Biochmical changes in liver enzymes in male rats treated with *Borago officinalis* extract

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ABSTRACT

Borago officinalis in the current experiment, assessment of a borage extract treatment on hepatic enzyme activity in male albino rats with special attention to the (ALT, AST), and (APL). Forty-one rats (150-300 g) were allocated into six experimental groups: 2 control groups ,and four treatment groups receiving seed or flower extracts. Seed extract at 300 mg/kg resulted in conspicuous hepatotoxicity with a rise of ALT activity and a decrease of ALP activity, while seed extract at 400 mg/kg had a contrasting effect on ultrasonic parameters that increased ALP activity and caused no hepatocellular damage. In comparison, flower extracts at both concentration (300,400) exerted only slight effects,no statistically significant differences in (ALP,AST,ALP).These data would reveal that the administration of borage seeds' secondary metabolites could provide a potentially markedly toxicological profile in the liver in a dose-related manner, whereas extracts obtained from its flowers no changes appeared.

1. Introduction

Plants are considered an important source of biologically active compounds involved in both traditional and modern medicine. The plants have one or more pharmacologically effective ingredients in various parts or derivatives, for example, roots, stems, leaves, flowers, and seeds. The amount of these compounds is extremely variable but bioactive. They are synthesized in planta to regulate various physiological and ecological roles such as plant defense against herbivores, fungi, and pathogens, and modulating plant–environment interactions [1].

1.1 Borage

Borago officinalis L. (family: Boraginaceae), known as borage or "starflower," is an annual herb and has been utilized for medicinal and culinary purposes since ancient times [2,3] .The plant, being native to the Mediterranean area, is widely distributed in many countries across the world because of its versatility and importance, 718 as both traditional medicines as well a commercial cultivation [4].

2.1 Secondary Metabolites

Plant metabolites are generally divided into primary and secondary compounds, both necessary for plant survival and ecological fitness. These are known as primary metabolites comprising carbohydrates, lipids, proteins, amino acids, and vitamins, which take direct part in respiration, growth, and reproduction at the cellular level [5] . In contrast, secondary metabolites are not necessary for primary metabolism but play important ecological roles such as defense mechanisms, responses to abiotic stresses, and inter-species signaling [6,7].numerous studies have documented the effects of plant secondary metabolites [8,9].

B. officinalis is abundant in various secondary metabolites: resins, tannins, ascorbic acid, niacin, β-carotene, riboflavin, silicic acid and thiamine, choline, arabinose, polyphenols, and unsaturated pyrrolizidine alkaloids (e.g., ampaline, lycopsamine, supinine),[10,11]. These compounds are responsible for the pharmacological actions of the plant, such as antioxidant and anti-inflammatory hepatoprotective effects.

3.1 Borage on Liver Enzymes

The liver, which serves as the master metabolic and detoxifying organ of the body, is extremely vulnerable to both xenobiotic and phytochemical insult. Its function includes carbohydrate and lipid metabolism, apolipoprotein synthesis, and cholesterol disposal through the production of bile [12]. Hepatic injury is traditionally diagnosed by changes observed in the serum levels of alanine

aminotransferase (ALT) and/or aspartate aminotransferase (AST) or alkaline phosphatase (ALP), which are generally used as biochemical markers for hepatocellular integrity and biliary function, respectively.

Borage Oil, being rich in γ-linolenic acid (GLA), has been extensively investigated. Antioxidants and free radical scavenging are well known that GLA presents a potential role in the antioxidation [13,14]. In the laboratory, GLA prevents lipid accumulation in hepatocytes but does not always restore liver architecture to control levels in ethanol-pretreated animals [15]. However, borage oil exhibited protective properties in mouse models of ethanol-mediated fatty liver [16]. Moreover, ethanolic borage extract suppressed pro-inflammatory mediators (TNF-α, NF-κB) and improved liver histology in CCl_4-induced toxic hepatitis rats by reducing serum ALT and AST levels, as previously described by Hamed and Wahid.numerous researchers have emphasized the significant role of plant extracts [17,18,19]

Altogether, these data indicate that *B. officinalis* is endowed with a double pharmacological risk: hepatotoxic or hepatoprotective effects depending on the plant organs tested, dose administered, and metabolic background.[20,21], but comprehensive systematic studies focusing on the role of seed and flower secondary metabolites in the control of liver enzymes remain scarce.

