Original Article

Assessment of Changes in Serum Concentrations of Liver Function (ALT, ALP, AST, LDH, GGT) After the Intake of Narcissus Bulbs

Morovvati H*, Armand N

Department of Basic Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

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Abstract

Background & Objective: Narcissus bulb is an antiphlogistic drug, effective for asthma treatment, shortness of breath and skin burns. The aim of this study was to investigate the effect of Narcissus tazetta bulb extract on serum concentrations of liver function test enzymes (ALT, ALP, AST, LDH, GGT) in male rats.

Materials & Methode: In this study, 20 male rats were divided into 4 groups of 5 series. They were divided into control groups and experimental groups and received the alcoholic extract 50, 100 and 150 mg/kg of Narcissus bulb. The extract was fed for 6 days and blood samples were taken from the animals on the seventh day and serum levels of liver enzymes were measured. Statistical analysis was performed with SPSS software (version 21.0) using t-test.

Results: The activity of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) enzymes in 150 mg/kg extract of Narcissus bulb significantly increased ($P<0.01$). Also, at the same dose, the enzymes of Alkaline phosphatase (ALP), Lactate Dehydrogenase (LDH) and Gamma Glutamyl Transferase (GGT) showed a significant increase compared to the control group ($P<0.05$). ALT at 100 - mg/kg dose significantly increased, but AST at 50 mg/kg dose showed a significant decrease compared to the control group ($P<0.05$).

Conclusion: Considering the significant increase in liver enzymes at high doses of extract, it is necessary to study the histopathological effects of the extracts of this plant on liver.

Keywords: Narcissus, Rat, liver enzyme.

Introduction

Narcissus flower, scientifically named Narcissus tazetta L., belongs to the family Amaryllidaceae. It is a bulbous plant and is used as cut flowers, flower pots or ornamental in the open air (1). Common with the name "Narjes", the plant has white and yellow flowers that appears from January to April and has a pleasant aroma. Amaryllidaceae family plants are often tropical or subtropical, while N. tazetta is found in southwestern Europe and the Mediterranean region, Spain and Portugal as well as in Iran and Kashmir (2,3). The four varieties of Narcissus flower in Narcissar Behbahan car include Shahla (SB), Shastpar, Meskin (MB) and Panjehgorbei (PGB) varieties (4). Narcissus is a valuable herb for cosmetic, medicinal and therapeutic properties. Its volatile constituents are largely composed of monoterpene, cisco terpenes, benzoyl compounds and other minor compounds (5). From 106 species of Narcissus flowers 61 alkaloids and 2 protoalkaloids (2 protoalkaloids) were identified. The leaves have higher content and more complex properties of alkaloids. The

***Corresponding Author: Morovvati Hasan,** Department of Basic Science, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Email: hmorovvati@ut.ac.ir
https://orcid.org/0000-0003-0275-1636

