



Effect of *Aloe vera* extract on some parameter liver functions changes and complete blood count induced by Azathioprine in male rats

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Received: June 17, 2023	Abstract The present study aimed to reveal the biological vitality of the immune suppressant medicine azathioprine (AZA), which has been used to treat immunological illnesses like acute lymphoblastic leukemia (ALL), inflammatory bowel disease, and organ transplants, successfully lowers immune reactivity for liver and bone marrow. The goal of the current study was to evaluate the preventive effects of Aloe Vera extract in treating hematological and biochemical side effects brought on by azathioprine. There were selected four groups of adult male rats. A mixture of azathioprine (50 mg/kg) and Aloe Vera (500 mg/kg) was given to group 4 in addition saline that group 1 received. Group 2 received azathioprine (50 mg/kg) orally for during a 30 days period. The findings show that azathioprine significantly reduced blood test results (RBC , WBC , PCV). While AST and ALT levels significantly increased in the biochemical test in contrast to the control group , Conclusions the Hepatotoxicity, or liver damage from immunosuppressive medications like azathioprine, is a possible side effect and Due to the damage that has happened to the hepatic tissue, the enzymes that assess the activities of the liver are raised.
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Introduction

The liver is essential for several physiological processes, including the intrahepatic detoxification of xenobiotics and the production of hormones such insulin-like growth factors and angiotensinogen [1]. The liver is a metabolic organ that contains important routes for the production of energy and the metabolism of proteins, lipids, and carbohydrates [2,3]. Instead of controlling blood clot factors, the liver makes vital proteins such albumin, bile acid, very-low-density lipoprotein (VLDL), cholesterol, stores glucose as glycogen and lipids as triglycerides (TG), in addition to producing other vitamins and minerals , In addition to these functions, the liver is regarded as an

immunological organ since it contains immune cells that are prepared to remove the pathogen from the gastrointestinal system (GI) [4].

A thiopurine medication having corticosteroid-sparing qualities is azathioprine (AZA), also known as 6-mercaptopurine [5]. It was first used as an immune suppressive medication in the 1960s for immunological illnesses such leukemia, systemic lupus erythematosus, rheumatoid arthritis (RA), inflammatory bowel disease (IBD), autoimmune hepatitis (AIH), and organ transplant, AZA can successfully reduce inflammation, but its adverse drug response (ADR) has become a hurdle and a justification for discontinuing medication [6].

Aloe Vera' One of the most popular medicinal plants has been utilized for a variety of reasons for thousands of years in many cultures, belongs to the plant family Liliaceae [7]. Aloe Vera has been demonstrated to have antioxidant effects in both people and animals, It contains antioxidants, vitamins, enzymes, minerals, polysaccharides, phenolic compounds, and organic acids. It has a wide range of biological effects, including anti-inflammatory, arthritis, dermatitis, gout reduction, anti-cancer, antioxidant, UV protection, and anti-diabetes [8]. By encouraging the growth of epithelial and fibrous tissue, which grows in a dry, warm environment and has thick leaves with an outside and inner area, it can also speed up the healing of wounds, The inner portion of each leaf has vital components accumulating within the gelatinous part (the gel), Using aloe vera as Due to its significant medical therapeutic properties, Aloe vera is used as an alternative medicine to treat various diseases, Particularly the active ingredient polysaccharides are found in the parenchyma cells of the inner layer of Aloe vera, Compounds in aloe vera have anti-inflammatory properties [9] and The current study attempted to assess the protective activity of Aloe Vera extract in alleviating hematological, Biochemical and histopathological changes caused by Azathioprine.

Materials and Methods

Preparation of Azathioprine (AZA)

Drug: Azathioprine (AZA) (Imuran®, aspen, Ireland). A commercially available formulation of AZA 50 mg/ tablet were purchased from a local private pharmacy. It was dissolved in normal saline and administered at 50mg/kg body weight.