4.1 Objective of the Study

In the current study, we have investigated the potential hypolipidemic and hepatoprotective effects of Bo seed/flo extracts on serum ALT, AST, and ALP in rats. Through profiling such biochemical responses, the present study aims to illustrate the potential hepatoprotective or hepatotoxic effects of plant parts as well as pave the way for their future therapeutic uses.

2. Experimental

2.1 Synthesis of Samples

Borago officinalis seeds and flowers were purchased from local markets and collected in Basrah province, Iraq. Sealed containers of the plant material were kept at room temperature for later use. The samples were ground finely (electric grinder) to achieve a uniform powder, and extracts were prepared for further use, which were stored under controlled temperature to protect the bioactive components of the samples.

2.2 Extraction Procedure

B officinalis seeds and flowers were extracted as per (Harborne,1998),[22] in a Soxhlet apparatus. The powder of the plant was placed into extraction thimbles ,using different solvents were used to obtain all pharmacologically active compounds in collection flasks. The extracts were then concentrated under reduced pressure on a rotary evaporator at 40 °C to remove the solvent, and a crude extract was obtained. The extracts were subsequently air-dried to a constant weight, and then they were stored for use in the preparation of concentrates (300,400).

2.3 Experimental Animals

Forty-one adult male rats (Rattus norvegicus), weighing 150–300g, were used in this research. The animals were kept in sterile plastic cages maintained under standard laboratory conditions (temperature, 25-30°C; 12-hour light/dark cycle) and given access to food and water *ad libitum*. The animals were divided into six experimental groups (Table 1):

1. Control group: Received water only.

2. DMSO control Received dimethyl sulfoxide (GablerGmbH) along with standard diet and water.

3. Treatment group 1: Given B. officinalis seed extract (300mg/kg).

4. Treatment group 2 - Received B. officinalis seed extract at a dose of 400mg/kg.

5. Group 3 (treatment): Treated with B. officinalis flower extract in a dose of 300mg/kg.

6. Group 4: Received B. officinalis flower extract (400mg/kg).

Table 1: Concentrations and doses used in the experiment

Group	Concentration	Dose
Seeds 300 mg/kg	6 g of extract + 100 ml of DMSO	1 ml
Seeds 400 mg/kg	8 g extract + 100 ml DMSO	1 ml
Flowers 300 mg/kg	6 g of extract + 100 ml of water	1 ml
Flowers 400 mg/kg	8 g of extract + 100 ml of water	1 ml

Control DMSO	at a concentration of 99.5 ml	0.5 ml
Control Natural	Water	0

The therapies were orally given three times a week for 6 weeks. At the end of the study, the animals were anesthetized, and blood samples were obtained directly from cardiac puncture. The serum was separated from whole blood by centrifugation and tested for hepatic enzyme activities, namely AST (aspartate aminotransferase), ALT (alanine aminotransferase), GOT, and GPT using an enzymatic assay kit (according to the manufacturer's instructions).

2.4 Statistical Analysis

SPSS Statistics was used for data analysis. Differences between groups were analyzed by one-way analysis of variance (ANOVA). $P<0.05$ was statistically significant.

3. Results

The action of the extracts from the Borago officinalis seed and flower on the activity of hepatic enzymes is presented in Table 2. Out of all experimental groups, 300mg/kg borage seed extract produced the greatest changes in liver enzemes. In particular, serum alanine aminotransferase (ALT) was significantly higher than the ALT of the control group (64.46±4.68 vs. 29.08±2.66; $p<0.001$) and DMSO controls ($p=0.004$), suggesting hepatocellular damage and increased membrane permeability resulting in enzyme leakage. By contrast, the activity of ALP was significantly reduced (11.00±4.76 compared to controls: 48.77±12.38; $p=0.008$) with a trend to difference also in comparison to the DMSO group ($p=0.004$), indicating either bile duct dysfunction or inhibition of synthesis of membrane-bound enzymes as its causes may be present). No significant difference was found for the level of Aspartate aminotransferase (AST) between 300mg/kg borage seed extract and the control group (71.55±18.47 vs. 100,11±35,93; $p=0.288$), but its level was different from the DMSO group($p=0.014$),.suggesting that AST may be less sensitive than ALT to detect liver damage, and solvent effects are contributory for changes observed here. Taken together, the ANOVA results confirm that a dose of 300mg/kg borage seed extract consistently induces significant hepatotoxicity with several enzymatic markers.