dominant alkaloids in the leaves are lycorin and bulbs are galantamine (6). Galantamine is used in the treatment of Alzheimer's disease as a cholinesterase inhibitor (7). A new alkaloid, belonging to the pretazettine group of the Amaryllidaceae alkaloids, was isolated from the dried bulbs of Narcissus jonquilla and renamed it jonquailine. Initial biological evaluation revealed significant antiproliferative effects against glioblastoma, melanoma, uterine sarcoma, and lung cancer cells (8). Isolated from the flower bulb of Narcissus flavan, coumarin (β-coumaranone) and flavonoids. These compounds have shown strong antioxidant activity against H2O2-induced impairment in human SH-SY5Y neuroblast cells (9). Hydrogen peroxide (H2o) causes neuroblastoma cell death, and can cause cell cancer (10). A peptide with a molecular mass of about 9 kDa (NTP) has been isolated and purified from the bulbs Narcissus tazetta variety Chinensis L., which can inhibit the protein synthesis of reticulocytes. NTP can significantly inhibit plaque formation by respiratory syncytial virus (RSV) and the cytopathic effect of influenza A virus (H1N1) as well as proliferation of human acute promyelocytic leukemia (APL = Acute promyelocytic Leukemia) cells (HL-60). (11) Also, the effect of Narcissus extract on antiviral activity, prophylaxis, antibacterial, antifungal, anti-malarial, insecticidal, cytotoxic, anti-tumor, anti-bacterial, anti-platelet, antihypertensive, acetylcholinesterase inhibitor. In vitro disinfectants have been studied and reported (12). Determination of serum levels of hepatic marker enzymes including: aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) have been proposed as liver function tests (13). Lactate dehydrogenase (LDH) is also a cytoplasmic enzyme, which is used to evaluate cellular damage and as an indicator to assess the toxicity of a chemical and cell lysis (14). Gamma glutamyl transferase (GGT) has a predictor of cellular antioxidant deficiency and for alcohol-related liver disease. (15). The effect of medicinal plants on the liver and its enzymes has been studied by many researchers. The effects of aqueous-alcoholic extract of Mongoose leaf on liver functional factors in male rats were investigated by Mokhtari et al. (2008). The results of this study showed that the aqueous-alcoholic extract of the medicinal leaf of Pomona reduces the liver enzymes ALT and AST (16). (2010) In a study, hepatotoxicity and renal toxicity of methanolic extract of Capparis spinosa in rats were studied. The results showed a significant increase in serum ALP at 200 mg/kg (17). Also, Zahedi et al. (2004) investigated the effect of two herbs of Valeriana Officinalis and Echium Amoenum on liver and kidney function in rats, and its results showed that E. Amoenum has more hepatotoxic effects and all three enzymes alanine aminotransferase and aspartate aminotransferase. Also, alkaline phosphatase at high dose (200 mg/kg) showed a significant increase compared to control (18). In recent years, the prevalence of plant poisoning has been increasing due to the increasing tendency of people to use herbs for medicinal and medical applications, largely due to the unconscious and arbitrary use of toxic herbs. They think it has therapeutic value. On the other hand, self-medication with herbs and herbs are considered as causing factors such as hallucinations, including causes of poisoning caused by plants in adults. The statistics of drug and poisoning information centers in the country also show that there are poisonings with herbs and herbal medicines among the population (19). However, according to reliable sources and international scientific profiles, no research has been reported on the effect of Narcissus flower on the liver and due to the increasing use of Narcissus flower, it is necessary to carry out scientific and research studies on this plant. The aim of this study was to assess the changes in serum concentrations of liver function (ALT, ALP, AST, LDH, GGT) after the intake of Narcissus bulbs.

Materials & Methods

Animals: 20 adult male rats weighing approximately 185 to 213 g were obtained from the School Pet, under 12 h light/dark conditions, appropriate temperature 22 °C to 26 °C, optimal ventilation. The animals were kept in the laboratory of Pars Animal Feed Factory for 6 days. This research was approved by the University by No. 2523/2/12 on 9/4/97. Plant Specimen Collection: Behbahan city is located in southeast of Khuzestan province with an area of 3195 km2. According to Behbahan weather station statistics, the average rainfall in this area is 350 mm, with an average annual temperature of 24.5 ° C, and the climate is semi-arid according to the Domartan method. Wind directions predominate in the western and northwestern regions (20). Narcissus bulbs was

prepared from Narcissus of Behbahan. Preparation of herbal extract: 200 g of chopped bulbs Narcissus was soaked in methanol at 40 °C for 72 h, repeated again to dissolve the remaining active ingredients in methanol extract. It was then concentrated by vacuum distillation in methanol extract. Preparation of edible extract from the extract: Dissolve 50, 100 and 150 mg of the dried extract individually in 10 ml of physiological serum, and then an oral solution was prepared at doses of 50, 100 and 150 mg per kg (mg/kg). Animals were fed 2 ml of each solution for 200 g of rat weight.

Study design: In this study, 20 rats were randomly divided into 4 groups: one control group, and three groups receiving Narcissus bulb extract at concentrations of 50, 100 and 150 mg/kg.

Control group: The rats received 2 ml of normal saline orally, daily for 6 days Experimental

group 1: The rats received 2 ml of 50 mg/kg Narcissus bulb extract orally, daily for 6 days Experimental

Group 2: The rats received 2 ml of 100 mg/kg Narcissus bulb extract orally, daily for 6 days Experimental

Group 3: The rats received 2 ml of 150 mg/kg Narcissus bulb extract orally, daily for 6 days The extract was fed for 6 days, and on the seventh day, ethyl ether was used to anesthetize the rats. Blood samples were then taken from the animals in order to separate serum and their blood was centrifuged at 300 rpm for 10 min and kept in a freezer 80 until enzyme assay.

Measure enzymes: Measurement of ALT, ALP, AST, LDH and GGT by photometric method (Pars Test Enzymatic Diagnosis Kits) and based on the recommendation of International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) by Biotechnical Company 3000 BT Auto-analyzer. occurred.

Statistical analysis: Data were analyzed by SPSS software, one-way ANOVA and t-test. Results were reported (Mean ± SD), $p<0.05$ and $p<0.01$ significant. It was considered.

Results

The activity of the enzymes was assayed at 50, 100 and 150 mg/kg doses of Narcissus bulb extract are presented in Table (1). the results showed that:

AST enzyme at 150 mg/kg dose of bulbs extract of Narcissus showed a significant increase compared to the control group ($P<0.01$), but at 50 mg/kg dose it showed a significant decrease compared to the control group ($P<0.05$).

