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RESEARCH ARTICLE

Atractyloside Levels in *Xanthium strumarium* and Atractyloside Concentrations in the Serum of Rats Given *Xanthium strumarium*

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Abstract

Atractyloside (ATR) may cause severe liver and kidney damage. However, there is a lack of information about levels of ATR in parts of Xanthium strumarium and the concentrations and kinetics of ATR in the serum. In this study, we determined atractyloside levels in *Xanthium strumarium* parts, its serum concentrations and some kinetic parameters concerning time with the gas chromatography-mass selective (GC-MS) method. After administering (80 mg/kg ATR) *X. strumarium* seed extract to rats through gastric gavage, blood samples were collected at the 0th, 4th, 6th, 8th, 12th, 24th, 36th, 48th, 60th, 72nd, 96th, and 120th h. After extraction, hydrolysis and derivatization of the plant and serum samples were analyzed by the GC-MS instrument. The ATR level in the *X. strumarium* seeds was 3.043 mg/g in August, 3.502 mg/g in September and 3.800 mg/g in October. ATR was not detected in other parts of the plant. After ATR administration to rats, the C_{max} value of ATR was calculated as 10.77 µg/mL at T_{max} of 48 h and $t_{1/2}$ of 6.07 h. A thorough understanding of ATR circulation in the blood will aid in determining the course of its toxic effects in the bloodstream, the onset of symptoms and the general management plan for ATR poisoning. Moreover, the results obtained from this study will contribute to the antidote studies for ATR poisoning.

Keywords: *Atractyloside, GC-MS, Rat, Serum, Xanthium strumarium L.*

Xanthium strumarium'da Atraktilozid Seviyeleri ve *Xanthium strumarium* Verilen Sıçanların Serumunda Atraktilozid Konsantrasyonları

Öz

Atraktilozid (ATR) karaciğer ve böbrekte ciddi hasarlar yapabilen bir toksindir. Buna karşın bileşiğin kandaki dağılımı ve kinetiği hakkındaki bilgiler yetersizdir. Bu çalışmada gas kromatografi-kütle spektrofotometre (GC-MS) ile *Xanthium strumarium*'daki atraktilozid seviyelerini, serum konsantrasyonunu ve zamana bağlı bazı farmakokinetik parametrelerini belirledik. Sıçanlara (80 mg/kg ATR) *X. strumarium* tohum ekstresinin mide sondası ile uygulanmasından sonra 0, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, ve 120. saatlerde kan alındı. Serum ve bitki örneklerinin ekstraksiyonu, hidrolizi ve türevlendirilmesi yapıldıktan sonra GC-MS cihazı ile analizi yapıldı. *X. strumarium* tohumlarında ATR seviyesi Ağustos'ta 3.043 mg/g, Eylül'de 3.502 mg/g ve Ekim'de 3.800 mg/g saptanmıştır. Bitkinin diğer kısımlarında ATR saptanmamıştır. ATR'nin C_{max} değeri 10.77 µg/mL, T_{max} 48 saat ve $t_{1/2}$ 6.07 saat olarak hesaplandı. Kandaki ATR seviyesinin dağılımının bilinmesi zehirlenmelerde semptomların başlangıcını, toksik etkilerin seyrini ve sağaltım planını belirlemeye yardımcı olacaktır. Ayrıca, bu çalışmadan elde edilen bulgular, ATR zehirlenmesi ile ilgili antidot çalışmalarına katkı sağlayacaktır.

Anahtar sözcükler: *Atraktilozid, GC-MS, Serum, Sıçan, Xanthium strumarium L.*

INTRODUCTION

Xanthium strumarium L. (Cocklebur) belonging to the Asteraceae family, which is poisonous and common in many parts of the world, is an annual plant species. It is 20-90 cm in height, its leaves are green, and each fruit of

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this plant is 1-3.5 cm long brown, hard, woody and hook-shaped thorny fruits contains two seeds [1,2].

