Original Article	*Curr. Appl. Sci.*, **2023**, *1*(2):97-104 DOI: 10.22034/cas.2022.334686.1011	Current Applied Sciences

Effects of Long-Term *Aloe vera* (L.) Burm.f. Consumption on Histopathology and Blood Biochemistry Factors in Male Wistar Rats

Mehdi Dehghani Kazemi

Department of Biology, Faculty of Science, University of Zabol, Zabol, Iran

Corresponding author's e-mail: *dehghanimehdi55@uoz.ac.ir*

Article Information

Received: 19 March 2023
Revised: 14 April 2023
Accepted: 15 April 2023
Published online: 28 April 2023

Keywords

Aloe vera
Blood biochemistry
Hepatic steatosis Liver histology
Medicinal plant

Abstract

There is a general belief that herbal supplements and remedies have no harmful effects on the human body. However, liver problems caused by the overuse of medicinal plants in recent decades are a growing concern. This study aims at investigating the effects of long-term administration of *Aloe vera* extracts on liver histology and blood parameters of rats. Three groups of 10-week-old male Wistar rats were treated with *Aloe vera* leaf pulp, leaf gel, and a mixture of both for 56 consecutive days. Then the liver histology and blood biochemistry of control and experimental groups were analyzed. The hematological study indicated that long-term consumption of *Aloe vera* extracts affected the levels of AST and ALT enzymes meaningfully in experimental groups compared with the control group. Changes in other blood parameters, including fasting blood sugar, cholesterol, and Lactate Dehydrogenase (LDH) levels, were also detected in test groups in comparison with the control group. The histopathological findings demonstrated that hepatic steatosis was the most prominent effect of long-term consumption of *Aloe vera* fractions, which was consistent with the rise in the levels of ALP and ALT enzymes in the blood of test groups compared with the control group. The findings of this study are of importance, as they warn against the harmful effects of the overuse of the curative medicinal plant, *Aloe vera*, on mammals' liver.

1. Introduction

Aloe vera (L.) Burm.f. is a leaf succulent evergreen perennial plant belonging to the family Asphodelaceae in the order Asparagales [1]. *Aloe* L. is a polyphyletic genus containing over 500 species around the world. Some molecular phylogenetic studies suggested it be split into six separate genera [2]. *Aloe vera* is native to the southeast Arabian Peninsula, but it is cultivated widely worldwide for commercial or ornamental purposes [3].

Aloe vera has been used widely as a valuable medicinal plant since ancient times. The specific epithet of the species name (vera) is Latin for true, designated it as the right medicinal plant with high health benefits. More than 200 different types of biologically active compounds have been isolated from the species, including polysaccharides, nonessential and essential amino acids, minerals, proteins, lipids, phenolic compounds, vitamins, and saponins [4-6]. Numerous biological activity and pharmacological uses have also been documented for the species in the literature, including antiviral, antiseptic, anticancer, anti-aging, anti-inflammatory, anti-diabetic, anti-atherosclerotic, anti-hypertensive, wound healing, skin protection, etc. so that it covers most of the folk remedies used to treat human disease [6-10]. Due to the wide beneficial effects of *Aloe vera,* it has been increasingly consumed as food, beverage, cosmetic, or medicine worldwide. However, contrary to the popular belief that medicinal plants have no adverse effects, they can be toxic sometimes, especially to the liver. Several studies have demonstrated that aqueous, methanolic, and supercritical carbon dioxide extracts of different organs of *Aloe vera* cause no acute toxicity [11-13]. However, several subacute and chronic toxicity have been reported due to *Aloe vera* administration in animals [7]. Oral administration of *Aloe vera* plus and fresh leaf juice caused pathological effects on the kidney and heart tissues of the treated Wistar rats [14-15]. Also, preclinical toxicological evaluation of *Aloe vera* health drinks led to an increase in red and white blood cell count, liver enzymes, and creatinine levels in the examined Wistar rats [16]. In a long-term study, the administration of aqueous leaf extract of *A. vera* resulted in the hyperplasia of the digestive tract and mesenteric lymph nodes of treated F344/N rats and B6C3F1 mice [16].