In contrast, at 400mg/kg of borage seed extract, the second group showed a distinctly different threshold. ALT level was higher than control subjects but did not reach statistical significance (48.84±27.25, p=0.105). AST was reduced (58.68±30.01) compared with the control, suggesting it to be trend-like significance (p=0.094), whereas it showed highly significant against DMSO (p<0.001), which means AST modulation is more likely to depend on the solvent rather than treatment group treatments. More interestingly, there was increased ALP activity (73.00±16.35 vs 48.77±12.38; p=0.053) with a statistically significant difference to DMSO (p=0.017), which would suggest a compensatory upregulation of bile duct-related enzyme activity or membrane protein synthesis by this modification during the cholestatic period in f/tGRP. Taken together, the abovementioned results show that 400 mg borage seed extract/kg has a major impact on ALP activity level, while changes in ALT and AST levels are more modest.

Compared to the control group, there were no statistically significant differences in ALT (36.65±7.93 vs 29.08±2.66, p=0.181), AST (104.70±18.19 vs 100.11±35.93, p=0.831), or ALP (58.99 ±13.29 vs 48.77 ±21-12, p =0.348) levels of rats treated with 300mg/kg borage flower extract, where no clear signs of liver damage were recorded.

The borage flower extract 400mg/kg group showed ALT (31.08±4.32; p=0.516) and AST (90.74±8.89; p=0.627) values resembling those of the control, and an increased ALP activity (70.49±13.24 vs 48,77±12,38; p=0.079), although not significantly between them all .

Table 2: Statistical results for liver enzymes

Groups	**GPT (ALT) Mean ± Std. Deviation**	**p-value**	**GOT (AST) Mean ± Std. Deviation**	**p-value**	**ALP Mean ± Std. Deviation**	**p-value**
Male/seedG1	64.458 ± 4.681	0.000**	71.545 ± 18.467	0.288	11.000 ± 4.763	0.008**
Control normal	29.083 ± 2.666		100.113 ± 35.929		48.767 ± 12.379	
Male/seedG1	64.458 ± 4.681	0.004**	71.545 ± 18.467	0.014*	11.000 ± 4.763	0.004**
DMSO	31.410 ± 8.725		161.895 ± 0.524		52.800 ± 0.275	
Group2	48.841 ± 27.248	0.105	58.681 ± 30.011	0.094	73.001 ± 16.346	0.053

Control normal	29.083 ± 2.666		100.113 ± 35.929		48.767 ± 12.379	
Group 2	48.841 ± 27.248	0.168	58.681 ± 30.011	0.000**	73.001 ± 16.346	0.017*
DMSO	31.410 ± 8.725		161.895 ± 0.524		52.800 ± 0.275	
Group3	36.645 ± 7.933	0.181	104.700 ± 18.190	0.831	58.988 ± 13.289	0.348
Control normal	29.083 ± 2.666		100.113 ± 35.929		48.767 ± 12.379	
Group 4	31.083 ± 4.324	0.516	90.740 ± 8.898	0.627	70.492 ± 13.241	0.079
Control normal	29.083 ± 2.666		100.113 ± 35.929		48.767 ± 12.379	