Xanthium species have been used as traditional herbal medicines for centuries in Eastern countries [3,4]. The whole plant has been used to treat bacterial infections, skin pruritus, diabetes, rhinitis, rheumatoid arthritis, as well as for its analgesic, anti-inflammatory, antitumor and cyto-toxicity activity [5-8].

Atractyloside (ATR) is a compound found in *X. strumarium* that leads to poisoning in humans and animals. ATR-induced intoxication could be lethal to pigs, sheep, cattle and humans. In animals, *X. strumarium* toxicity causes fatigue, weak pulse, nausea, ataxia, convulsion, depression, anorexia, vomiting, dyspnea, abdominal pain, weakness and recumbency resulting in death between 6 and 96 h after ingestion [5,9]. The liver toxicated by ATR shows the most distinctive microscopic lesions, which are characterized by the acute diffuse necrosis of the centrilobular hepatocytes accompanied by congestion and hemorrhage [10-13].

By preventing oxidative phosphorylation in cells, ATR leads to the disruption of the energy metabolism, opening of mitochondrial permeability transfer pore (mPTP) channels and necrotic/apoptotic cell death [12,14-16].

In this study, ATR levels found in the *X. strumarium* and some kinetic parameters of ATR given rats through gastric gavage were investigated. Determining ATR levels in *X. strumarium* and its kinetics in rat poisoned by will contribute to determining the course of intoxication and treatment methods.

Material and Methods

Ethical Statement

Ethics committee approval for this experimental study was obtained from Bingol University Animal Experiments Local Ethics Committee, with the decision dated 21.02.2018 and numbered 02/01.

Animals

This study included a total of 72 male Wistar Albino rats (6-7 weeks old, 200-250 g) with six animals in each group. The experimental animals used in the study were obtained from Bingol University, Experimental Research Center. All rats were housed in special cages under standard conditions (at a fixed temperature and in ventilated rooms; 12 h of daylight and 12 h of dark). Fresh water and feed were given to the rats *ad libitum*.

Reagents and Materials

Atractyloside (potassium salt) was obtained from Cayman (Cas no: 102130-43-8, purity ≥95), whereas trimethylsilyl imidazole (TMSI), pyridine, ethyl acetate, acetone and other chemicals to be used in the GC-MS analysis were purchased from Merck (Darmstadt, Germany).

Preparation of Stock Standard Solution

Atractyloside (potassium salt) was prepared in a methanol solution, and the standard solution was stored at +4°C. The concentration of ATR was 12.5 mmol/L.

Preparation of X. strumarium Extract Applied to Rats

The plant material was collected from the Elazig region in October, and the seeds were taken out of the fruits. A 100 g amount of *X. strumarium* (voucher number: Soberats, TR9008, CIFMT, Fuentes, 4785, ROIG) of seed was weighed with a precision scale and heated-refluxed twice with purified water (1:5, w/v) for 2 h each time. The extract was then filtered, combined and concentrated under reduced pressure in a vacuum rotary evaporator. The contents of the extract were measured using the GC-MS method, and it was found to contain 10 mg/mL of ATR. Due to the lack of dose studies of ATR in the literature, nontoxic 80mg/kg dose of ATR used in this study. That amount of dose is inferentially determined on the base of Bouabid et al.[17] study's results about oral LD_{50} dose of 1000 mg/kg. 80 mg/kg dose of ATR was given to the rats as *X. strumarium* seed extract via oral.

Preparation of Serum Samples

An aliquot of 0.5 mL of rat serum sample was spiked with 1 mL of acetone placed and vortexed. The mixture was then centrifuged (3500xg. 5 min, 4°C), and the supernatant was removed [18]. The supernatant was dried under a stream of N_2 at 40°C. The dried extract was reconstituted with 1 mL distilled water and acidified with 2 mL of hydrochloric acid (2 mol/L). The tubes were vortexed and stored overnight. The hydrolysates were extracted five times with 2 mL ethyl acetate. The combined organic extracts were dried under a stream of N_2 at 40°C. The dried extract was reconstituted with 200 µL of TMSI, and 200 µL of pyridine was added. Derivatization was performed at 100°C for 2 h before the GC-MS analysis [19].