Herbal-induced liver injury has been a growing concern, causing liver toxicity in past years [17]. *A. vera* is one of the most common supplements that initiate liver problems with symptoms of abdominal pain, jaundice, fatigue, and nausea and produce hepatocellular and cholestatic patterns of the lesion [18]. This paper aims at throwing light on the effects of long-term use of *A. vera* on liver histology and blood biochemistry factors related to the liver in male Wistar rats.

2. Materials and Methods

The fresh leaves of *Aloe vera* were obtained from Baqiyatallah Al-Azam Educational and Recreational Complex, belonging to the University of Zabol, Zabol, Iran. A herbarium specimen of the studied species was prepared and deposited in the herbarium of the University of Zabol. The collected leaves were washed with distilled water, and the leaf gel was extracted using a knife and a spoon. The leaf pulps were cut into pieces and homogenized with Phosphate-buffered saline (PBS) using a Moulinex Masterchef blender. The obtained extract was stored at 4 °C for 12 hours. The extract was then filtered through a cloth, followed by centrifuging at 20000 rpm for half an hour at 2° C in a refrigerated centrifuge (Cryofuge 20-3 Heraeus-Christ). Next, the supernatant was lyophilized to give a powder using a Labconco apparatus, and the green pellet was thrown away. The final powder was dissolved in PBS using a magnetic stirrer in order to prepare 7.5% pulp extract from the *A. vera* leaf. To prepare the gel of leaf extract, the obtained gel was homogenized using a laboratory blender, followed by dilution with an equal volume of PBS and homogenization one more time. The obtained extract was kept overnight at 4°C and filtered through a cloth afterward. The final clear filtrate was divided into small portions and stored at -20°C.

2.1 *Animals and treatments*

Ten-week-old male Wistar rats were purchased from Razi Institute from Mashhad, Iran. The animals were kept in the laboratory for seven days to adapt to the new environment. Then the rats were divided randomly into four separate groups with eight repetitions: the control group (treated with normal saline, 150 mg/kg/day), experimental group 1 (treated with gel extract, 150 mg/kg/day), experimental group 2 (treated with pulp extract, 150 mg/kg/day) and experimental group 3 (treated with a mixture of gel and pulp extract, 150 mg/kg/day). The leaf pulp extract, gel extract, and an equal mixture of pulp and gel extracts were administered to the three experimental groups for 56 consecutive days.

2.2 *Tissue preparation*

Pentobarbital sodium was applied to anesthetize and sacrifice the animals after the treatment period. The blood samples taken from the abdominal aorta of all studied groups were transmitted to the Dr. Ahmadi Medical Laboratory in Zabol, where a Hitachi 717 biochemistry auto-analyzer was used for analyses. The blood biochemistry parameters were quantified according to the normal blood test procedures. The livers of all groups were also removed and fixed in formalin after erasing the adhering tissues, followed by sectioning and slide preparation. The slides were observed and photographed with an optical Nikon H III Ophtiphot-2 microscope.

3. Results and Discussion

3.1 *Blood biochemistry*

The results of blood biochemistry analyses of the control and experimental groups of male Wistar rats have been shown in Table 1. The cholesterol concentration rose significantly in the group treated with gel extract compared to the control group, while triglyceride concentration showed no significant change among all groups. There were no significant differences among the control and experimental groups in terms of total, direct, and indirect bilirubin, alkaline phosphatase, total protein, and albumin levels (Table 1).

The Fasting Blood Sugar (FBS) level has dropped remarkably in the test group treated with pulp extract, although the FBS test varies in a normal range in the other two test groups. The LDH level slightly dropped in the bloodstream of rats treated with gel extract but rose twice and three times in groups treated with pulp extract and gel +pulp extract, respectively, compared to that of the control group. The concentrations of Serum Glutamic-Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) in blood tests of groups being tested raised insignificantly compared to that of the comparison group. High levels of SGOT, also called aspartate aminotransferase (AST), are a diagnostic parameter for liver damage. However, serum glutamic-oxaloacetic transaminase is not a liver-specific enzyme and could leak into the bloodstream from other parts of the body, such as kidneys, heart, and muscles. To overcome this problem, the amount of SGPT or alanine aminotransferase (ALT), which is more concentrated in the liver, was also measured. The high levels of AST and ALT indicated potential liver damage in test groups.

