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Original article

Nephrotoxicity of Sodium Benzoate and Ameliorating Role of *Ephedra alata* Aqueous Extract on Some Biochemical Parameters in Male Rats

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Abstract
A common preservative, *sodium benzoate*, is used in a variety of foods, such as salads, fruit juices, jams, and carbonated drinks, as well as in the pharmaceutical industry to preserve liquid medications. Ephedra alata is utilized for its anti-cold and anti-hay fever, and asthmatic effects. The purpose of this study is to evaluate Ephedra alata aqueous extract's ability to protect against sodium benzoate-induced kidney injuries, including urea, creatinine, uric acid, sodium, potassium, and chloride in the blood sera in male rats. In this study, twenty male albino rats weighing between 195 and 300 g were employed. The rats were divided into 4 groups, each with 5 rats. Animals in the control group were given distilled water orally every day for two weeks. In the group of sodium benzoate, rats were administered 100 mg/kg/b. w. of sodium benzoate orally daily for two weeks. Ephedra alata group, included rats that were administered aqueous extract of Ephedra alata daily at a dose (1g/kg b.w.) for two weeks. Animals received an oral dose of ephedra aqueous extract with sodium benzoate for two weeks. Twenty-four hours after the two-week treatment period ended, the rats from the reference and experimental groups were weighed and sacrificed by jugular decapitation. Their blood samples were collected for biochemical investigation, including urea, creatinine, uric acid, sodium, potassium, and chloride in the blood sera. According to the study's findings, when compared to the control group, none of the groups' serum urea, creatinine, uric acid, Na^+, K^{+2}, and Cl levels changed significantly after two weeks; however, the sodium benzoate rats' potassium levels significantly decreased. In conclusion, the study's findings support sodium benzoate's little harmful effects on the kidneys and no effects of ephedra when it is administered as a protective agent.
Keywords. Sodium Benzoate, Ephedra Alata, Rat, Kidney Biochemical.

Introduction

Sodium benzoate, which has the number E211, is the sodium salt of the non-essential amino acid glutamic acid. It has the chemical formula $C_7H_5NaO_2$ and is benzene carboxylic acid sodium salt (benzoic acid) [1,2]. People with urea cycle abnormalities who have acute hyperammonemia have been treated with sodium benzoate [3]. In the early stages of zebra fish larval development, sodium benzoate can cause teratogenicity, neurotoxicity, and nephrotoxicity [4]. Additionally, the kidney excretes sodium benzoate as hippuric acid after it has been conjugated with glycine in the liver [5].

Sodium benzoate caused a decrease in Hb and WBC, while significant increases in Na^+, K^+, $HCO_3^{-,}$ and Cl^- showed no significant changes [6]. Blood total protein, albumin, creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and cholesterol all increased as a result of sodium benzoate's subchronic toxicity. While, non-significant increase in blood urea nitrogen, uric acid, also, non-significant decrease in glucose, plasma globulin levels [7]. The male albino rats administered with sodium benzoate caused a decrease in total protein, albumin, cholesterol, triglycerides, urea, creatinine, uric acid, ALP, AST, and ALT [8].

Sodium benzoate markedly increased serum levels of AST, ALT, urea, uric acid, and creatinine [9]. Lipid peroxidation and GSH level in kidney tissues were markedly elevated by sodium benzoate, but CAT activity in the kidney tissues was markedly decreased [10]. Compared to the control group, sodium benzoate increased the activity of ALT, AST, ALP, albumin, bilirubin, and MDA while decreasing GSH, CAT, and SOD [11]. Rats given sodium benzoate showed no discernible changes in the weight of body, liver, kidney, heart, testis, and relative weights of liver, kidney, heart, or testis [12]. Ephedra alata (E. alata) pertains to the Ephedraceae family. These plants are widely distributed over North Africa, Palestine, Libya, Egypt, Saudi Arabia, and Iraq. E. alata has been used extensively in traditional medicine in Libya and other Arabian countries. E. alata has been used to treat asthma, hay fever, and common colds [13]. Significant variations in the overall body weight and the relative weight of the organs were brought on by E. alata, with varying differences in the oxidative stress parameters [14]. In traditional medicine, many diseases, including cancer, are commonly treated with E. alata [15,16]. Additionally, some study groups recommended E. alata as a therapy for diabetes mellitus.