4. Discussion

Treatment with 300mg/kg of Borago officinalis seed extract resulted in significant hepatocellular injury, reflected by a greater rise in serum ALT (64.46±4.68 vs 29.08±2.66 in controls, $p<0.001$). This rise indicates the leakage of enzymes from hepatocytes as a result of loss of cellular membrane integrity, which might be due to the effects of oxidative stress or direct toxicity of bioactive components. These results agree with previous studies that have shown ALT to be the most sensitive biomarker of HCC damage [23] and increased levels, a marker of acute hepatocyte injury [24]. On the other hand, alkaline phosphatase (ALP) activity was decreased significantly (11.00 ± 4.76 vs 48.77 ± 12.38; $p=0.008$), which is an unusual profile for classical cholestatic injury where ALP usually rises. It has been suggested that ALP levels decrease under chronic circumstances or suppression of gene expression, as reported earlier by Singh et al. and Iluz-Freundlich et al.[25,26]. Also, solvents, which could include the DMSO inhibition of bile acid synthesis pathways [27], can result in transient decreases in ALP. There was no significant change in serum aspartate aminotransferase (AST) compared to the control group, which matches the lower hepatic specificity of AST because it is found both within extrahepatic tissue, like cardiac and skeletal muscle tissues [28] .Other reports, however, suggest that there can be simultaneous rises in ALT and AST during severe hepatic injury [29]. Taken together, these data suggest that an oral dose of 300mg/kg borage seed extract causes acute hepatocellular injury, and the suppression of ALP may represent bile duct damage or altered enzyme expression.

In the seed extract 400mg/kg group, only ALP changes were marginal, showing a pattern of an early compensatory relationship rather than true markers of hepatotoxicity, as they were not statistically significant. This course of events is also consistent with the finding that early biliary perturbation could lead to an up-regulation of ALP because of cholangiocyte activation or the start-up of repairing processes [30,31]. The dissociation between ALT and ALP is distinct from published associations of elevated ALP concentration with more advanced biliary obstruction or fibrosis [32], indicating that these responses are likely to indicate a hepatic adaptive rather than direct cytotoxic effect.

The results of the groups treated with Borago officinalis flower extract confirm no statistically significant changes in liver enzymes (AST, ALT, and ALP). However, there is a significant histological disruption in the livers of the treated animals. This outcome can be interpreted by Fu et al. (2002), who demonstrated that borage extracts can promote DNA mutations and genotoxic effects, causing substantial hepatocellular injury even in the absence of increased liver enzyme levels. This result can be attributed to the capability of certain compounds in Borago flowers to downregulate the pathways of gene expression that are responsible for the synthesis of these enzymes, hence limiting their release into the circulation. Additionally, these compounds can exert mutagenic effects with prolonged use and at high doses [33].

4. Conclusions

The effect of oral administration of 300 mg kg (-1) borage seed extract was pronounced and resulted in an increase in ALT-level as well as a decrease in ALP. On the other hand, 400mg/kg seed extract produced compensatory elevations in ALP, with little shift in ALT and AST. Borage flower extracts at 300mg/kg and 400 mg/kg concentrations caused few and inconsistent changes in the enzymes.

References

[**1**]Zaït I, Khalfawi A, Dbaib R. Ethnobotanical study of some medicinal plants in the Meftah region. Graduation dissertation for the degree of Secondary Education Teacher. École Normale Supérieure, Kouba–Algeria; 2020.

[2]Zając M, Duda I, Skoczylas Ł, Tabaszewska M. Potential use of Hyssopus officinalis and Borago officinalis as curing ingredients in pork meat formulations. Animals (Basel). 2020;10(12):2327.

[3]Adamczyk-Szabela D, Wolf WM. The influence of copper and zinc on photosynthesis and phenolic levels in basil (Ocimum basilicum L.), borage (Borago officinalis L.), common nettle (Urtica dioica L.) and peppermint (Mentha piperita L.). Int J Mol Sci. 2024;25(7):1-13. https://doi.org/10.3390/ijms25073612

[4]El-Rahman A, Ali AF, Amer EH. Physiological effects of organic and bio fertilizers on borage (Borago officinalis L.) plants. Arch Agric Sci J. 2023;6(2):141-164.

[5]Jeyasri R, Muthuramalingam P, Karthick K, Shin H, Choi SH, Ramesh M. Methyl jasmonate and salicylic acid as powerful elicitors for enhancing the production of secondary metabolites in medicinal plants: An updated review. Plant Cell Tissue Organ Cult. 2023;53:447-458.