GC-MS Conditions

A Shimadzu GC-MS device equipped with a Shimadzu auto-injector and a Shimadzu mass-selective detector was used for the GC-MS analysis (Shimadzu, GCMS-QP2010) with a DB-1 capillary column (30 mx250 µm I.D., film thickness: 0.1 µm).

For injection in the pulsed splitless mode, the injector temperature was 250°C, the ion source temperature was 200°C, the helium carrier gas flow rate was 1.9 mL/min, and the oven temperature was programmed to rise from 215 to 310°C at 2.30/min. The run time was 45 min under these conditions.

Preparation of X. strumarium Extract

Xanthium strumarium samples were collected from the Elazig

region from May to October. ATR levels were measured in the roots, stems, leaves and seeds of *X. strumarium* from May to October while the plant was growing. One g amount of *X. strumarium* seeds (Voucher number*:* Soberats, TR9008, CIFMT, Fuentes, 4785, ROIG; authenticated by the Department of Biology, Firat University) was powdered and extracted in 10 mL water at 100ºC for 15 min. 1 mL of the plant extract was acidified with 2 mL of hydrochloric acid (2 mol/L). The extract samples were vortexed and stored overnight. The hydrolysates were extracted five times using 2 mL ethyl acetate. The combined organic extracts were dried under a stream of N_2 at 40ºC. 200 µL of TMSI and 200 µL of pyridine were added to the dried extracts, and derivatization was performed at 100ºC for 2 h. Then, 2 µL of derivatives were injected into the GC-MS device [19].

Bioanalytical Method Validation

The method was validated in the whole serum and plants according to the FDA Bioanalytical Method Validation Guidance for Industry (2018 edition).

Selectivity, Specificity, and Carryover

Selectivity was assessed by comparing blank rat serum from six individual sources, spiked samples with ATR at low limit of quantification (LLOQ) and a real serum sample obtained from *X. strumarium* extract-treated rats. The analyte responses in the blank should be less than 20% LLOQ of spiked samples. Carryover between samples was evaluated in five circles by detecting the blank samples immediately after upper LOQ samples.

Calibration Curve

The calibration curves for ATR were comprised of a blank (no analyte), a zero calibrator, and seven non-zero calibrator levels covering the quantitation range on three consecutive days. The linearity of every calibration curve was obtained by evaluating the concentration-response relationship using a weighted ($1/x^2$) least squares linear regression. Non-zero calibrators should be ±15% of nominal concentrations, except at LLOQs (±20%).

Accuracy and Precision

Evaluating the accuracy and precision across the quantitation range involves analyzing the performance of ATR in a calibration curve and at the LLOQ, low, medium, and high QCs in five replicates per QC level in three independent runs. The precision within-run and between runs (described as RSD%) must be within 15%, except 20% at LLOQ. The accuracy (described as relative error, RE) should not deviate ±15% of nominal concentrations, except ±20% at LLOQ.

Extraction Recovery and Matrix Effect

The recoveries of ATR were calculated by comparing the peak areas between extracted samples with post-extracted spiked samples at low, medium, and high QC concentrations. The matrix effects were determined by the peak-area ratio between post-extraction samples with water-substituted samples at three QC levels

Stability

The stability study was performed at three replicates at low and high concentrations of QC samples including stock solution stability, bench-top stability, autosampler stability, post-preparation stability, freeze-thaw stability, and long-term stability. The method was considered to be stable when the accuracy (% nominal) at each level was ±15%.

Pharmacokinetic and Statistical Study

Blood samples (1 mL) were collected from the orbital venous plexus at 0, 4, 6, 8, 12, 24, 36, 48, 60, 72, 96, and 120 h after the rats received a single intragastric administration of the *X. strumarium* extract. The pharmacokinetic parameters of the samples were calculated using non-compartmental analysis with the PKSolver program. All analyses were performed using IBM SPSS Statistics Version 22.0 statistical software package.