ALT in the dose of 150 mg/kg extract of Narcissus bulb extract showed a significant increase compared to the control group ($P<0.01$), but at the dose of 100 mg/kg it showed a significant increase compared to the control group ($P<0.05$).

ALP only significantly increased at 150 mg / kg dose of Narcissus bulb extract ($P<0.01$).

Table 1. Effect of methanol extract of Narcissus bulbs on liver function enzymes

Compare groups	GGT U/l	AlkP U/l	ALT U/l	AST U/l	LDH U/l
Control group	2/560±0/670	401/27±39/37	69/24±7/89	192/11±23/17	1675/00±252/639
50 mg/kg	2/578 ± 0/901	395/31±74/30	67/39±11/59	179/92±14/47*	1679/535±80/32
100 mg/kg	2/780±0/568	429/05±132/19	84/73±9/54*	214/43±28/51	1766/00 ± 269/20
150 mg/kg	3/229±1/296*	517/82 ±51/98*	112/73± 11/34**	295/86 ±29/76**	2014/57± 29/095*

- The values are "Maen ± SEM". The presence of * in the table indicates a significant difference ($P<0.05$), and the presence of ** in the table indicates a significant difference ($P<0.01$) compared to the control group.

LDH enzyme showed a significant increase only in the dose of 150 mg/kg extract of Narcissus bulb (P<0.01).

GGT enzyme showed a significant increase only in the dose of 150 mg / kg extract of Narcissus bulb (P<0.01).

Discussion

Nowadays, due to the side effects and high cost of chemical drugs, the study of herbs used in traditional medicine, with the aim of achieving further advancement in medical science, is a priority. Medicinal herbs contain natural ingredients that are less likely to have side effects. Many of these plants have rich sources of natural antioxidants, which can reduce the effects of oxidants or some diseases (21). Narcissus bulb is one of the medicinal herbs used in traditional medicine, it is warm and dry in nature, and has been suggested as a purifier, absorbent and anti-cream (Anthelmintic) (2, 3). There has been no scientific report on the validity of Narcissus flower bulbs and its effect on the liver. The present study resulted in a significant increase in ALT and AST in extracts of bulbs, due to hepatocyte necrosis, increased membrane permeability (22), hepatocyte membrane damage, lack of proper cell membrane function, and cell leakage. (23). In a study similar to the results of this study, Zarei et al. (2012) investigated the effect of hydroalcoholic extract of Jasmine on blood indices of kidney and liver function. The control group had a significant decrease (24).

Consistent with this study, the results of studies by Marzban et al. (2017), Trouhideh et al. (2015) and Mohammadi et al. (2013) showed that, liver when exposed to toxins, the AST, AlkP and ALT enzymes significantly increased. Compared to the control group (22, 23, 25). Zahedi et al (2004) studied of the "Effect of Valeriana Officinalis and Echium Amoenum on Liver and Renal Function Tests in Rats". The results showed that the dose of 100 mg/kg extract did not cause an increase in ALT, AST enzymes, indicating no hepatotoxicity at the above dose, following administration of 200 mg/kg of both enzymes. AST, ALT increased significantly, indicating damage to the parenchyma and hepatocytes at this dose. Also, doses of 100 mg/kg and 200 mg/kg of bamboo extract significantly increased the AlkP enzyme, which may indicate toxicity of the extract to bile duct epithelium and cholestasis. In our study, however, it did not increase significantly at low doses, but it did show a significant increase at high doses (150 mg/kg) (18). Results of Banaee studies et al. (2016) entitled "Effect of administration of Mint Extract (Mentha longifolia) on Blood Biochemical Parameters and growth performance in Common Carp (Cyprinus carpio). The results showed that mongoose extract was toxic to fish at concentrations of 1 and 0.5% and could lead to poisoning. On the other hand, LDH enzyme significantly increased under 1% concentration of the extract. In our study, LDH enzyme showed a significant increase at high dose (150 mg/kg). This may be indicative of the metabolic changes caused by the toxicity of the phytochemicals in the extract, and the process of glycogen and glucose catabolism towards lactate formation in more muscles, leading to increased LDH levels (26). Khosravi et al. (2013) investigated the effect of salvia officinalis hydroalcoholic extract on liver enzymes in male rat and showed that the highest dose of sage extract (400 mg/kg) with isoniazid resulted in an increase in GGT. This was consistent with our research results. Probably, because of cholestasis or bile duct blockage, the concentration of this enzyme increased (27). In this study, the dose of 50 mg/kg improved the performance of all enzymes, and showed a protective effect, although only AST at this dose showed a significant decrease compared to the control group. Plants contain compounds such as alkaloids, flobotanin, tannins, terpenoids, glycosides, and anthracutinone and flavonoids, which can, through their antioxidant activity, maintain cell membrane stability, and improve tissue damage (25). The mechanism of antioxidant activity of flavonoids is due to their reductive properties, and therefore can act as antioxidants by depriving reactive oxygen species, decomposing peroxides, scavenging radicals, neutralizing radicals, and inhibiting enzymes producing reactive oxygen species to be oxidized. On the other hand, flavonoids can inhibit leukotrienes and protect cells and tissues by inhibiting enzymes such as lipoxygenase. The antioxidant properties of phenolic compounds depend on their ability to give electrons to trap free radicals by the formation of stable phenoxyl compounds (21).

