Effects of Avocado Aqueous Seed Extract on Liver Biochemical Markers in Rats with Hepatotoxicity Induced by Isoniazid

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Abstract

Objective: To observe the improvement in serum biomarkers of liver function (LFTs) by using aqueous Avocado seed extract with isoniazid (INH) in Albino rats.

Method: It was an experimental research conducted at KEMU from January to June 2019. The study included thirty six male Sprague Dawley Albino rats divided into 4 group using lottery method, each group having 9 animals. All animal groups were administered treatment by gavage method for 30 days. Blood sample of each animal for biochemical markers analysis was collected 24 hours after the last dose of drugs by cardiac puncture. The collected data from the four groups was entered in Statistical package for social sciences (SPSS) version 26 for analysis. Mean and standard deviation were calculated for quantitative characteristics. One way ANOVA was used to make the comparison between all groups. Pair wise comparison was performed using Least square difference (LSD) Test. P-value <0.05 was considered significant.

Results: Serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP), and serum bilirubin showed a significant difference upon co-administration of aqueous Avocado seed extract with INH among all the groups.

Conclusion: The current study indicates a significant dose-dependent improvement in serum biomarkers of liver function when INH and avocado aqueous seed extract are used together.

Keywords: Avocado seed, Isoniazid-induced hepatotoxicity, LFTs, Albino rats.

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Introduction

Avocado is a dicotyledonous plant belonging to the order Ranales and the family Lauraceous.[1] Avocado seeds make up a significant portion of the total fruit. Phenolic acids, falvinoids and condensed tannins are essential phytochemicals present in avocado seeds.[2] Ethanol extracts of leaf and fruit contain important phytochemicals that produce apoptosis of tumor cells by inhibiting growth signals within these cells.[3] The biological benefits of aqueous seed extracts of Avocado have been implicated in hypertension observed on rat models by reducing heart rates.[4] The aqueous seed extract has also been found effective in the treatment of hyperglycemia in diabetic rats.[5]

Tuberculosis (TB) is a major contributor to the disease burden in Pakistan and 75% of TB patients are in productive age group.[6] Prompt and complete treatment of TB is strongly indicated. Isoniazid (INH) is one of the most important drugs used to treat TB. But it has been associated with severe hepatotoxicity and fatal liver injury by causing necrosis and steatosis of hepatocytes.[7,8]

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The reported liver toxicity with isoniazid is 1.6%.[9]

The research on biological effects of Avocado seed is still in its early stages. The present study was conducted to monitor the improvement in serum biomarkers of liver function by using aqueous Avocado seed extract and INH together in INH-induced hepatotoxic albino rats.

Material and Method

It was an experimental study conducted on thirty six 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 at Experimental Research Laboratory (Animal House) of Postgraduate Medical Institute, Birdwood Road, Lahore in collaboration with Anatomy department and Histopathology laboratory of KEMU from January 2019 to June 2019.

A total of 36 male Sprague Dawley Albino rats of 8-12 weeks age, weighing between 200-250 grams were randomly divided into 4 groups by lottery method. Each group had 9 animals and each group was housed in a separate cage labeled according to the animal groups. Animals were allowed to acclimatize for 1 week before start of experiment. Any rats that became inactive or stopped eating during acclimatization were excluded. The animals were fed tap water ad libitum and standard diet.

Group 1, Control group (CG) received only1 ml/kg/day distilled water in morning.10 Group 2, Isoniazid group (INHG), received only isoniazid 100 mg/kg/day dissolved in 1ml distilled water as a single dose in the morning.[11] 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,[11] and Avocado seed extract (aqueous) 250mg/kg/day dissolved in 2 ml distilled water as a single dose in the morning, one hour after INH.[10] Group 4, Isoniazid-Avocado (high dose) group (INHAVhigh) received Isoniazid 100 mg/kg/day dissolved in 1 ml distilled water as a single dose in the morning,[11] 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 the doses were given by gavage method for 30 days.

Blood sample of each animal for biochemical markers analysis was collected 24 hours after the last dose of drugs by cardiac puncture. Almost 2 ml blood was collected in sterile syringe & transferred to vial containing sterile gel. The vials were transported to Biochemistry laboratory, KEMU with utmost care. Blood was allowed to clot for 1 hour. Serum was separated and centrifuged at 3000 revolutions per minute for 10 minutes. The centrifuged serum samples were stored at -20°C in autoclaved tubes till they were used for LFT's estimation (Serum ALT, AST, ALP and Total Bilirubin for all groups). Statistical Package for the Social Sciences version (SPSS) 26 for data analysis. Mean and standard deviation were calculated for quantitative characteristics. One way ANOVA was used to make the comparison between all groups. A pair wise comparison was performed using the Least square difference (LSD) Test. P-value <0.05 was considered significant.