Results

Standard Curve

The standards were prepared at different concentrations from the stock solution, and chromatograms were determined. The linear equation was f(x)=5466X+1771, r^2=0.946 [f(x)= peak area and X=concentration (µg/µL)]. The chromatograms of the ATR standard solution (8 µg) and the rat blood samples are demonstrated in *Fig. 1-A,B*. The retention time was 36.2 min for ATR.

Sensitivity, LOD, LOQ and Recovery

For the method used in the analysis, the limit of detection (LOD) was 17 ng/mL, and the limit of quantification (LOQ) was 50 ng/mL.

Recovery was examined from the QCs for 20 ng/mL, 35 ng/mL and 60 ng/mL concentration ranges in the serum and plant samples. The mean recovery was 89.50±5.1% in the serum samples and 92±6% in the plant samples.

Distribution of ATR in the Serum of Rats

As seen in *Table 1*, after the administration of *X. strumarium* through gastric gavage, the absorption of ATR began at the 6th h, and its presence in the serum remained even at the 96th h. The mean plasma ATR concentrations in all groups are shown in *Table 1*.

Some kinetics parameters obtained after the intragastric administration of the *X. strumarium* extracts to the rats included a peak plasma concentration (C_{max}) of 10.77 µg/mL, and the time for the drug concentration to reach the peak value (T_{max}) was 48 h. Some pharmacokinetic parameters

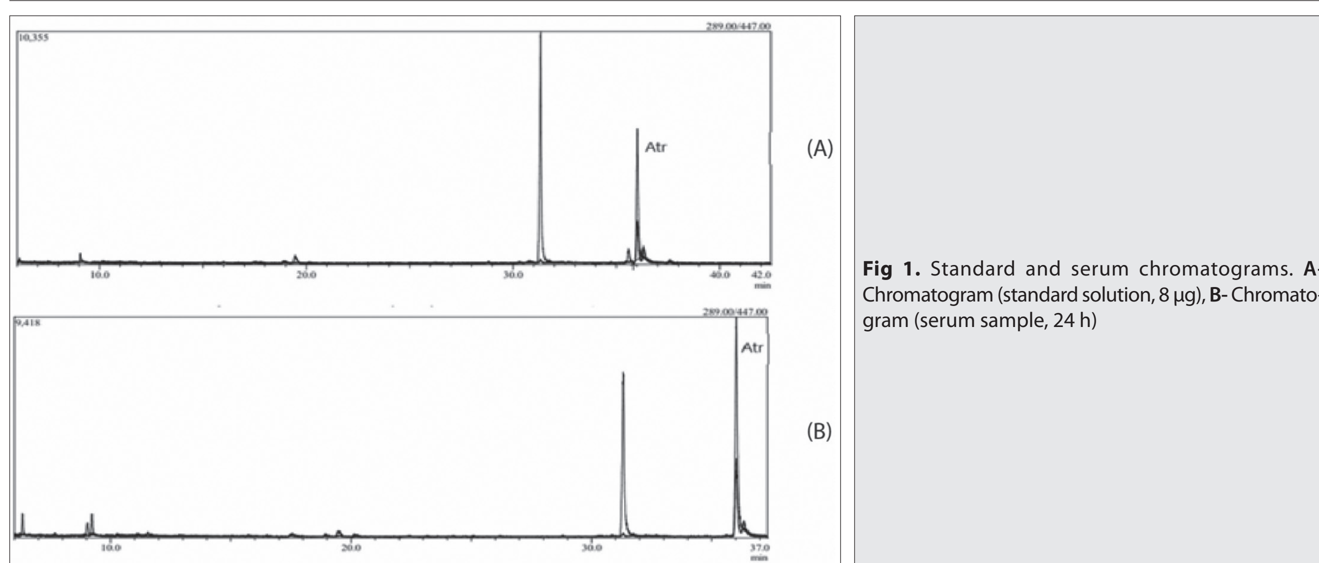


Fig 1. Standard and serum chromatograms. **A-** Chromatogram (standard solution, 8 µg), **B-** Chromatogram (serum sample, 24 h)