Table 1. Blood biochemistry results of the control and experimental groups treated with gel extract, pulp extract, and gel+pulp extract

Test	units	control	Gel extract	Pulp extract	Gel+pulp extract
FBS	MG/DL	135	145	44	138 H
Cholesterol	MG/DL	29	59	29	33L
Triglyceride	MG/DL	66	57	55	67L
SGOT	U/L	660	779	890	750 H
SGPT	U/L	166	198	199	188 H
Alkaline phosphatase	U/L	95	88	92	93 L
LDH	U/L	22	15	44	60 L
Total bilirubin	MG/DL	0.32	0.4	0.31	0.22
Direct bilirubin	MG/DL	0.15	0.16	0.19	0.18
Total protein	MG/DL	2.90	2.90	2.50	2 L
Albumin	G/DL	1.8	2.1	2	1.8 L
Indirect bilirubin	MG/DL	0.2	0.2	0.1	0.0 L

3.2 *Histological studies*

In the control group and all test groups, bile canaliculi was normal, and piecemeal necrosis, interface hepatitis, and fibrosis were not observed (Table 2). Cholestasis, a liver disease resulting from the blockage or reduction of bile flow from the liver, was not found in liver tissues of the control and treated groups, which is confirmed by the concentration of direct bilirubin in hematological tests (Tables 1 and 2).

Microscopic cross-section findings demonstrated that lipid accumulation in the liver increased meaningfully in experimental groups compared to the control group (Figure 1 B and C). Fatty liver disease or hepatic steatosis, also called nonalcoholic fatty liver disease (NAFLD), is a serious health problem worldwide, affecting around 10% of Asians and 30% of people in Western countries [19]. Lipid accumulation in the liver may lead to lipotoxicity, which can cause immune response in the Kupffer cells and hepatic stellate cells, resulting in nonalcoholic steatohepatitis (NASH), cirrhosis, and even carcinoma [20, 21]. There is a consistent connection between fatty liver disease and over-nutrition [22], which is in accordance with the results of this study. It appears that long-term use of *Aloe vera* fractions could lead to fatty liver disease in Wistar rats. While the liver biopsy is the standard criterion for diagnosing steatohepatitis, the elevation of the level of liver enzymes, such as aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT), and especially alanine aminotransferase (ALT) in blood samples, is also a typical marker characterizing a fatty liver [23-25]. In this study, it was shown that the ALP levels in blood samples are positively correlated with hepatic steatosis progression, diagnosed in the liver histological assay. This finding supports the results of previous studies regarding the correlation between rising ALP levels in blood samples and hepatic steatosis [26-29].

Only a mild portal inflammation was observed in the test group treated with *Aloe vera* gel (Figure 1 D). Portal inflammation, a common feature in fatty liver diseases, is associated with clinical and histological characteristics that indicate advanced disease [23]. Ballooning degeneration of hepatocytes was only seen in the group treated with gel pulp (Figure 1 E). Ballooning degeneration of hepatocytes is a kind of hepatocyte death followed by cell enlargement [24]. The rearrangement of the intermediate filament cytoskeleton, fat aggregation in the cytoplasm, and inflammation of the endoplasmic reticulum are among the factors contributing to hepatocyte ballooning [25]. Hepatocyte ballooning and hepatic fibrosis are characteristics in diagnosing nonalcoholic fatty liver disease [30].

Altogether, the results of this study agree with several previous studies emphasizing on negative effects of integral *Aloe vera* or its fractions on animal or human liver [14-16, 31]. Some authors claim that the anthraquinone Aloe-emodin is responsible for these negative effects of *Aloe* species on the liver [31-34].