Preparation of Aloe Vera gel

Mature, healthy and fresh Aloe Vera leaves about 75 to 90 cm long were washed with fresh water. The leaves were cut transversely into pieces. Thickened epidermis has been selectively removed. Natural Aloe Vera gel was seen, the outer part of the Aloe Vera leaf was peeled off to obtain Aloe Vera gel directly, using a small spoon, the entire gel was extracted. Then the gel was transferred to a blender to obtain a mixture and foam ready to be dosed to the animals under study [10].

The Dosage calculation of Azathioprine and *Aloe vera* gel for experimental animals is calculated as the following equation [11].

$$\text{Dosage in mg} = \frac{\text{Body weight of animal(g)} \times \text{dose (mg)}}{1000\text{g}}$$

The Experimental Design

In the current investigation, 40 male white rats were employed They were split into 4 groups (n = 10) as listed in the points below:-

- 1) **1-Group (G1):** 10 rats were administrating sterile water and introduce as single daily dose administered orally for four weeks.
- 2) **Group (G2):** 10 rats were administrating azathioprine (50mg/kg. b. w) was dissolved in sterile water and introduce as single daily dose administered orally for four weeks [12].
- 3) **Group (G3):** 10 rats were administrating Aloe vera gel extract (500mg/kg b.w) was dissolved in sterile water and introduce as single daily dose administered orally for four weeks [13].
- 4) **Group (G4):** 10 rats were administrating azathioprine (50mg/kg. b. w) and Aloe vera gel extract (500mg/kg b.w) was dissolved in sterile water and introduce as single daily dose administered orally for four weeks.

Results and Discussion

As can be seen in Table (1), the main value of RBC , WBC and PCV were decreased in significant value (P<0.05) in azathioprine group comparatively to control group and aloe vera groups while there were elevation in value (P<0.05) for RBC , WBC and PCV in combination aloe vera and azathioprine group in comparison with azathioprine group. Azathioprine has the ability to Myelosuppression and a reduction in thymus size were anticipated side effects of azathioprine therapy and can cause the decrease in WBC , RBC and PCV this result by a process that decreases 6-mercaptopurine (6-MP), a thiopurine, which results in an inhibition of RBC , PCV and WBC count as a result of the bone marrow's inability to produce blood cells [14]. cytotoxicity is thought to be caused by the incorporation of thiopurine metabolites into cellular nucleic acids, which damages RBC and WBC and lowers their count [15,16]. Furthermore, that Azathioprine has harmful effects on the liver, including drug-induced hepatotoxicity that can cause release reactive oxygen species (ROS) and is consequently ascribed to hepatotoxicity. Oxidation may play a role in these effects that lead to decrease in RBC , PCV and WBC count [17]. In this study aloe vera extract has been showed improvement in hematological parameters include WBC , RBC and PCV this may be due to purported ability of aloe vera extract to stimulate bone marrow and promote erythropoiesis , It

may be related to the stimulation of factors that help stimulate erythrocyte proliferation and differentiation, such as interleukins [18].

Additionally, Aloe vera is known to contain minerals such as iron, copper, and folic acid, which are the building blocks for red blood cell production, as well as vitamins such as A, C, E, B1, B2, B5, B6, and B12 [19].

Table (1): Effect of *Aloe vera* extract 500 (mg/kg/BW) and Azathioprine 50 (mg/kg/BW) on WBC , RBC and PCV in male rats.

Groups	WBC Microliter	RBC million /mi- croliter	PCV %
Control group	12.52±0.36a	7.19±0.17a	39.29±0.53a
Aloe Vera group	13.04±0.29a	7.38±0.12a	41.39±0.33a
Azathioprine group	4.19±0.10c	4.74±0.15b	30.76±0.45c
Aloe Vera & Azathioprine group	9.68±0.37b	7.14±0.24a	37.10±1.40b
LSD	0.91	0.53	2.41

Data represented as mean ± SD different letters significant differences at P-value (P<0.05).

The data in Table (2) showed a significant increase in AST , ALT and Arginase I level in azathioprine group as compare to control and aloe vera group on the other hand there were observed a significant decrease in AST , ALT and Arginase I level in combination aloe vera and azathioprine in comparison with azathioprine group.