[6]Tariq A, Zeng F, Graciano C, Ullah A, Sadia S, Ahmed Z, Zhang Z. Regulation of metabolites by nutrients in plants. In: Plant Ionomics: Sensing, Signaling, and Regulation. Hoboken (NJ): John Wiley & Sons Ltd; 2023. p. 1-18.

[7]Chen D, Mubeen B, Hasnain A, Rizwan M, Adrees M, Naqvi SAH, Shahzad SM, Hussain M, Alharby HF, Irshad MK, et al. Role of promising secondary metabolites to confer resistance against environmental stresses in crop plants: Current scenario and future perspectives. Front Plant Sci. 2022;13:881032.

[8] Al-Saadi SA, Al-Derawi KH, Abd Alrasool S. The effect of steroidal extract of Nerium oleander on the liver tissue structure of albino mice. IOSR J Pharm Biol Sci. 2017;12(2):52–5.

[9] Al-Saadi SA, Al-Derawi KH, Al-Azzem DA. Variation in essential oil content and composition (Pimpinella anisum L.). J Biol Agric Healthc. 2016;6(20):43-52.

[10] Gudej P, Tomczyk M. Chromatographic analysis of polyphenolic compounds from the herbs of Borago officinalis (L). Herba Polonica. 1996;42:252-256.

[11] Bandoniene D, Murkovic M. The detection of radical scavenging compounds in crude extract of borage (Borago officinalis L.) by using an on-line HPLC-DPPH method. J Biochem Biophys Methods. 2002;53(1-3):45-49.

[12] Singh M, Kamal TY, Khan AM, Parveen R, Ahmad S. In vitro antioxidant activity and HPTLC analysis of Borago officinalis Linn. Indian J Pharm Educ Res. 2013;47.(4)

[13] Leung PS. The Gastrointestinal System: Gastrointestinal, Nutritional and Hepatobiliary Physiology. New York: Springer; 2016. p. 24-36.

[14] Nabavi SY, Silva AS. Nonvitamin and nonmineral nutritional supplements. In: Tewari D, Bawari S, Patni P, Sah AN, editors. Borage (Borago officinalis L.). 1st ed. London (UK); San Diego (CA): Academic Press; 2018. p. 165-170.

[15] Singh H, Du J, Yi TH. Green and rapid synthesis of silver nanoparticles using Borago officinalis leaf extract: anticancer and antibacterial activities. Artif Cells Nanomed Biotechnol. 2017;45:1310-1316.

[16] Segarnick DJ, Cordasco DM, Agura V, Cooper NS, Rotrosen J. Gamma-linolenic acid inhibits the development of the ethanol-induced fatty liver. Prostaglandins Leukot Med. 1985;17:277-282.

[17] Thuwaini MM, Al-Derawi KH, Kadhem HS. Nephroprotective activity of Matricaria chamomile and Curcuma longa aqueous extracts on tetracycline-induced nephro-toxicity in albino rats. Int J Pharm Ther. 2016;9(4):161-7.

[18] Al-Malaak MK, Al-Derawi KH, Hassan WN. Histological study on effect of Solanum nigrum dried fruits extract on induced stomach ulcers in experimental rats. J Thi-Qar Sci. 2012;3(3):21-6.

[19] Ali SA, Alderawi KH, Al-Mansour NA. Toxic effects of lead-induced damage in liver of rats male and the role of ethanolic ginseng extract as protective agents (histological and physiological). Merit Res J Med Med Sci. 2018;6(9):307-15.

[20] Lukivskaya OY, Naruta E, Sadovnichy V, Kirko S, Buko VU. Reversal of experimental ethanol-induced liver steatosis by borage oil. Phytother Res. 2012;26:1626-1631.

[21] Hamed A, Wahid A. Hepatoprotective activity of Borago officinalis extract against CCl_4-induced hepatotoxicity in rats. SSRN Electron J. 2021. https://doi.org/10.2139/ssrn.3776362

[22] Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd ed. London; New York: Chapman and Hall; 1984.