Table 1. *Periods of blood drawing and average serum ATR levels (µg/mL, n=6)*

Groups	Time (hour)	Mean±SEM
Control	0	0±0
Group 1	4	0±0
Group 2	6	0.031±0.004
Group 3	8	0.053±0.011
Group 4	12	0.072±0.192
Group 5	24	4.983±1.258
Group 6	36	7.126±1.599
Group 7	48	10.776±2.601
Group 8	60	7.200±1.333
Group 9	72	3.433±0.944
Group 10	96	0.134±0.034
Group 11	120	0±0

Table 2. *Main pharmacokinetic parameters after a single gastric administration of 80 mg/kg ATR (n=6)*

Kinetic Parameters	Unit	Oral ATR
$T_{1/2}$	h	6.071
T_{max}	h	48
C_{max}	µg/mL	10.77
MRT	h	47.807
V_{dss}	L/kg	1.623
Cl	L/kg/h	0.185
AUC	µg/h/mL	430.231

$t_{1/2}$: *elimination half-life,* ***C_{max}:*** *maximum concentration attained,* ***T_{max}:*** *time at which C_{max} is attained,* ***MRT:*** *mean residince time,* ***V_{dss}:*** *volume of distrubition at steady state,* ***Cl:*** *clearance of drug,* ***AUC:*** *total are under the concentration time-curve,* ***AUMC:*** *total area under the first moment concentration-time-curve*

of ATR are shown in *Table 2*, and the concentration-time curve of ATR is presented in *Fig. 2*.

Distribution of ATR levels in X. strumarium Parts by Months

ATR was not present in the root, stem or leaves of the plant in May-October as shown in *Fig. 3*. As of August, when the *X. strumarium* plants began to seed, the ATR levels in the seed were tested. In August, the mean ATR concentration in the seeds was 3.043 mg/g, whereas it was 3.502 mg/g in September and 3.800 mg/g in October. In September and October, the ATR concentrations in the seeds were higher than those in August ($P<0.05$, $P<0.001$ respectively). However, there was no significant difference between the values in September and October ($P>0.05$).

Discussion

Ingestion of *X. strumarium* plant contains ATR causes poisoning in humans and other animals. By preventing oxidative phosphorylation in cells, ATR leads to the disruption of the energy metabolism, stimulates the opening of mPTP channels and brings about necrotic/apoptotic cell death. Studies that had concentrated on the ATR levels in blood and various parts of the plant in cases of poisoning in animals are not sufficient. It is believed that the reason for this is that analyzing ATR is difficult, as stated by some authors [20-22].

In the literature, there is no study on ATR concentrations in various parts of the *X. strumarium* plant on monthly basis. In this study, ATR was detected in the seeds of the plant as at 3.01 mg/g in August, 3.4 mg/g in September and 3.9 mg/g in October, while it was not detected in the other parts of the plant. The absence of ATR in leaf, stem and root of the plant, also the existence of ATR in seed correlates with higher levels of ATR in seed in August-September-October months which is the seeding period. In addition to that, the analysis held in November due to the rotting of the plant, ATR levels could not be measured.

