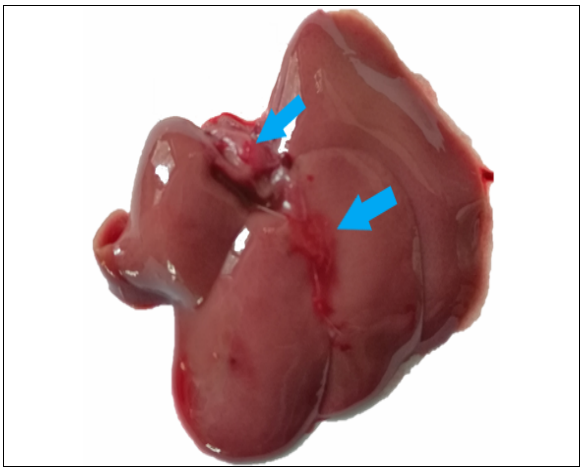

Figure-2: Pale red liver with haemorrhagic spots

DISCUSSION

In the current study, variations were observed in final body weights when the weights of experimental animals were measured at the start and end of experiment were statistically analysed. In control group, the mean weight increased, while in the INHG a decrease in body weight was seen. This effect was in accordance with a previous study conducted by Cavanagh[16] on a rat model where INH was given in a dose of 250 mg/Kg/day by oral and subcutaneous routes. It concluded that oral INH produced failure to gain weight because of more pronounced absorptive inhibitory effects on digestive system than subcutaneous route in absorption of amino acids through intestinal wall.[16] In the group that received avocado seed extract in low dose along with INH ($INHAV_{low}$), the final body weight was also found to be reduced but this decrease in weight was less than that seen in INHG. It could most likely be due to co-administration of avocado seed extract. The experimental animals which were administered high dose of extract along with INH ($INHAV_{high}$) showed a significant increase in final body weight as compared to other groups. This indicated an effective dose dependent role of avocado seed extract on weight gain even in presence of INH. This finding is in concordance to the observations of Alhassan *et al*[17]. They observed the effects of avocado seed extract on diabetic rats. There was a significant increase in body weight of rats in comparison to those which did not receive extract. It was probably because the extract overcame the INH induced inhibitory effects on intestinal amino acid absorption, thus enhancing proper utilization of nutrients.[17] This corresponds to the results of our study in terms of weight gain seen in $INHAV_{high}$.

The weight of the liver was in accordance with the final body weight of rats in all groups when the mean liver weight and RTWI for each liver was calculated. However, mean liver weight was lowest in INHG and it was noted that weight of the liver in

$INHAV_{high}$ was significantly higher in comparison to other groups. The reduced liver weight in INHG is in agreement to the finding of Humayun *et al*[18] who studied the hepatoprotective effects of propolis ethanolic extract in INH induced hepatotoxicity in albino mice.[18] The gain in liver weight with high dose of avocado seed extract in the present study was similar to the results observed by Uchenna *et al* who studied the effects of avocado ground seeds on carbohydrate and lipid metabolism in a rat model. An increase in weight of liver was documented on giving diet containing 8% of avocado seeds.[19]

The texture of the liver was found to be smooth showing no deleterious effects of INH on liver surface as reported by Humayun *et al*[20]. In the present study, variations of colour were observed. In CG, all livers were reddish in colour reflecting good vascularity of tissue. In INHG, most of the livers were pale red and a few had haemorrhagic spots. This pale colour change was also reported by Enriquez-Cortina *et al*[21] who studied the hepatoprotective effects of hepatocyte growth factor (HGF) on INH and Rifampicin (RIF) induced hepatotoxicity on mice. The explanation given by Groossman for this effect was because of INH induced oxidative stress, there was decreased liver vascularity due to vasoconstriction secondary to thickening of tunica media of blood vessels by oxidative stress induced smooth muscle cell proliferation and collagen deposition.[22] In the present study, in $INHAV_{low}$ group, the colour of liver was found to be reddish in most rats and a few haemorrhagic spots were observed. In $INHAV_{High}$ group, the frequency of healthy reddish coloured livers was more than $INHAV_{low}$ indicating the possible role of avocado seed extract in maintaining liver vascularity. Two animals of each of $INHAV_{low}$ and $INHAV_{High}$ groups showed reddish brown liver colour.

The size of hepatocytes was found to be smallest in the CG and largest in INHG. Similar results were reported by Humayun *et al* who studied the changes in liver histology produced by INH in mice.[20] Liu *et al* also reported increase in size of hepatocytes after 30 day treatment with ATT drugs.[23]

According to Simon *et al*[24], INH induced oxidative stress is believed to disturb the Na^+ equilibrium across cell membrane mainly affecting Na^+ efflux, ultimately resulting in Na^+ retention which leads to cellular swelling and death. The same mechanism might have been responsible for this observation in the current study. A significant dose-dependent decrease in size of hepatocytes was observed in $INHAV_{low}$ and $INHAV_{High}$ groups as compared to INHG. This was probably due to anti-oxidant effects exerted by avocado seed extract to reduce cellular swelling by inhibiting oxidative stress. Similar results were reported by Hamidian *et al*[25]. They investigated the effects of methanolic avocado seed extract on liver of diabetic mice and at the end of the experiment, the results indicated an increase in size of hepatocytes in diabetic mice.

CONCLUSION

The concomitant use of aqueous avocado seed extract helps to prevent the INH-induced decrease in body and liver weights in a dose-dependent manner. It is also beneficial in maintaining the gross features of liver and size of hepatocytes in INH affected albino rats.

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