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ORIGINAL ARTICLE

The Novel Role of β-Aescin in Attenuating Isoniazid (INH)- Induced Hepatotoxicity in Rats

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ABSTRACT

Objective: To investigate the hepatoprotective effect and underlying mechanisms of β-Aescin in Isoniazid (INH)-induced liver damage.

Methodology: This experimental study was carried out at the Pharmacology department and Postgraduate Laboratory of Isra University Hyderabad with the collaboration of Sindh Agricultural University Tando Jam. Forty adult Albino Wister rats, weighing between 200 and 250 grams were taken under a well-ventilated & hygienic environment at 26◦C of room temperature and light/dark cycle of 12 hours and divided into 5 groups, each group containing 8 rats. After experiment animals were sacrificed via midline incision, the heart was identified and blood was drawn by cardiac puncture into a plain vial. The blood was allowed to clot and serum separated and stored at 20○C till further use. The liver was identified, removed and rinsed with normal saline and weighed. Serum levels of alanine aminotransferase (ALT), aspartate amino- transferase (AST) and bilirubin total were assessed using a commercially available kit. All the data were collected via study proforma, and analysis was done using SPSS version 26.

Results: A total of 40 animals after dividing in 5 groups as per study protocol were studied; to observe the hepatoprotective effect of β-Aescin in Isoniazid (INH)-induced liver damage. The body weight of the animal was significantly decreased in Isoniazid (INH) control group, experimental group C and D, while it was cured by β-Aescin 3.6 mg/kg. The findings are showing that the Isoniazid (INH) drug also significantly decreased the body weight and β-Aescin 3.6 mg/kg showed the significant protective effects in the body weight. The weight of the liver was significantly increased in Isoniazid (INH) group and experimental group C and D in contrast to the normal control (p-0.001), while experiment group E (β-Aescin 3.6 mg/kg) showed almost equal liver weight compared to the control normal group (p-0.345). β-Aescin 3.6 mg/kg showed the significant protective effects of hepatotoxicity induced by Isoniazid (INH), as the average of ALT, AST, total bilirubin and direct bilirubin were significant decreased almost near to control normally in the β-Aescin 3.6 mg/kg treated group (p-0.001).

Conclusion: As per study conclusion the β-Aescin 3.6 mg/kg was observed to be a protective agent against Isoniazid (INH)-induced hepatic toxicity. It was observed as the antioxidative and anti-inflammatory and antifibrotic to cure the liver. Hence, it can be used as a promising hepatoprotective agent.

Key words: Hepatotoxicity, tuberculosis drug, β-Aescin, effectiveness

INTRODUCTION

The liver is the major glandular and important organ in the body, and it is responsible for drug processing. It aids in the detoxification and excretion of several xenobiotics and medicinal substances.[1] Injury or destruction of its functions has serious consequences for the affected person's health.[1] Hepatotoxicity is becoming a primary cause of death throughout the world, and its incidence is expanding at an exponential rate and the hepatotoxicity can be caused by several factors.[2] Hepatotoxicity is defined as liver malfunction or damage caused by an overabundance of medicines or xenobiotics. Hepatotoxins and hepatotoxicants are synthetic chemicals that cause liver injury. Exogenous hepatotoxicants include overdoses of some drugs, industrial chemicals, plants, and dietary supplements, among other things.[2,3] Drugs undergo oxidation, hydrolysis, hydration, reduction, conjugation, condensation, or isomerization in the liver. Although the liver metabolizes medications, hepatotoxicity can occur if these systems are disrupted. Although drug-induced liver damage due to various medicines somewhat differs, the clinical manifestations of INH-induced hepatic injury.[4] One of the most serious side effects associated with anti-TB medications are drug-induced hepatotoxicity (DIH). Hepatic injury is usually asymptomatic and could only be discovered by detecting hepatocyte injury markers like aspartate aminotransferase (AST) and alanine aminotransferase (ALT). A transitory increase in serum alanine aminotransferase (ALT) occurs in about 10%–20% of cases who consume INH. The majority of patients adjust to it and whose serum ALT concentrations return to normal without treatment, but a small percentage of individuals (less than 1%–3%) acquire serious hepatic injury and even liver failure.[6,7] The hepatotoxicity of INH has been studied using several animal studies, including rats. Nevertheless, no animal model has been established to mimic the human patterns of INH-induced liver injury.[8,9] Hepatic damage is a significant condition that has ramifications on many levels, including specific patient health, pharmaceutical regulatory choices, and therapeutic development plans. Due to lack of complete cure, multiple unpleasant effects, decreased safety, and other factors, currently available medication

treatments are insufficient to meet clinical demand.[10] As a result, the usage of herbal medications, which have a higher safety and efficacy profile, is becoming more popular. β-Aescin is the main active ingredient isolated from the Aesculus hippocastanum L horse chestnut tree.[10] Furthermore, β-Aescin's anti-inflammatory and antioxidant properties have been shown to protect against endotoxin-induced hepatic injury.[10] This study has been done to assess the hepatoprotective action of β-Aescin and underlying processes in Isoniazid (INH)-induced hepatic damage.