Serum levels of Aspartate Aminotransferase and Alanine Aminotransferase, These result about AST and ALT agreement with [20] that suggested that the elevation in liver function tests such as AST and ALT have close relationship with induction of liver dysfunction by azathioprine and can be sign for hepatotoxicity. Azathioprine is one of many medications used to treat cancer and immunological disorders, and it is the most popular , Numerous reports suggest that azathioprine has hepatotoxic side effects, which manifest as an increase in serum AST and ALT [21]. The harmful effects of azathioprine on liver cells are part of its mechanical toxicity, which reduces levels of free radicals that disrupt hepatocyte mitochondria, lowers ATP levels, and eventually causes necrosis, which leads to liver damage [22,23]. While the result revealed a substantial reduction in Arginase I , The opposite of its major function is regulation, which is linked to the role of the promoter azathioprine, due to the variable regulation of arginase-specific oxidative stress in different degenerative illnesses via modifying NO [24].

Table (2): Effect of *Aloe vera* extract 500 (mg/kg/BW) and Azathioprine 50 (mg/kg/BW) on serum AST , ALT and Arginase I level in adult male rats.

Groups	AST IU/ML	ALT IU/ML	Arginase I
Control group	50.00±1.8a	28.36±0.6a	3.37±0.10a
Aloe vera group	48.00±1.3b	30.00±0.2b	4.67±0.10a
Azathioprine group	128.00±3.9c	40.61±2.3c	9.33±0.33b
Aloe vera & Azathioprine group	100.00±2.3d	34.81±0.4d	6.65±0.30c
LSD	4.85	3.71	1.07

Means with a different letter in the same column are significantly different (P<0.05).

Rats with liver toxicity had lower arginase activity, which may have been caused by a decline in antioxidant activity , Arginase activation could also have resulted in the uncoupling of eNOS due to an increase in ROS , As a result, the uncoupled eNOS produces superoxide using molecular oxygen, further reducing the NO level [25,26]. By decreasing the availability of arginine to endothelium nitric oxide synthase (eNOS), increased arginase activity in the body has been shown to reduce endothelium dependent vasorelaxation, which ultimately results in a decrease in NO production and subsequently clogs the blood arteries , Arginase activity is found to increase when toxicants such azathioprine medications are administered by phenolic-rich dietary plants to inhibit [27,28,29]. Arginase has been shown to be superior to ALT and AST in the acute and chronic rat liver damage caused by toxicants , The mitochondria are important targets for drug toxicity, either directly or indirectly through the creation of reactive metabolites, according to an analysis of the mechanisms causing drug-induced liver damage and the release of soluble products like AST, ALT, and arginase , These abnormalities typically result in mitochondrial oxidative stress and the production of peroxynitrite [30].

Which affects the structural integrity of proteins and mitochondrial DNA , Apoptosis-inducing factor and other intermembrane proteins are also released , Nuclear DNA fragmentation results from the nuclear translocation of and endonuclease G , These things happen together to cause necrotic cell death. On the other hand, mitochondrial release of cytochrome C and other proapoptotic elements can encourage caspase activation and apoptotic cell death [31,32]. The results of several articles indicates the hepatoprotective effect of aloe vera against hepatotoxicity there are many medical plants available to mend the hepatotoxicity of liver damage ,The treatment with herbals

like Aloe Vera might be utilized instead of some drugs to get rid of their toxic activity as well as employed to treat their adverse effects [33].

The treatment with herbals like Aloe vera could be used instead of some drug to rid of their toxic activity as well as used to treat their side effects, Aloe vera gel has been shown to play a protective effect in preventing liver damage, which is indicated by elevated AST and ALT levels and a considerable decline in those values [34]. The results of the current study shown that the biological system's numerous organs depend on NO production, which is increased when arginase activity is inhibited by aloe vera and azathioprine treatment [35]. Arginase is a crucial mediator in the etiology of vascular disease, injury, and inflammation, according to mounting evidence, Hepatotoxicity illness problems associated with inflammation, hypertension, and bodily function failure have all been linked to increased arginase activity [36].

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