Acute administration of ephedrine and/or caffeine, either independently or in combination, stimulated cardiovascular and central nervous system parameters [17]. Rats treated with carbon tetrachloride showed a substantial decrease in ALP and bilirubin due to the hepatoprotective action of 500 mg/kg dosages of E. foliate (whole plant) crude extract. [18]. Fatty acids, sterols, neutral lipids, phospholipids, and total lipid in

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Aspergillus flavus were considerably reduced by the use of E. alata extracts [19]. The administration of E. pachyclada extract considerably reduced the serum's ALT and AST activities [20]. The animal receiving carbon tetrachloride also had a higher survival rate. In mice, E. pachyclada extract significantly decreased serum AST and ALT levels [21]. The aqueous extracts of E. alata caused a non-significant increase in potassium and sodium loss in comparison to the control group [22]. Rats given E. alata aqueous extracts showed no discernible changes in the weight of body, liver, kidney, heart, testis, and relative weights of liver, kidney, heart, or testis [12].

Methods

Animals

Twenty male albino rats (*Rattus norvegicus*) weighing between 195 and 300 g were used in this investigation. They were acquired from the animal home of the Zoology Department at the University of Omar Al-Mukhtar's Science Faculty. The animals were housed in four groups of cages in identical rooms with constant environmental conditions, such as humidity (50–60%) and temperature (22±3°C). *Ad libitum* water and sufficient rat feed were provided to them. To the study's two-week start, all rats were given 2 weeks to become acclimated to the surroundings.

Experimental chemical and medical plant

Sodium benzoate is a compound with the chemical formula C_6H_5COONa was utilized. BDH Chemicals Ltd. (England) provided it. Ephedra alata: On the east coast of Libya, in the Al-Jabal Al-Akhdar district, leaves of the plant were collected. The extraction process for E. alata was carried out using the methodology described by [23].

Preparation of sodium benzoate

For two weeks, sodium benzoate was taken orally at a dose of 100 mg/kg/b. w. dissolved in newly prepared distilled water, according to the group distribution [24].

Preparation of Ephedra alata

After being weighed, the leaves were cleaned with water, dried, and then chopped into small pieces, weighed again. Hit the quantity in the mixer for an hour, filtered with a funnel. The solvent from the samples was removed using a rotary evaporator, and the heavy extract was gathered. For a total of two weeks during the trial, E. alata was given orally daily at a dose of 1 g/kg/b. w [25]. To avoid harming the buccal and oral interior linings, the two dosages were given orally via a customized gastric tube with a smooth tip.

Experimental design

For this experiment, a total of twenty male albino rats were used. Using the following procedure, the rats were randomly assigned to four equal groups, each including five male rats: 1- Control group (G1): The animals in this group received daily oral gavages of distilled water for two weeks. 2- Sodium benzoate treated group (G2): Rats were administered 100 mg/kg/b. w. of sodium benzoate orally daily for two weeks. 3- E. alata treated group (G3): For two weeks, the rats in this group were given a daily dose of 1g/kg b. w. of E. alata. 4- Combination Group (G4): The animals in this group received an oral dose of ephedra (1 g/kg b. w.) and sodium benzoate (100 mg/kg b. w.) for two weeks.

Serum samples preparation

The animals were slaughtered at the end of the 2nd week. Glass tubes were used to collect separate blood samples from each of them. Centrifugation was used to separate the serum for ten minutes at 3000 rpm. The resulting sera were gathered for examination using biochemistry.

Serum urea level measurement

Using a commercial kit acquired from Randox, U.K., blood urea was measured using the Fawcett and Scott method [26].

Serum creatinine level measurement

The Stanbio, U.S.A. kit and the Seeling and Wust technique [27] were used to measure serum creatinine.

Serum uric acid levels measurement

The Walker et al. method was used to measure blood uric acid [28].