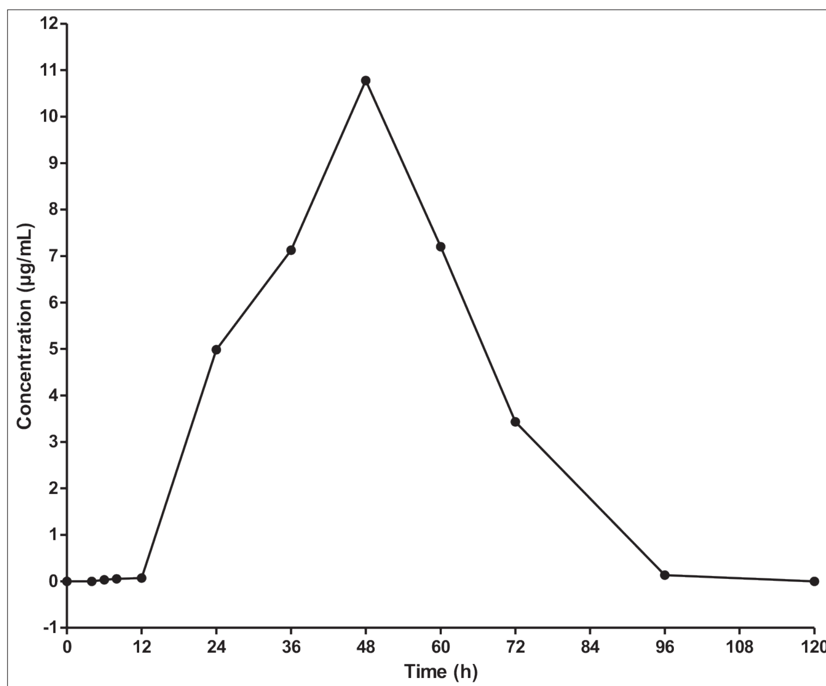


Fig 2. Serum concentration-time curve of ATR

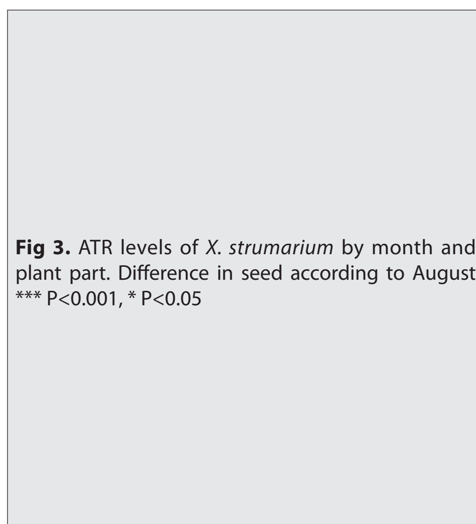
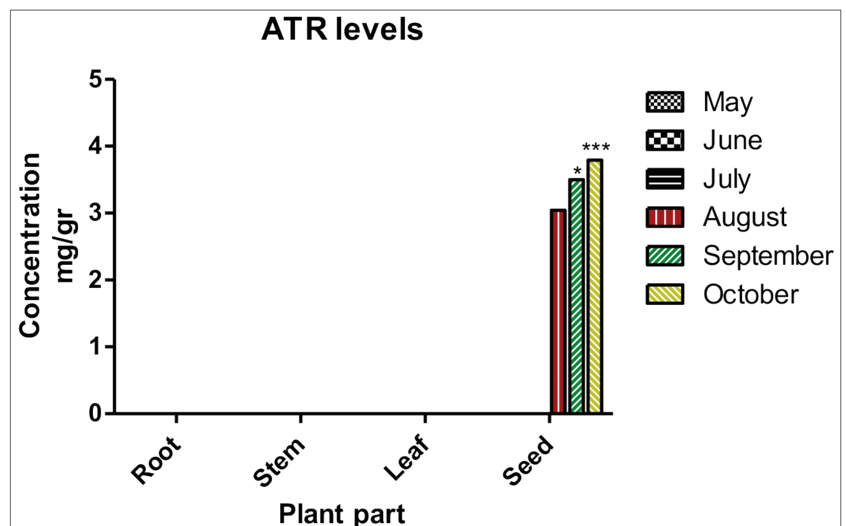


Fig 3. ATR levels of *X. strumarium* by month and plant part. Difference in seed according to August *** P<0.001, * P<0.05



In our study, for ATR, the C_{max} value was found as 10.77 µg/mL, T_{max} was determined as 48 h, and $t_{1/2}$ was calculated as 6.07 h, where the concentration fell below the detection limit after the 96th h. Fan et al.[2] reported the values of $t_{1/2}$ as 13.64, 9.62 and 8.61 h, T_{max} as 0.38, 1.85 and 0.27 h and C_{max} as 41.98, 24.61 and 263.40 µg/mL after administering ATR at the respective concentrations of 11.4, 22.8 and 45.6 mg/kg. Carlier et al.[23] detected 0.883 µg/mL ATR in the blood of a woman poisoned with the *Atractylode gummifera* plant at the end of the 3rd day. The formation of the symptoms of ATR poisoning and the findings of Carlier et al.[23] showed that ATR stays in the bloodstream for a long time. This supported our findings on the T_{max}, C_{max} and $t_{1/2}$ values.