MATERIAL AND METHODS

This experimental study was carried out at the Pharmacology department and Postgraduate Laboratory of Isra University Hyderabad. Animal protocol was followed at the Animal House Department of Husbandry and Veterinary Sciences, Sindh Agricultural University Tando Jam. Forty adult Albino Wister rats, weighing between 200 and 250 grams were taken and divided into 5 groups, each group containing 8 rats. The animals were kept in plastic cages and equipped with plastic drinkers with nozzles of stainless steel and feed containers of stainless steel. They were free to access a standard chow diet and water before and following the experiments. The animals were kept under a well-ventilated & hygienic environment at 26°C of room temperature and light/dark cycle of 12 hours.

Animals were divided into five groups (8 animals in each group) as the following:

Group A (Control) were given normal chow diet along with clean water ad libitum.
Group B (Isoniazid (INH) control) group.
Group C (Experimental, β-Aescin 0.9 mg/kg treated group).
Group D ((Experimental β-Aescin 1.8 mg/kg treated group).
Group E ((Experimental β-Aescin 3.6 mg/kg treated group).

The weights of the control and experimental animals were measured after the experiment. All the animals were sacrificed via cervical dislocation. The blood was obtained using the cardiac puncture procedure for biochemical examination. On an automatic modular analyzer, liver function tests (AST, ALT, and total bilirubin) were performed using a diagnostic kit approach. The livers of rats were removed, and gross morphological characteristics (weight and size) were recorded after washing using normal saline. A Petri dish was balanced at zero on an electronic precision balance (sartorex) to determine the hepatic weight. The liver size was determined using a measuring scale, and the livers were subsequently fixed in 10% formalin for histological investigation. Tissues were processed through a series of ethyl alcohol grades before being cleared in xylene. The tissues were treated using the paraffin embedding procedure to create paraffin blocks. Using a microtome, 4 micrometer sections were cut for slides and stained with hematoxylin and eosin for observation at 100 and 400 magnifications under a light microscope. All the data were collected via study proforma, and analysis was done using SPSS version 26.

RESULTS

A total of 40 animals after dividing in 5 groups as per study protocol were studied; to observe the hepatoprotective effect of β-Aescin in Isoniazid (INH)-induced liver damage. The body weight of the animals was significantly decreased in Isoniazid (INH) control group, experimental groups C and D, while body weight was increased in the normal control group and experimental group E.

Table. 1 Mean of difference between initial and final body weight in all study groups n=40

Study groups	N	Pre to post experimental difference in body weight (grams)	
		Mean	Std. Deviation
Normal control	8	15.21	3.144
Isoniazid (INH) control	8	-19.17	7.264
β-Aescin 0.9 mg/kg treated	8	-8.40	2.003
β-Aescin 1.8 mg/kg treated	8	-3.84	3.324
β-Aescin 3.6 mg/kg treated	8	2.54	2.891

Table.2 Liver weight comparison of control and INH groups with experimental groups n=40

Groups	weight of liver (grams)		p-value	F-value
A vs B	4.23±0.175	7.11±0.645	0.001	40.475
A vs C	4.23±0.175	5.48±0.560	0.001	
A vs D	4.23±0.175	5.37±0.399	0.001	
A vs E	4.23±0.175	4.66±0.400	0.345	
B vs C	7.11±0.645	5.48±0.560	0.001	
B vs D	7.11±0.645	5.37±0.399	0.001	
B vs E	7.11±0.645	4.66±0.400	0.001	

Table.3 Lipid profile comparison of control and INH groups with experimental groups n=40

Groups	ALT(IU/L)		p-value	F-value
A vs B	45.88±9.465	206.54±18.790	0.001	233.606
A vs C	45.88±9.465	152.03±9.257	0.001	
A vs D	45.88±9.465	91.57±9.756	0.001	
A vs E	45.88±9.465	72.06±10.020	0.001	
B vs C	206.54±18.79	152.03±9.257	0.001	
B vs D	206.54±18.79	72.57±9.756	0.001	
B vs E	206.54±18.79	55.06±10.020	0.001	
AST(IU/L)				
A vs B	44.59±7.315	170.88±19.533	0.001	190.531
A vs C	44.59±7.315	128.14±6.286	0.001	
A vs D	44.59±7.315	83.19±3.675	0.001	
A vs E	44.59±7.315	71.25±6.037	0.001	
B vs C	170.88±19.53	128.14±6.286	0.001	
B vs D	170.88±19.53	83.19±3.675	0.001	
B vs E	170.88±19.53	71.25±6.037	0.001	
Total bilirubin				
A vs B	0.22±0.282	1.35±0.299	0.001	23.991
A vs C	0.22±0.282	0.95±0.044	0.001	
A vs D	0.22±0.282	0.64±0.181	0.020	
A vs E	0.22±0.282	0.40±0.365	0.610	
B vs C	1.35±0.299	0.95±0.044	0.034	
B vs D	1.35±0.299	0.64±0.181	0.001	
B vs E	1.35±0.299	0.40±0.365	0.001	
Direct bilirubin				
A vs B	0.18±0.115	1.07±0.641	0.001	0.8552
A vs C	0.18±0.115	0.66±0.193	0.060	
A vs D	0.18±0.115	0.42±0.335	0.619	
A vs E	0.18±0.115	0.27±0.165	0.979	
B vs C	1.07±0.641	0.66±0.193	0.145	
B vs D	1.07±0.641	0.42±0.335	0.005	
B vs E	1.07±0.641	0.27±0.165	0.001	