Determination of serum sodium ion (Na^+) levels

Sodium was determined using commercial kits purchased from Randox, U.K. according to the method of Tietz [29].

Determination of serum potassium ion (K^+) levels

A commercial kit from Randox, U.K., and the Tietz method [30] were used to measure the potassium concentration.

Determination of serum chloride ion (Cl^-) levels

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Using Skeggs and Hochstrasser's approach, blood chloride was measured [31].

Analytical Statistics

Finding the difference between means was done using one-way analysis of variance (ANOVA). Tukey's test was used to distinguish means at P<0.05. When two means are compared, the t-test is also utilized. The Minitab statistical package application (Minitab version 21) was used to collect all of the data above.

Results

Concentrations of serum urea, creatinine, and uric acid

Blood urea, creatinine, and uric acid levels are measured, and the results are shown in Table 1 and Figures 1-3. Throughout the investigation, control rats displayed essentially consistent levels. Furthermore, when compared to the control group, no discernible changes in serum urea, creatinine, or uric acid concentrations were seen in any of the groups after two weeks. However, after two weeks, the serum urea, creatinine, and uric acid concentrations of the sodium benzoate group were 49.00±3.84 (8.4%), 1.20±0.08 (0.24%), and 6.90±0.34 (1.52%), respectively, and were not statistically different from those of the reference group.

In the *E. alata* group, the blood urea, creatinine, and uric acid concentrations recorded a non-statistical increase, where recorded as 40.80±4.66 (0.2 %), 1.20±0.25 (0.24 %), and 5.96±0.97 (0.58 %), respectively, when compared with the control. In the combination group, the levels of serum urea showed a slight decline. Where reached 39.80±1.88 at percentage (-0.8 %), while creatinine and uric acid recorded a non-significant increase where reaching 1.02±0.07 at percentage (0.06 %) and 5.98±0.30 at percentage (0.6 %) respectively, after two weeks, in contrast to the control group.

Table 1. Aqueous extract from E. alata protects blood urea (mg/dl), creatinine (mg/dl), and uric acid (mg/dl) levels from sodium benzoate toxicity.

Parameters	Duration	Group	G1- control	G2- Sodium benzoate Group	G3- *E. alata* Group	G4- Comb. Group
Urea	2nd week	Mean ± S.E. % of change	40.60^{A}±1.86	49.00^{A}±3.84 8.4	40.80^{A}±4.66 0.2	39.80^{A}±1.88 -0.8
Creatinine	2nd week	Mean ± S.E. % of change	0.96^{A}±0.08	1.20^{A}±0.08 0.24	1.20^{A}±0.25 0.24	1.02^{A}±0.07 0.06
Uric Acid	2nd week	Mean ± S.E. % of change	5.38^{A}±0.34	6.90^{A}±0.34 1.52	5.96^{A}±0.97 0.58	5.98^{A}±0.30 0.6

A, B: The groups in the same row with different letters are statistically significant (p < 0.05).

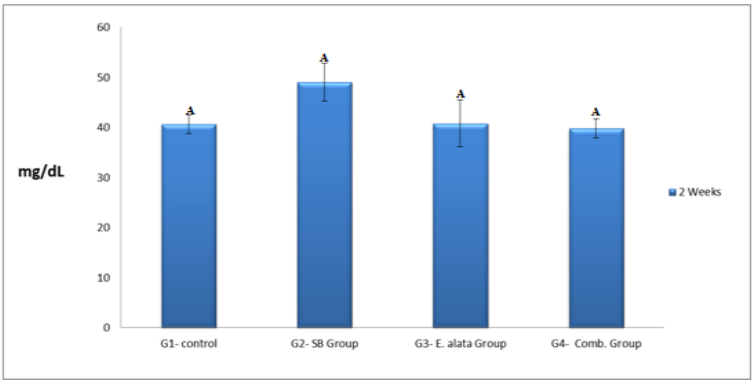

Figure 1: The preventive effect of E. alata aqueous extract on urea levels (mg/dl) against sodium benzoate toxicity. Similar letters indicate no significant differences between the means (p > 0.05).

