Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 04/11/2022;
Date Revised: 28/02/2022;
Date Published Online: 31/03/2023;

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How to Cite:
Irshad N, Hameed H, Mumtaz M, Aslam Z, Batool A, Sohail I (2023). *Terminalia arjuna* restores the levels of alkaline phosphatase and aspartate aminotransferase of acetaminophen intoxicated mice. Adv. Life Sci. 10(1): 104-108.

Keywords:
Hepatoprotective; *Terminalia arjuna*; ALP; AST

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Terminalia arjuna restores the levels of alkaline phosphatase and aspartate aminotransferase of acetaminophen intoxicated mice

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Abstract

Background: Herbal medicines are natural and comparatively safer than conventional treatments and are well-documented for carrying little potential for harm. Therefore, phytonutrients have occupied a central stage in the therapeutics. *Terminalia arjuna,* a medicinal plant, has been reported to have homeostatic, laxative, diuretic, antidiabetic, anticancer and cardiotonic actions.

Methods: The current study was designed to investigate the protective role of *T. arjuna* leaf extract at three dose levels (100, 250, 500 mg/kg body weight) against acetaminophen (250 mg/kg body weight) induced liver damage.

Results: The administration of hepatotoxin (Acetaminophen) resulted in disturbance of hematological and serological profile including alkaline phosphatase (ALP) and aspartate aminotransferase (AST) which was assayed in control and drug treated experimental models. Treatment with *T. arjuna* leaf extract for 7 days restored the normal levels of markers and response was dose dependent.

Conclusion: This study adds to the very limited existing literature regarding hepatoprotective effect of *T. arjuna* against acetaminophen toxicity. It is also important to get a step closer to development of accessible, authoritative, and independent information resources about herbal medicines and wide-ranging health disorders, which are currently lacking in Pakistan.

Introduction

Liver is a major detoxifying and metabolizing organ and is continuously exposed to variety of environmental pollutants, drugs and other highly reactive substances [1-3]. Wide range of exogenous and endogenous stresses and inability of cellular defence to counteract these cellular insults predispose the cells to undergo pathogenesis [4]. Research over the years has refined the knowledge regarding phytonutrients and nutrigenomics [5]. It has been documented that the Terminalia species act as a potential medicinal plant against variety of diseases. The members of Terminalia species are widely used because of their homeostatic, laxative, cardiotonic, antidiabetic, anticancer, antioxidant, anticoagulant, antihypertensive, antithrombotic, antiviral, antifungal, antibacterial and diuretic activities [6-10]. Different parts of plant have been investigated for the presence of phytoconstituents and pharmacological actions [11]. Phytochemical extract have been attributed to possess antimicrobial and antioxidant activity [12]. The experiment showed that *T. arjuna* (200 mg/kg) significantly reversed the effect of cadmium induced toxicity and proved that it has hepatoprotective and antioxidant potential [13]. *Terminalia arjuna* extracts in both alcohol and water significantly reduced the activity of the CYP3A4, CYP2D6 and CYP2C9 enzymes. According to an enzyme kinetics investigation, treatments resulted in the fast, reversible, non-competitive inhibition of all three enzymes in human liver microsomes [14]. Aqueous and ethanol extracts of *Terminalia arjuna* bark were found to have hepatoprotective potential against the liver damage caused by paracetamol/CCl_4 in Wistar albino rats [15]. Additionally, it was noted that the purified flavonoids in the methanolic extract of *Terminalia arjuna* stem bark have hepatoprotective effects against CCl_4-induced hepatic damage [16]. Interestingly the hepatoprotective role of *Terminalia arjuna* leaf is still incompletely defined. Therefore, we tested whether or not *Terminalia arjuna* leaf extract had hepatocurative potential by taking into account the serological and hematological parameters.

Methods

Preparation of plant extract:

Leaves of *Terminalia arjuna* were collected from Scholars Garden of Government College University Lahore, Pakistan and were identified by an expert (Professor Dr. Zaheer-Ud-Din Khan, Ex-Chairperson Botany Department, Government College University, Lahore, Pakistan). Collected leaves were washed thoroughly with distilled water. Then they were shade dried for two weeks. Using electric blender the dried leaves were pulverized and 500 grams of powder was soaked in 4 liters of absolute ethanol (95%) for 15 days with sporadic shaking. After filtration, the filtrate was subjected to rotary evaporator at 42-45°C to evaporate the solvent (ethanol). After the distillation process crude extract was obtained. The extract was then stored at 4°C until further use.