Determining the ATR levels *X. strumarium* and MRT, T_{max}, C_{max}, $t_{1/2}$ and CL etc. values in rat serum will provide valuable information about preventing, diagnosis and treatment of ATR poisoning. The results of this study could enlight the way to discovery of the antidote of *X. strumarium* poisoning and will be a leading source for further investigations.

Availability of Data and Materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

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This manuscript received no grant from any funding.

Conflict of Interest

The authors have declared that no conflicts of interest.

Author Contributions

Tanyildizi S, Dagoglu G, Baykalir BG planned, designed and supervised the research procedure, the samples were collected by Ozturk Y, Keskin Z, Korkak FA performed the analysis, and Hark BD conducted the statistical analysis.

References

1. Cesur C, Coşge Şenkal B: Pıtrak *(Xanthium strumarium* L.) bitkisinin kültüre alınma potansiyelinin incelenmesi. *KSÜ Doğa Bil Derg*, 19 (1): 72-75, 2016. DOI: 10.18016/ksujns.03494

2. Fan W, Fan L, Peng C, Zhang Q, Wang L, Lin L, Wang J, Zhang D, Peng W, Wu C: Traditional uses, botany, phytochemistry, pharmacology, pharmacokinetics and toxicology of *Xanthium fundngstrumarium* L: A review. *Molecules*, 24 (2): 359, 2019. DOI: 10.3390/molecules24020359

3. Pan H, Yang F, Xiang D, Shi F: Simultaneous quantification of atractyloside and carboxyatractyloside in rat plasma by LC-MS/MS: Application to a pharmacokinetic study after oral administration of Xanthii Fructus extract. *J Sep Sci*, 43 (3): 590-597, 2020. DOI: 10.1002/jssc.201900888

4. Scherer R, Godoy HT: Effects of extraction methods of phenolic compounds from *Xanthium strumarium* L. and their antioxidant activity. *Rev Bras Plantas Med,* 16 (1): 41-46, 2014. DOI: 10.1590/S1516-05722014000100006

5. Amin S, Barkatullah, Khan H: Pharmacology of Xanthium species. A review. *J Phytopharmacol*, 5 (3): 126-127, 2016.

6. Kiem PV, Hoang NH, Thu VK, Tai BH, Nhiem NX: Diterpene glycosides and phenolic compounds from the fruits of *Xanthium strumarium*. *Vietnam J Chem,* 58 (5): 648-653, 2020. DOI: 10.1002/vjch.202000061

7. Lin B, Zhao Y, Han P, Yue W, Ma XQ, Rahman K, Zheng CJ, Qin LP, Han T: Anti-arthtritic activity of *Xanthium strumarium* L. extract on complete Freund's adjuvant induced arthritis in rats. *J Ethnopharmacol,* 155 (1): 248-255 2014. DOI: 10.1016/j.jep.2014.05.023

8. Qi L, Song F, Han Y, Zhang Y, Ding Y: Atractyloside targets cancer-associated fibroblasts and inhibits the metastasis of colon cancer. *Ann Transl Med*, 8 (21): 1443, 2020. DOI: 10.21037/atm-20-1531

9. Turgut M, Alhan CC, Gürgöze M, Kurt A, Doğan Y, Tekatli M, Akpolat N, Aygün AD: Carboxyatractyloside poisoning in human. *Ann Trop Paediatr*, 25 (2): 125-134, 2005. DOI: 10.1179/146532805X45728

10. Saidi H, Mofidi M: Toxic effect of *Xanthium strumarium* as an herbal medicine preparation. *EXCLI J,* 8, 115-117, 2009. DOI: 10.17877/DE290R-691

11. Kamboj A, Saluja AK: Phytopharmacological review of *Xanthium strumarium* L. (Cocklebur). *Int J Green Pharm,* 4 (3): 129-139, 2010. DOI: 10.22377/IJGP.V4I3.133