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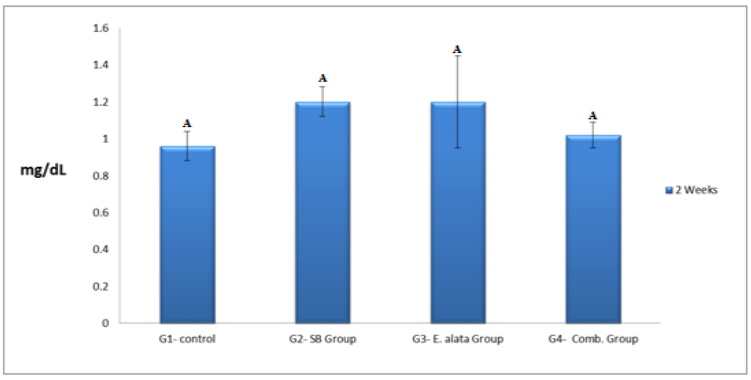

Figure 2: The preventive effect of E. alata aqueous extract on creatinine levels (mg/dl) against sodium benzoate toxicity. Similar letters indicate no significant differences between the means (p > 0.05).

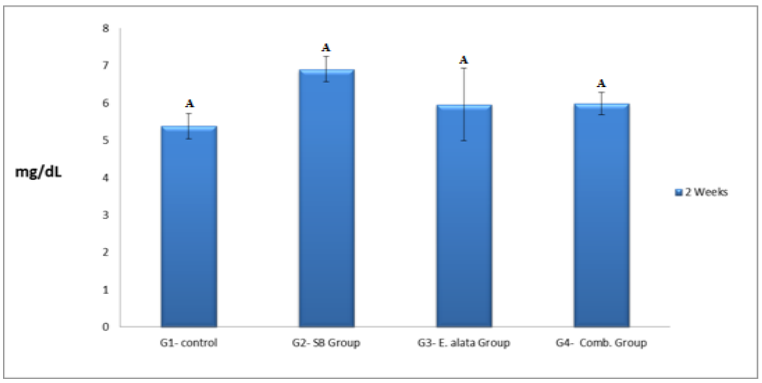

Figure 3: The preventive effect of E. alata aqueous extract on uric acid levels (mg/dl) against sodium benzoate toxicity. Similar letters indicate no significant differences between the means (p > 0.05).

Serum Na^+, K^{+2}, and Cl^- cOncentrations

The concentrations of serum Na^+, $K^{+2,}$ and Cl^- are shown in (Table 2) and (Figures 4, 5 and 6). In the sodium benzoate and E. alata groups, a non-significant decrease in blood Na^+ concentrations was observed when compared with the reference group at 2 weeks, reaching 126.80±9.06 (-22.2 %) and 140.80±0.49 (-8.2 %), respectively, at 2weeks (Table 2) and (Figure 4). In combination, rats demonstrated a non-statistical rise in Na^+ levels, where they recorded 153.20±5.37 (4.2 %) following two weeks. In E. alata and combination rats demonstrated a non-statistical decrease in K^{+2} levels, reaching 5.04±0.08 (-0.54 %) and 5.20±0.22 (-0.38 %) respectively. While sodium benzoate group showed a significant decrease in K^{+2} levels after 2 weeks where reaching 4.30±0.19 (-1.28 %) (Table 2) and (Figure 5). In blood chloride concentrations, no remarkable alterations were reported in all groups in comparison with the reference group following two weeks, where it reached 109.00±2.28 (6.6 %) in the sodium benzoate group, 103.20±1.77 (0.8 %), and the combination group 108.20±2.35 (5.8 %) (Table 2) and (Figure 6).