This study, like any other study, had shortcomings. In this study, histological studies were not performed. The concentration of only three doses of alcoholic extract (methanol) was

investigated. In this study, hydroalcoholic extract should not be used in similar conditions to compare the results. It is also suggested that in future research the effects of essential oils, total alkaloids, flavonoids and other secondary metabolites of Narcissus flowers should be studied along with histological studies.

Conclusions

Significant increase in ALT, ALP, AST, LDH, GGT in high doses of Narcissus bulbs extract (150 mg/kg), and decrease in the amount of enzymes in 50 mg/kg dose showed that Narcissus bulbs in high doses had toxic effect. Has, and probably has a protective effect at low doses. Therefore, histological studies should be performed for further investigation.

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Conflict of Interests

There is no conflict of interest.

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مقاله پژوهشی

بررسی تغییرات غلظت سرمی آنزیم‌های مربوط به فونکسیون کبد (ALT ، ALP، AST، LDH ، GGT) متعاقب از مصرف عصاره پیاز گل نرگس

حسن مروتی*، نظام آرمند

گروه علوم پایه، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

تاریخ دریافت مقاله: ۱۳۹۸/۰۸/۲۰ تاریخ پذیرش مقاله: ۱۳۹۸/۱۰/۱۵

چکیده

زمینه و هدف: پیاز گل نرگس به عنوان داروی Antiphlogistic، موثر در درمان آسم، تنگی نفس و سوختگی‌های پوستی است. با توجه به عدم بررسی تاثیر عصاره آن بر تست های عملکردی کبد، هدف از انجام این تحقیق تاثیر عصاره پیاز گل نرگس بر تغییرات غلظت سرمی آنزیم‌های مربوط به فونکسیون کبد (ALT، ALP، AST، LDH، GGT) در موش صحرایی نر می باشد.

مواد و روش ها: در این مطالعه از ۲۰ موش صحرایی نر در ۴ گروه ۵ سری استفاده شد. گروها به یک گروه شاهد و سه گروه دریافت کننده عصاره پیاز گل نرگس با غلظت های ۵۰، ۱۰۰ و ۱۵۰ mg/kg تقسیم شدند. عصاره به مدت ۶ روز خورانده و در روز هفتم از حیوانات خونگیری انجام و سطح سرمی آنزیم های کبدی مورد اندازه گیری قرار گرفت. داده های با نرم افزار SPSS نسخه ۲۱ جمع آوری و محاسبات آماری با استفاده از آزمون تی (t- test) انجام گردید.

نتایج: نشان داده است که فعالیت آنزیم های آسپارتات آمینوترانسفراز (AST) و آلانین آمینوترانسفراز (ALT) در دوز ۱۵۰ mg/kg عصاره پیاز گل نرگس افزایش معنی داری نسبت به گروه شاهد ایجاد نمود($P<0/01$)، همچنین در همین دوز آنزیم های آلکالن فسفاتاز (ALP)، لاکتات دهیدروژناز (LDH) و گاماگلوتامیل ترانسفراز (GGT) افزایش معنی داری نسبت به گروه شاهد نشان دادند ($P<0/05$). ALT در دوز ۱۰۰ mg/kg افزایش معنی داری ولی AST در دوز ۵۰ mg/kg کاهش معنی داری را نسبت به گروه شاهد نشان داد ($P<0/05$).

نتیجه گیری: با توجه به افزایش معنی داری آنزیم های کبدی در دوزهای بالای عصاره لازم است تاثیرات هیستوپاتولوژی عصاره های این گیاه بر کبد مورد بررسی قرار گیرد.

کلمات کلیدی: گل نرگس، موش، کبد

* **نویسنده مسئول: حسن مروتی،** استاد گروه علوم پایه، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران
Email: hmorovvati@ut.ac.ir
https://orcid.org/0000-0003-0275-1636