Table 1: *Comparison of LFTs among Study groups (One Way ANOVA)*

LFTs		Sum of squares	df	Mean square	F	P-value
Serum Alanine aminotransferase (ALT)	Between groups	69806.779	3	23268.926	85.732	0.000*
	Within groups	8685.245	32	271.414		
	Total	78492.024	35			
Serum Aspartate aminotransferase (AST)	Between groups	80544.673	3	26848.224	37.190	0.000*
	Within groups	23101.447	32	721.920		
	Total	103646.120	35			
Serum Alkaline phosphatase (ALP)	Between groups	95465.715	3	31821.905	58.772	0.000*
	Within groups	17326.233	32	541.445		
	Total	112791.948	35			
Serum Bilirubin	Between groups	.530	3	.177	15.212	0.000*
	Within groups	.372	32	.012		
	Total	.902	35			

*Significant P-value <0.05

Results

The mean serum Alanine aminotransferase (ALT) for the four groups was 17.50±5.17u/l (CG), 140.97±28.04 u/l (INHG), 89.66±9.97 u/l (INHAVlow) and 73.69± 13.16 u/l(INHAVhigh). Serum ALT was highest in INHG and lowest in CG. When compared among groups, serum ALT was significantly different among groups (P value= 0.000 (Table-1).

The mean serum Aspartate aminotransferase (AST) for the four groups was 50.49±12.29 u/l (CG), 172.48±36.12 u/l (INHG), 136.31±32.19u/l (INHAVlow) and 158.03± 19.88u/l (INHAVhigh). Serum AST was highest in INHG and lowest in CG. One-way ANOVA showed that the difference between the groups is significant (P value= 0.000) (Table-1).

The mean serum Alkaline phosphatase (ALP) for the four groups was 91.26±8.33 u/l (CG), 221.79±22.35 u/l (INHG), 205.32±30.93 u/l (INHAVlow) and 198.63± 25.29 u/l (INHAVhigh). Serum ALP was highest in INHG and lowest in CG. Difference in serum ALP among groups was significant (P value= 0.000) by One Way ANOVA (Table-1). The mean serum bilirubin for the four groups was 0.40±0.07 mg/dl (CG), 0.74±0.09 mg/dl (INHG), 0.59±0.14mg/dl (INHAVlow) and 0.52 ±0.10 mg/dl (INHAVhigh). Serum bilirubin was highest in INHG and lowest in CG. Comparison between groups by one way ANOVA showed that the difference in serum bilirubin between groups was significant. (P value= 0.000) (Table-1).

Discussion

The Avocado seed makes up about 13-18% of the whole fruit which is discarded as such despite being a natural and good source of carbohydrates, fats, proteins and important minerals like calcium, potassium, magnesium and phosphorus.[12,13] Although, it is mainly used for germination but it is also a rich source of nutrients and phytochemicals.[14]

This study was performed to observe the hepatoprotective effects of avocado seed aqueous extract on hepatic biochemical markers in isoniazid-induced hepatotoxicity in albino rats. Levels of serum transaminases and bilirubin were the highest in INHG, indicating severe hepatic insult. Several studies have proved the hepatic injury evident by elevated liver enzymes and serum bilirubin by using INH alone or in combination with other anti-TB drugs.[15-18] However, the levels of serum transaminases (ALT &AST) and serum bilirubin were found significantly reduced in INHAVlow (P value < 0.05) when compared with INHG but the reduction in serum ALP levels was not statistically significant. In INHAV high group, serum biochemical markers showed significant improvement (P value < 0.05) in comparison to INHG. It is worth emphasizing that there was an improvement in serum AST level in INHAVlow group more than INHAVhigh group which was quite unexpected. These findings in current study point towards a hepatoprotective role of avocado seed extract (aqueous) in both low and high doses.

The results of our study are consistent with the results of a study by Brai et al (2014) on aqueous leaf extract of avocado. They tried to find out what role it plays in liver damage from carbon tetrachloride in albino rats. Results indicated significantly low levels of liver enzymes and serum bilirubin in rats pretreated with aqueous leaf extract when compared to other groups which did not receive the extract.[19] In another study done by Jibril et al., (2015), the effects of avocado seed homogenates on liver enzymes were studied in albino rats as a co-treatment with first line Anti-TB drugs. Result showed a significant reduction in hepatic enzymes in the presence of avocado seed homogenate when compared with rats which received only Anti-TB drugs.[10] These results are also in accordance with findings in current study. A study by Cemaluk et al., conducted in 2018 showed that serum AST and ALT activity decreased in rats liver damaged by Monosodium glutamate (MSG) following administration of ethanolic Avocado seed extract. The study also reported similar decreasing levels of alkaline phosphatase and total serum Bilirubin upon administration of ethanolic Avocado seed extract. Tugiyanti et al. demonstrated in their study that powder supplement of Avocado seed improved the liver function in culled female quail. These studies strengthen the findings of our study.[20,21]

In a study by Zakariya et al., the hepatotoxic effects of aqueous and phenolic extracts from avocado seeds were compared in Wistar Albino rats. The study reported that serum levels of ALT, AST, and ALP were significantly higher in the aqueous and phenolic extract groups than in the control group with no extracts given.[22] The study results are in contrast to the present study.

Conclusion

There is a significant dose-dependent improvement in serum biomarkers of liver function when INH and avocado aqueous seed extract are used together showing its hepatoprotective effects.

Conflict of Interest: *None*
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Authors Contribution

ZB, SQA, RT: Conceptualization of Project
ZB, SQA, AA: Data Collection
SQA: Literature Search
ZB, RT: Statistical Analysis
GM, SK: Drafting, Revision
ZB, SQA: Writing of Manuscript