Table 2: Aqueous extract from E. alata protects serum Na^+ (mmol/l), K^{+2} (mmol/l), and Cl^- (mmol/l) concentrations from sodium benzoate toxicity

Parameters	Duration	Group	G1- control	G2- Sodium benzoate Group	G3- *E. alata* Group	G4- Comb. Group
Sodium	2nd week	Mean ± S.E. % of change	149.00^{AB}±3.64	126.80^{B}±9.06 -22.2	140.80^{AB}±0.49 -8.2	153.20^{A}±5.37 4.2
Potassium	2nd week	Mean ± S.E. % of change	5.58^{A}±0.24	4.30^{B}±0.19 -1.28	5.04^{AB}±0.08 -0.54	5.20^{A}±0.22 -0.38
Chloride	2nd week	Mean ± S.E. % of change	102.40^{A}±1.17	109.00^{A}±2.28 6.6	103.20^{A}±1.77 0.8	108.20^{A}±2.35 5.8

A, B: The groups in the same row with different letters are statistically significant (p < 0.05).

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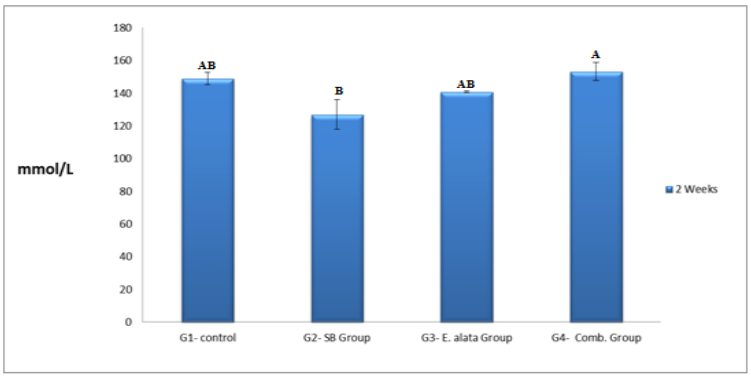

Figure 4: The preventive effect of E. alata aqueous extract on sodium levels (mmol/L) against sodium benzoate toxicity. Different letters indicate significant differences between the means (p < 0.05). Similar letters indicate no statistical differences between the means (p > 0.05).

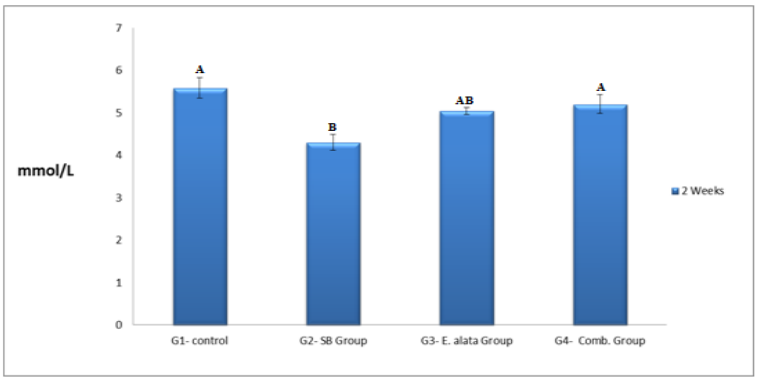

Figure 5: The preventive effect of E. alata aqueous extract on potassium levels (mmol/L) against sodium benzoate toxicity. Different letters indicate significant differences between the means (p < 0.05). Similar letters indicate no statistical differences between the means (p > 0.05).

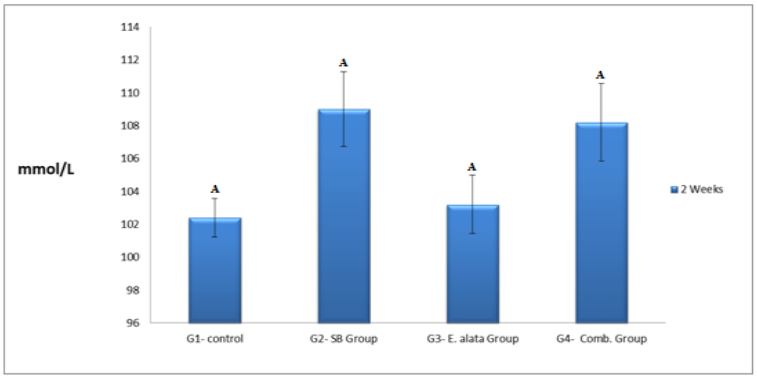

Figure 6: The preventive effect of E. alata aqueous extract on chloride levels (mmol/L) against sodium benzoate toxicity. Similar letters indicate no statistical differences between the means (p > 0.05).