[23] Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S, Snyder R. The current state of serum biomarkers of hepatotoxicity. Toxicol. 2008;245(3):194-205. https://doi.org/10.1016/j.tox.2007.11.021

[24] Giannini EG, Testa R, Savarino V. Liver enzyme alteration: A guide for clinicians. CMAJ. 2005;172(3):367-379. https://doi.org/10.1503/cmaj.1040752

[25] Singh A, Bhat TK, Sharma OP. Clinical biochemistry of hepatotoxicity. J Clin Toxicol. 2011;S4:001. https://doi.org/10.4172/2161-0495.S4-001

[26] Iluz-Freundlich D, Uhanova J, Grubert Van Iderstine M, Minuk GY. The impact of primary biliary cholangitis on non-alcoholic fatty liver disease. Eur J Gastroenterol Hepatol. 2021;33(4):565-570. https://doi.org/10.1097/MEG.0000000000001782

[27] Chen X, et al. Dimethyl sulfoxide inhibits bile acid synthesis in healthy mice. PMC Open Access. 2023.

[28] Gowda S, Desai PB, Hull VV, Math AAK, Vernekar SN, Kulkarni SS. A review on laboratory liver function tests. Pan Afr Med J. 2009;3(1). https://doi.org/10.4314/pamj.v3i1.51713.

[29] Ali R, Cusi K, Hecht L. Clinical utility of serum transaminases for the detection of hepatocellular injury. Clin Liver Dis. 2015;19(2):183-194. https://doi.org/10.1016/j.cld.2014.09.002

[30] Pratt DS, Kaplowitz N. Biochemical liver tests. In: Kaplowitz N, DeLeve LD, editors. Drug-Induced Liver Disease. New York: Marcel Dekker; 2000. p. 21-39. https://doi.org/10.1016/B978-012387817-5.00002-1

[31] Nathwani RA, Pais S, Reynolds TB, Kaplowitz N. Serum alanine aminotransferase in skeletal muscle diseases. Hepatology. 2005;41(2):380-382. https://doi.org/10.1002/hep.20548

[32] Carey EJ. Noninvasive tests in cholestatic liver diseases. Clin Liver Dis. 2015;19(1):97-110. https://doi.org/10.1016/j.cld.2014.09.006

[33] Fu, P. P., Xia, Q., Lin, G., & Chou, M. W. (2002). Genotoxic pyrrolizidine alkaloids—mechanisms leading to DNA adduct formation and tumorigenicity. International Journal of Molecular Sciences, 3(9), 948-964.

التغيرات البيوكيميائية في انزيمات الكبد لذكور الجرذان المعاملة بمستخلص نبات لسان الثور (*Borago officinalis*)

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المستخلص

يُعتبر نبات لسان الثور (Borago officinalis) من النباتات الطبية الشائعة التي تحتوي على مجموعة واسعة من المركبات الثانوية منها الراتنجات والتانينات وغيرها وقد أظهرت العديد من الدراسات أن هذه المركبات تمتلك خصائص دوائية متعددة، من بينها التأثيرات المضادة للأكسدة والمضادة للالتهابات. تهدف هذه الدراسة إلى دراسة تأثير مستخلص نبات لسان الثور في الجرذان المختبرية على تراكيز بعض إنزيمات الكبد (AST, ALT, ALP) .

تم استخدام 41 جرذًا مختبريًا ذكريًا بوزن 150–300 غرام، قُسمت إلى 7مجاميع: 3 مجاميع سيطرة واربع مجموعات معالجة بتراكيز مختلفة

حيث بينت النتائج إن المجموعة المعاملة بمستخلص بذور نبات لسان الثور بتركيز 300 ملغم/ كغم هي الوحيدة التي سببت ضررًا كبديًا حقيقيًا ارتفاع ALT وانخفاض ALP بينما المجموعة المعاملة بمستخلص البذور بتركيز 400 ملغم /كغم أظهرت تغيرًا في اتجاه مختلف يتمثل بارتفاع .ALP أما مجاميع المعاملة بالازهار بتراكيز 300 و400 ملغم/كغم فالتغيرات فيهما طفيفة ، لم تظهر تغيرات معنوية في (ALT,AST,ALP) تشير هذه النتائج إلى أن المركبات الثانوية في بذور نبات لسان الثور قد تسبب تلف كبدي بينما ازهار نبات لسان الثور لم تظهر تغيرات واضحة .