Experimental animals

The albino mice weighing 25-27g were purchased from University of Veterinary and Animal Sciences Lahore. The animals were housed in Government College University animal house under standard conditions (22 ± 2°C; 12:12 hours light dark period) and fed with standard diet (poultry feed) and water. All the experimental work in animals such as administration of drug and plant extract, and blood collection were performed according to the national standards under appropriate regimes with veterinary services. The ethical permission to use animal has been taken from institutional ethical committee (GCU-IIB-114).

Assay kits and Chemical Reagents:

Acetaminophen (Paracetamol) was used for liver intoxication. Test kits for measuring AST, and ALP were supplied by synchron® systems (Ireland).

Experimental Design:

In this experiment, twenty-five male albino mice were randomly allocated into five groups, each consisting of five animals (n=5).

Group I: Normal healthy control

The animals were fed on standard diet and water ad *libitum*.

Group II: Untreated Acetaminophen control

Animal was given orally 250mg/kg of acetaminophen, suspended in distilled water for seven days. Blood sampling was done on 8th day. The blood was analyzed for various serological and hematological parameters.

Group III: Acetaminophen+ 100mg/kg body weight of Plant Extract

The animals of this group were treated with 100mg/kg body weight plant extract as well as 250mg/kg acetaminophen per day for 7 days. The dose was given orally.

Group IV: Acetaminophen+ 250mg/kg body weight of Plant Extract

The animals of this group were treated orally with 250mg/kg body weight plant extract as well as 250mg/kg acetaminophen per day for 7 days.

Group V: Acetaminophen+ 500mg/kg body weight of Plant Extract

The animals of this group were treated orally with 500mg/kg body weight plant extract as well as 250mg/kg acetaminophen per day for 7 days.

Blood Sampling

On 8th day the mice were anesthetized and dissected. Approximately one milliliter blood was collected directly from the heart by cardiac puncture. Half of which was transferred into EDTA sample tubes for hematological studies, while the other half was transferred into 1.5 ml polypropylene tube-(Eppendorf SE, Hamburg, Germany) which was then allowed to clot by leaving it undisturbed for half an hour. The latter was centrifuged at 4000 rpm for 10 minutes to separate the serum for serological studies.

Data Analysis

Data was analyzed by using SPSS software version 25. The values represent mean ± SD. Statistical significance was determined by ANOVA followed by Tukey's post-hoc. A *P* value ⩽ 0.05 was considered as significant.

Results

Hematological and Serological Parameters

The values of various hematological and serological parameters are shown in Table 1. The table shows the comparison of mean±SD of white blood cells (WBCs), granulocytes (GRA), lymphocytes (LYM), aspartate transaminase (AST) and alkaline phosphatase (ALP). The number of WBCs observed in normal healthy control was 2.7 ± 0.16 $x10^3$/µl. In untreated acetaminophen control the number of WBCs increases and the value observed was 3.61 ± 0.67 $x10^3$/µl. In the treatment groups III, IV and V, WBCs number decreases with administration of extract. The WBCs values observed in group III, IV and V were 2.51 ± 0.035 $x10^3$/µl, 2.37 ± 0.06 $x10^3$/µl and 2.29 ± 0.025 $x10^3$/µl, respectively.

The number of lymphocytes observed in the control group was 3.28 ± 0.07$x10^3$/µl. This number is elevated in group II in which only acetaminophen was administered, and the value observed was 4.50 ± 0.50 $x10^3$/µl. The number of lymphocytes observed in group III, IV, and V which were treated with leaf extract was 1.67 ± 0.03 $x10^3$/µl, 1.58 ± 0.04 $x10^3$/µl and 1.53 ± 0.16 $x10^3$/µl, respectively (Table 1). The number of granulocytes in untreated healthy control was 1.32 ± 0.02 $x10^3$/µl. After acetaminophen was administered this value rises to 1.93 ± 0.07 $x10^3$/µl. The GRA count was found to be significantly decreasing in group III, IV, and V i.e., 0.66 ± 0.02 $x10^3$/µl, 0.32 ± 0.03 $x10^3$/µl and 0.29± 0.04 $x10^3$/µl.

The serum level of AST in untreated (Control) was 63.92 ± 3.2 U/l. After acetaminophen administration in group II, a significant elevation in serum AST level i-e 87.14 ± 3.15U/l was observed which indicate liver damage. In treatment groups III, IV, and V in which extract was given at the rate of 100, 250 and 500mg/kg of body weight along with acetaminophen the values observed for serum AST level were 83.09 ± 6.5 U/l, 76.49 ± 4.9 U/l and 69.70 ± 3.40U/l, respectively.