Table 2. histopathological results of the control and experimental groups treated with gel extract, pulp extract, and gel+pulp extract

groups	Body weight (g)	portal inflammation	hepatocytes	steatosis	cholestasis	bile canaliculi	Piecemeal necrosis	interface hepatitis	fibrosis
Control	276	negative	normal	<5%	negative	normal	negative	negative	negative
Gel	278	mild	normal	15-20%	negative	normal	negative	negative	negative
Pulp	277	negative	normal	20-25	negative	normal	negative	negative	negative
Gel-Pulp	275	negative	ballooning degeneration	20	negative	normal	negative	negative	negative

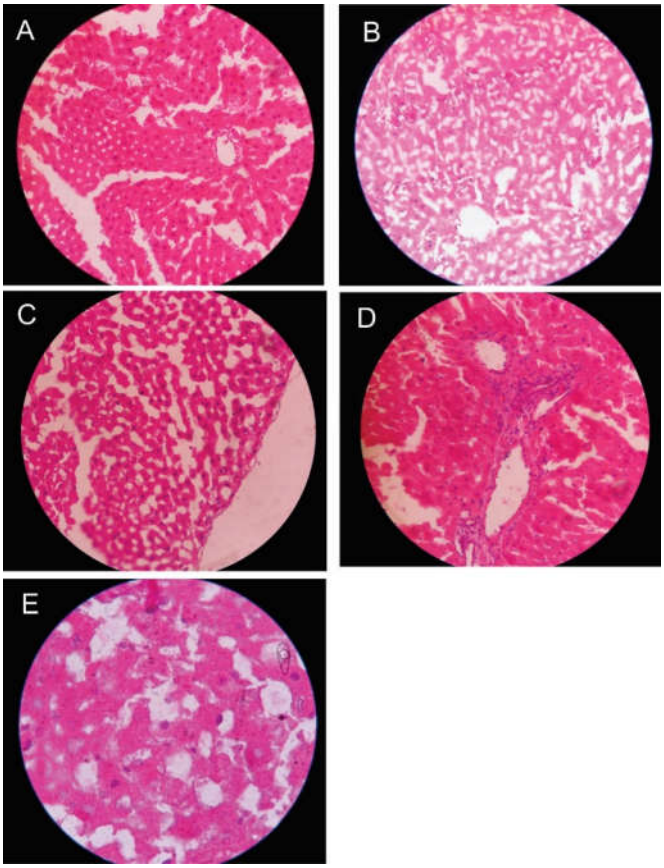

Figure 1. Histological changes in mice liver after long-term oral administration of *Aloe vera* fractions. (A) Normal liver in the control group; (B) and (C) Lipid accumulation in the groups treated with pulp and gel fractions, respectively; (D) Mild portal inflammation in the group treated with gel extract; (E) Ballooning degeneration in the group treated with gel+pulp fraction

4. Conclusions

This study demonstrated that *Aloe vera* treatment for 56 consecutive days affected blood biochemistry parameters and liver histopathology in ten-week-old male Wistar rats. It was concluded that the histopathological abnormalities observed in the livers of the treated Wistar rats with long-term consumption of *Aloe vera* gel, pulp, and gel-pulp include mild portal inflammation, ballooning degeneration of hepatocytes, and hepatic steatosis. However, fatty liver disease is the most prominent effect of long-term consumption of *Aloe vera* fractions, which

is consistent with the rise in the levels of ALP and ALT enzymes in blood samples of experimental groups compared with the control group. This finding is important as it warns against the negative effects of the overuse of curative medicinal plants on the liver, which is a vital organ in mammals, especially during the coronavirus pandemic era.

Conflicts of Interest

The author declares that there are no conflicts of interest regarding this article.

Acknowledgments

This paper was supported financially by the University of Zabol under grant number IR-UOZGR-0331. The author would like to thank Mr. Jasem Estakhr who provided the equipment for this study.

References

1. Chase MW, Christenhusz M, Fay M, Byng J, Judd WS, Soltis DE, Mabberley DJ, Sennikov AN, Soltis PS, Stevens PF. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.*, 2016, 181(1):1-20.