Discussion

Carbonated drinks, olives, sauces, relishes, jellies, jams, preserves, olives, syrups, salted margarine, fruit salads, prepared salads, low-fat salad dressing, and vegetable storage all include sodium benzoate [5,32]. In temperate regions of North and South America, Northern Africa, and Eurasia, ephedra is abundantly found. Ephedra's extensive history of therapeutic use is largely due to its presence in various alkaloids, including ephedrine. Ephedra's medical history dates back at least 2700 B.C., when the Chinese used E. sinica to treat bronchitis, cough, and asthma. [33,34,35].

This investigation revealed that, as compared to the reference rats, there were no appreciable alterations in the serum concentrations of uric acid, urea, or creatinine in any of the groups after 2 weeks. This result is similar to that of Dewangan in rats [7] and disagrees with Aziz and Zabut; Oyewole *et al.*; Tawfek *et al.* [8,9,24] in rats when treated with sodium benzoate. The differences between these results may be due to different doses and durations. From this study no effects of ephedra and combination (sodium benzoate and ephedra) on concentrations of blood uric acid, urea, and creatinine after two weeks in comparison to the control group.

In sodium benzoate groups, the results indicate that non-significant decrease in serum Na^{+}. While it showed a significant decrease in K^{+2} levels. Furthermore, in serum chloride levels, no remarkable changes were

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reported comparing the control group after 2 weeks. These results agree with Ibekwe *et al.* in rats when studying the effects of sodium benzoate on K^{+2} and Cl^- levels [6] and Tawfek *et al.* in K^{+2} levels on rats [24], and disagree with Ibekwe *et al.* in sodium levels [6] and Tawfek *et al.* in Na^+ and Cl^- levels [24]. The differences between these results may be due to different doses and durations. The results showed that non-significant decrease in the ephedra group and a non-significant increase in the combination group for serum Na^+. While, showed K^{+2} levels lowered in the ephedra and combination rats, but not statistically. Furthermore, in serum chloride levels, non-significant changes were reported in the ephedra and combination groups after two weeks, when compared with the reference rats. These results are in agreement with the results of Rahhal *et al.,* who showed a non-significant increase in Na^+ and K^{+2} in mice when treated with ephedra [22].

Conclusion

In summary, the findings of this investigation support sodium benzoate's hazardous effects on the kidneys and the lack of impact of ephedra when administered as a kidney-protective agent to adult male rats.