12. Botha CJ, Lessing D, Rösemann M, Wilpe E, Williams JH: Analytical confirmation of *Xanthium strumarium* poisoning in cattle. *J Vet Diagn Invest*, 26 (5): 640-645, 2014. DOI: 10.1177/1040638714542867

13. Xue LM, Zhang QY, Han P, Jiang YP, Yan RD, Wang Y, Rahman K, Jia M, Han T, Qin LP: Hepatotoxic constituents and toxicological mechanism of *Xanthium strumarium* L. fruits. *J Ethnopharmacol,* 152 (2): 272-282, 2014. DOI: 10.1016/j.jep.2013.12.024

14. Nikles S, Heuberger H, Hilsdorf E, Schmücker R, Seidenberger R, Bauer R: Influence of processing on the content of toxic carboxy-atractyloside and atractyloside the microbiological status of *Xanthium sibiricum* fruits (Ceng'erzi). *Planta Med*, 81, 1213-1220, 2015. DOI: 10.1055/s-0035-1546207

15. Zhang P, Li L, Sun H, Zhang Y, Zhang G, Zhang T, Zeng C: Mitochondrial energy-regulating effect of atractyloside inhibits hepato-cellular steatosis through the activation of autophagy. *Front Pharmacol*, 11:575695, 2020. DOI: 10.3389/fphar.2020.575695

16. Takata N, Ohshima Y, Suziki-Karasaki M, Yoshida Y, Tokuhashi Y, Suziki-Karasaki Y: Mitochondrial Ca^{2+} removal amplifies TRAIL cytotoxicity toward apoptosis-resistant tumor cells via promotion of multiple cell death modalities. *Int J Oncol*, 51 (1): 193-203, 2017. DOI: 10.3892/ijo.2017.4020

17. Bouabid K, Lamchouri F, Toufik H, Faouzi MEA: Plant science today inventory of poisonings and toxicological studies carried out on *Atractylis gummifera* L.: A review. *Plant Science Today,* 6 (4): 457-464, 2019. DOI: 10.14719/pst.2019.6.4.582

18. Gaillard Y, Pepin G: Poisoning by plant material: Review of human cases and analytical determination of main toxins by high-performance liquid chromatography-(tandem) mass spectrometry. *J Chromatogr B Biomed Sci Appl*, 733 (1-2): 181-229, 1999. DOI: 10.1016/s0378-4347(99)00181-4

19. Laurens JB, Bekker LC, Steenkamp V, Stewart MJ: Gas chromato-graphy-mass spectrometric confirmation of atractyloside in a patient poisoned with *Callilepis laureola*. *J Chromatogr B Biomed Sci Appl*, 765, 127-133, 2001. DOI: 10.1016/s0378-4347(01)00410-8

20. Steenkamp PA, Harding NM, Heerden FR, Wyk BE: Identification of atractyloside by LC-ESI-MS in alleged herbal poisonings. *Forensic Sci Int*, 163 (1-2): 81-92, 2006. DOI: 10.1016/j.forsciint.2005.11.010

21. Miao J, Huang Z, Liu S, Li X, Jia P, Guo Y, Wu N, Jia D: Hydroxytyrosol protects against myocardial ischemia reperfusion injury by inhibiting mitochondrial permeability transition pore opening. *Exp Ther Med*, 17 (1): 671-678, 2019 DOI: 10.3892/etm.2018.7016

22. Witte ST, Osweiler G, Stahr H, Mobley G: Cocklebur toxicosis in cattle associated with the consumption of mature *Xanthium strumarium*. *J Vet Diagn Invest*, 2, 263-267, 1990. DOI: 10.1177/104063879000200402

23. Carlier J, Romeuf L, Guitton J, Priez-Barallon C, Bevalot F, Fanton L, Gaillard Y: A validated method for quantifying atractyloside and carboxyatractyloside in blood by HPLC-HRMS/MS, a non-fatal case of intoxication with *Atractylis gummifera* L. *J Anal Toxicol*, 38 (9): 619-627, 2014. DOI: 10.1093/jat/bku078