2. Manning J, Boatwright JS, Daru BH, Maurin O, van der Bank M. A molecular phylogeny and generic classification of Asphodelaceae subfamily Alooideae: a final resolution of the prickly issue of polyphyly in the alooids. *Syst. Bot.*, 2014, 39(1):55-74.

3. Reynolds T. Aloes: the genus *Aloe*. 2004, CRC Press.

4. Sharma P, Kharkwal AC, Kharkwal H, Abdin M, Varma A. A review on pharmacological properties of *Aloe vera*. *Int. J. Pharm. Sci. Rev. Res.*, 2 014, 29(2):31-37.

5. Banik S, Sharangi AB. Phytochemistry, health benefits and toxicological profile of *Aloe*. *J. Pharmacog. Phytochem*., 2019, 8(3):4499-4506.

6. Nalimu F, Oloro J, Kahwa I, Ogwang PE. Review on the phytochemistry and toxicological profiles of *Aloe vera* and *Aloe ferox*. *Future J. Pharm. Sci.* 2021;7(1):1-21.

7. Liang J, Cui L, Li J, Guan S, Zhang K, Li J. *Aloe vera*: a medicinal plant used in skin wound healing. *Tissue Eng. Part B Rev.*, 2021, 27(5):455-474.

8. Sabbaghzadegan S, Golsorkhi H, Soltani MH, Kamalinejad M, Bahrami M, Kabir A, Dadmehr M. Potential protective effects of *Aloe vera* gel on cardiovascular diseases: A mini-review. *Phytother. Res.*, 2021, 35(11):6101-6113.

9. Aida P, Cosmin P, Emil M, Graur F, Fetti A, Florea M. The phytochemical constituents and therapeutic uses of genus *Aloe*: A review. *Not. Bot. Horti Agrobot. Cluj Napoca*, 2021, 49(2):12332.

10. Rezazadeh F, Moshaverinia M, Motamedifar M, Alyaseri M. Assessment of anti HSV-1 activity of *Aloe vera* gel extract: an in vitro study. *J. Dent. Shiraz Univ. Med. Sci.*, 2016, 17(1):49-54.

11. Saritha V, Anilakumar KR. Toxicological evaluation of methanol extract of *Aloe vera* in rats. *Int. J. Pharm. Biol. Res*., 2010, 1(5):142-149.

12. Tanaka M, Yamada M, Toida T, Iwatsuki K. Safety evaluation of supercritical carbon dioxide extract of *Aloe vera* gel. *J. Food Sci.*, 2012, 77(1):T2-T9.

13. Sehgal I, Winters WD, Scott M, David A, Gillis G, Stoufflet T, Nair A, Kousoulas K. Toxicologic assessment of a commercial decolorized whole leaf *Aloe vera* juice, Lily of the desert filtered whole leaf juice with aloesorb. *J. Toxicol.*, 2013, 2013:802453.

14. Koroye O, Siminialayi I, Etebu EN. Effects of oral administration of *Aloe vera* plus on the heart and kidney: a subacute toxicity study in rat models. *Niger. J. Health*, 2010, 10(1-2):17-21.

15. Sodani IJ. Histopathological Changes of Male Mice Kidneys Treated with Fresh *Aloe vera* whole Leaf Extract. *Iraqi J. Med. Sci.*, 2015, 13(2): 160-166.

16. Sudhakar P, Prabhu VV, Jamuna B, Adithya RS, Joy A, Anand R. Preclinical toxicological evaluation of *Aloe vera* health drinks in Wistar rats. *Int. J. Res. Pharm. Sci.,* 2018, 1(1):27-32.

17. Woo SM, Davis WD, Aggarwal S, Clinton JW, Kiparizoska S, Lewis JH. Herbal and dietary supplement induced liver injury: Highlights from the recent literature. *World J. Hepatol.* 2021;13(9):1019-1041.

18. Ballotin VR, Bigarella LG, de Mello Brandão AB, Balbinot RA, Balbinot SS, Soldera J. Herb-induced liver injury: Systematic review and meta-analysis. *World J. Clin. Cases*, 2021, 9(20):5490-5513.