The concentration of ALP in untreated healthy control was 24.47 ± 3.6 U/l. After the administration of acetaminophen (250mg/kg body weight) for 7 days in group II this value rises to 51.90 ± 4.42 U/l indicating liver damage. The values of serum ALP level observed in group III, IV, and V which were treated with extract along with acetaminophen was 46.67 ±5.47 U/l, 39.19 ± 4.72 U/l and 28.25 ± 5.37 U/l, respectively.

Discussion

In this study the effect of *T. arjuna* leaf on serological and hematological parameters was evaluated. For this purpose, acetaminophen (paracetamol) was used to damage the liver in experimental model. Previous studies have proved that various parts of *T. arjuna* are effective against a diverse range of diseases. Fruits are used as tonic and deobstruent [17]. Moreover studies have also revealed that *T. arjuna* fruit extract has hepatoprotective and hepatocurative role against acetaminophen intoxication[18].In our study the level of hepatic enzymes i.e. AST and ALP increased in blood after administration of acetaminophen. The treatment with *Terminalia arjuna* leaf extract at all doses (100, 250 and 500mg/kg) normalized enzyme activities which is in concordance with the work of [19]. Similarly our data is in accordance with other research works in which levels of AST and ALP are elevated after administration of acetaminophen, CCl_4 (Carbon tetrachloride) or thioacetamide observed in mice[20]. Various other researchers also explored the likely correlation between pharmaceutical toxicology and phytonutrients and suggested that paracetamol induced pathology was reversed significantly by silymarin [21]. Our results are in line with [22], who demonstrated that hydroalcoholic extract of bark of *T. arjuna* possessed hepatoprotective effect

The blood related functions of plant extract and its product can be explained by investigating certain biochemical parameters [23]. The levels of WBCs, Lymphocytes and granulocytes increased dramatically with oral administration of acetaminophen which might be related to an immunological response upon liver damage when compared with control. However experimental mice treated with herbal extract displayed a decline in level of WBCs and other cell counts declined at all doses.This indicates that the extract might have some bioactive components that could harm or impair the production of white blood cells. WBCs and other cell counts were normalized especially at dose of 500 mg/kg body weight of extract

PARAMETERS	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
WBC ($x10^3/\mu l$)	2.7** ± 0.16	3.61 ± 0.67	2.51*** ± 0.035	2.37*** ± 0.06	2.29*** ± 0.025
LYM ($x10^3/\mu l$)	3.28*** ± 0.07	4.50 ± 0.50	1.67*** ± 0.03	1.58*** ± 0.04	1.53*** ± 0.16
GRA ($x10^3/\mu l$)	1.32*** ± 0.02	1.93 ± 0.07	0.66***± 0.02	0.32*** ± 0.03	0.29*** ± 0.04
AST (U/L)	63.92*** ± 3.2	87.14 ± 3.15	83.09 ± 6.5	76.49* ± 4.9	69.70*** ± 3.40
ALP (U/L)	24.47*** ± 3.6	51.90 ± 4.42	46.67 ± 5.47	39.19** ± 4.72	28.25*** ± 5.37

Table 1: Mean values of Hematological and serological parameters of Different Groups after Acetaminophen and plant extract treatment (Mean ± SD).The statistical significance is determined using one way ANOVA followed by Tukey's post-hoc. P≤ 0.5 is considered as significant. Group II. is compared with other groups. WBC: White blood cells; GRA: Granulocytes; LYM: lymphocytes; AST; aspartate transaminase; ALP: alkaline phosphatase.

along with paracetamol. This observation is in accordance with the work of [24], who reported the effect of ethanolic extract of *B. speatabilis* leaves on haematological and serum lipid variables in rats. Studies have shown that *T. arjuna* contains flavonoids, tannins and minerals. Flavonoids have been reported to exert antioxidant effect. The hepatoprotective role of *T. arjuna* in our findings might be due to enhanced antioxidant activity [12].

In conclusion, *T. arjuna* leaf extract was found to demonstrate hepatoprotective efficiency and also improves hematological abnormal profile against acetaminophen toxicity. Although these studies partially describe the underlying mechanisms which are targeted by herbal extracts to safeguard cells against cellular insults. Yet detailed information is necessary for a better understanding of the cellular mechanisms which are nonfunctional or which are inactivated during pathogenesis. Additionally, a greater awareness of the bioactive components of the plant is vital for its utilization and likely translation to an effective drug that can be used in clinical trials.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

The study was planned by Imran Sohail. Imran Sohail, Maryam Mumtaz, Zainab Aslam conducted experiments. Initial draft of the manuscript was written byImran Sohail, Hassan Hameed, Nida Irshad.Imran Sohail, Andleeb Batool, Nida Irshad, and Hassan Hameed participated in revisions.

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