The findings are showing that the Isoniazid (INH) drug also significantly decreased the body weight and β-Aescin 3.6 mg/kg showed the significant protective effects in the body weight. Table.1 Weight of the liver was significantly increased in Isoniazid (INH) group and experimental group C and D in contrast to the normal control (p-0.001), while experiment group E (β-Aescin 3.6 mg/kg) showed almost equal liver weight compared to the control normal group (p-0.345). Table.2 β-Aescin 3.6 mg/kg showed the significant protective effects of hepatotoxicity induced by Isoniazid (INH), as the average of ALT, AST, total bilirubin and direct bilirubin were significant decreased almost near to control normally in the β-Aescin 3.6 mg/kg treated group (p-0.001), the results shown in table.3

DISCUSSION

In this study, hepatotoxicity by acute administration of isoniazid was investigated; it could indeed be ameliorated by treatment with Aescin. With confidence and a rigorous search of the medical literature, this study being reported from the Pharmacology Department of Isra University. Isoniazid (INH) is a first-line drug of anti-tuberculosis drug regimens of the current era. INH is notorious of its liver damaging potential, culminating in severe acute liver failure and even death may occur in a few patients. Instead of this, it is being used in clinical practice because of no alternative effective drug agents against Mycobacterium tuberculosis.[11-14] The β-Aescin exerts anti-inflammatory effects through its inhibitory effects on the histaminergic and serotonergic receptors.[15] The β-Aescin inhibits the Phospholipase A2 (PLA2) enzyme that generates the arachidonic acid derived inflammatory mediators. The β-Aescin inhibits the inflammatory reaction in hypoxia induced human endothelial cell injury. This is the major anti-inflammatory pathway through which β-Aescin exerts clinical response and alleviation of edema and inflammation.[16] Liu et al[17] conducted study on the in vitro effects of β-Aescin against the periodontal ligament inflammation. They found a significant inhibitory effect on the expression of Toll-like receptor (TLR). The pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 were reduced in a lipopolysaccharide induced inflammatory animal model.[18] Aescin also improved the liver histology and protected the liver parenchyma against INH injury. The findings of our study are consistent with the previousthose of previous studies.[19,20] In a study by Wei et al the hepatoprotective effects of CoQ10 in isoniazid fed rats has been observed as determined by the liver enzymes and histology, serum bilirubin, ALT, AST, LDH, ALP, GGT, Albumin, GPX, TNF-α . Similar effects were obtained by concomitant Aescin + INH in our study. Wang et al[21] has reported an experimental rat study for the effectiveness of Aescin against the indomethacin induced gastric ulcer. The findings support the histological protective effects of aescin similar to this study. However, they used indomethacin instead of Isoniazid that is not similar to this study. Ezberrci F et al[22] reported the effects of Aescin in the pile/hemorrhoids. They reported that aescin is effective in vanishing the piles. The findings of the above study is a worth regarding the pharmacological effect of aescin as also observed in the present study. Gupta SC et al[23] reported the down regulation of tumor necrosis factor and other proinflammatory biomarkers by polyphenols of Aescin. The finding is in agreement with the present study as we have observed similar finding of down regulation of TNF-α. However; other pro- inflmmatory mediators were not analyzed in the present study that is in disagreement. Krause et al[24] and Roman et al[25] reported anti-fungal activity of Aescin on dermatophytes in *vitro*. This finding supports the present study that the Aescin has pharmacological effects. Zhang et al[26] studied the effects of aescin on the Tumor Necrosis Factor in Human Immunity. Above the previous study reported the Aescin down regulates the effects of TNF-α. This pharmacological finding was in agreement with the present study. However, other cytokine markers were also studied by Zhang et al and those were not analyzed in the present study; hence, other findings of cytokines are inconsistent. Elmas et al[27] analyzed the effects of Aescin on Diabetic nephropathy in a streptozotocin-induced diabetic rat model. They reported the antioxidant (GPX) was increased and TNF-α was decreased. They also reported the histoprotective effects of Aescin on rat kidneys. The present study used an experimental model of liver histology that was ameliorated by Aescin. The histological effects of Elmas et al[27] on the kidney, although contradict to the liver histology, but still support the present study.

CONCLUSION

After the study β-Aescin β-Aescin 3.6 mg/kg was observed to be protective agent against Isoniazid (INH)-induced hepatic toxicity. It was observed as the antioxidative and anti-inflammatory and antifibrotic to cure the liver. Hence, it can be used as a promising hepatoprotective agent. Hepato-protective role of Aescin might have been mediated partly due to its anti-inflammatory effect and anti-oxidative mechanisms as has been observed by Glutathione peroxidase and Tumor necrosis factor-α.

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