19. Singh S, Osna NA, Kharbanda KK. Treatment options for alcoholic and non-alcoholic fatty liver disease: A review. *World J. Gastroenterol.*, 2017, 23(36):6549-6570.

20. Koo S-H. Nonalcoholic fatty liver disease: molecular mechanisms for the hepatic steatosis. *Clin. Mol. Hepatol.*, 2013, 19(3):210-215.

21. Neuschwander-Tetri BA. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. *Hepatology*, 2010, 52(2):774-788.

22. Larter CZ, Chitturi S, Heydet D, Farrell GC. A fresh look at NASH pathogenesis. Part 1: the metabolic movers. *J. Gastroenterol. Hepatol.*, 2010, 25(4):672-690.

23. Rakha EA, Adamson L, Bell E, Neal K, Ryder SD, Kaye PV, Aithal GP. Portal inflammation is associated with advanced histological changes in alcoholic and non-alcoholic fatty liver disease. *J. Clin. Pathol.*, 2010, 63(9):790-795.

24. Lackner C, Gogg-Kamerer M, Zatloukal K, Stumptner C, Brunt EM, Denk H. Ballooned hepatocytes in steatohepatitis: the value of keratin immunohistochemistry for diagnosis. *J. Hepatol.*, 2008, 48(5):821-828.

25. Lackner C. Hepatocellular ballooning in nonalcoholic steatohepatitis: the pathologist's perspective. *Expert Rev. Gastroenterol. Hepatol.*, 2011, 5(2):223-231.

26. Burgert TS, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, Constable RT, Weiss R, Tamborlane WV, Savoye M, Seyal AA. Alanine Aminotransferase Levels and Fatty Liver in Childhood Obesity: Associations with Insulin Resistance, Adiponectin, and Visceral Fat. *J. Clin. Endocrinol. Metab.*, 2006, 91(11):4287-4294.

27. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am. J. Gastroenterol.*, 2003, 98(5):960-967.

28. Westerbacka J, Corner A, Tiikkainen M, Tamminen M, Vehkavaara S, Häkkinen A-M, Häkkinen AM, Fredriksson J, Yki-Järvinen H. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetol. Croat.*, 2004, 47(8):1360-1369.

29. Schindhelm RK, Diamant M, Dekker JM, Tushuizen ME, Teerlink T, Heine RJ. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus and cardiovascular disease. *Diabetes Metab. Res. Rev.*, 2006, 22(6):437-443.

30. Tokushige K, Ikejima K, Ono M, Eguchi Y, Kamada Y, Itoh Y, Akuta N, Yoneda M, Iwasa M, Yoneda M, Otsuka M. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. *J. Gastroenterol.*, 2021, 56(11):951-963.

31. Dong X, Fu J, Yin X, Yang C, Ni J. Aloe-emodin induces apoptosis in human liver HL-7702 cells through Fas death pathway and the mitochondrial pathway by generating reactive oxygen species. *Phytother. Res.*, 2017, 31(6):927-936.

32. Quan Y, Gong L, He J, Zhou Y, Liu M, Cao Z, Li Y, Peng C. Aloe emodin induces hepatotoxicity by activating NF-κB inflammatory pathway and P53 apoptosis pathway in zebrafish. *Toxicol. Lett.*, 2019, 306:66-79.

33. Nesslany F, Simar-Meintières S, Ficheux H, Marzin D. Aloe-emodin-induced DNA fragmentation in the mouse in vivo comet assay. *Mutat. Res. - Genet. Toxicol. Environ. Mutagen.*, 2009, 678(1):13-19.

34. Vath P, Wamer WG, Falvey DE. Photochemistry and Phototoxicity of Aloe Emodin. *Photochem. Photobiol.*, 2002, 75(4):346-352.

How to cite this article: Dehghani Kazemi M. Effects of Long-Term *Aloe vera* (L.) Burm.f. Consumption on Histopathology and Blood Biochemistry Factors in Male Wistar Rats. *Curr. Appl. Sci.*, 2023, 1(2):97-104. https://doi.org/10.22034/cas.2022.334686.1011