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References

1. Mpountoukas P, Pantazaki A, Kostareli E, Christodoulou P, Kareli D, Poliliou S, Mourelatos C, Lambropoulou V, Lialiaris T. Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food and chemical toxicology. 2010; 48(10): 2934-2944.
2. Shahmohammadi M, Javadi M, Nassiri-Asl M. An overview on the effects of sodium benzoate as a preservative in food products. Biotechnology and Health Sciences. 2016; 3(3): 7-11.
3. Brahmachari S, Jana A, Pahan K. Sodium benzoate, a metabolite of cinnamon and a food additive, reduces microglial and astroglial inflammatory responses. The Journal of Immunology. 2009; 183(9): 5917-27.
4. Tsay HJ, Wang YH, Chen WL, Huang MY, Chen YH. Treatment with sodium benzoate leads to malformation of zebrafish larvae. Neurotoxicology and teratology. 2007 ;29(5): 562-569.
5. Lennerz BS, Vafai SB, Delaney NF, Clish CB, Deik AA, Pierce KA, Ludwig DS, Mootha VK. Effects of sodium benzoate, a widely used food preservative, on glucose homeostasis and metabolic profiles in humans. Molecular genetics and metabolism. 2015; 114(1): 73-9.
6. Ibekwe SE, Uwakwe AA, Monanu MO. Effect of oral intake of sodium benzoate on some haematological parameters of wistar albino rats. Sci. Res. Essays. 2007; 2:6-9.
7. Dewangan D. *Studies on toxicopathology of sodium benzoate in rats* (Doctoral dissertation, Chhattisgarh Kamdhenu Vishwavidyalaya, Durg). 2009.
8. Aziz IA, Zabut BM. Blood indices of sodiumbenzoate-administrated albino rats: effect of olive oil and/or time-dependent recovery. Egyptian Journal of Biology. 2012; 14: 50-56.
9. Oyewole OI, Dere FA, Okoro OE. Sodium benzoate mediated hepatorenal toxicity in wistar rat: Modulatory effects of azadirachta indica (neem) leaf. European Journal of Medicinal Plants. 2012; 2(1):11.
10. Khodaei F, Kholghipour H, Hosseinzadeh M, Rashedinia M. Effect of sodium benzoate on liver and kidney lipid peroxidation and antioxidant enzymes in mice. Journal of Reports in Pharmaceutical Sciences. 2019; 8(2): 217-223.
11. Radwan EH, Elghazaly MM, Hussein HK, Aziz K, Barakat AI. The possible effects of sodium nitrite and sodium benzoate as food additives on the liver in male rats. J. Adv. Biol. 2020; 13: 14-30.
12. Awad FMA, Gheth EMM, Eldurssi IS, Tayeb AYA, Alhissade NAA, Alkeelani NKA, Hamad MHA. Effects of Sodium Benzoate and *Ephedra alata* on Body weight and some organs weight in Male Rats. AlQalam Journal of Medical and Applied Sciences, (2024); 7(1) Part (3): 409-419.
13. Eldawaki N, Alseah M, Altuhami M, Alshalmani S. Pharmacognostical and Phytochemical Investigation, Antimicrobial and Antioxidant Studies on *Ephedra alata* Growing in East of Libya. 2nd Libyan conference on Chemistry and Its Applications LCCA-2, Benghazi-Libya. 2017.
14. Djahra AB, Zoubiri F, Gouasmia S, Benkaddour M, Benkherara S, Zghib K, Ghania A. *Effect of natural products of Ephedra alata face intoxication by deltamethrin insecticide in albino wistar rat*, University of El Oued, Faculty of Natural and Life sciences,, El Oued, Algeria. (2019).
15. Shabana MM, Mirhom YW, Genenah AA, Aboutabl EA, Amer HA. Study into wild Egyptian plants of potential medicinal activity. Ninth communication: hypoglycaemic activity of some selected plants in normal fasting and alloxanised rats. Archiv fur experimentelle Veterinarmedizin. 1990; 44(3): 389-94.
16. Jaradat NA, Al-Ramahi R, Zaid AN, Ayesh OI, Eid AM. Ethnopharmacological survey of herbal remedies used for treatment of various types of cancer and their methods of preparations in the West Bank-Palestine. BMC complementary and alternative medicine. 2016; 16: 1-12.
17. Greenway FL, Heber D. Herbal and alternative approaches to obesity. Handbook of obesity. 2004, 329-358.
18. Alqasoumi SI, Al-Rehaily AJ, AlSheikh AM, Abdel-Kader MS. Evaluation of the hepatoprotective effect of Ephedra foliate, Alhagi maurorum, Capsella bursa-pastoris and Hibiscus sabdariffa against experimentally induced liver injury in rats. Natural Product Sciences. 2008; 14(2): 95-99.
19. Al-Qarawi AA, Abd Allah EF, Abeer H. Effect of Ephedra alata Decne. on lipids metabolism of Aspergillus flavus Link. Bangladesh Journal of Botany. 2013; 42(1): 45-50.
20. Azarnia M, Ghasemi M. Protective Effects of Ephedra pachyclada on Animal Models with Acute Liver Disease Induced by Carbon Tetrachloride. Journal of Cell & Tissue Research. 2013; 3: 351-362.

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21. Ghasemi M, Azarnia M, Jamali M, Mirabolghasemi G, Nazarian S, Naghizadeh MM, Rajabi M, Tahamtani Y. Protective effects of *Ephedra pachyclada* extract on mouse models of carbon tetrachloride-induced chronic and acute liver failure. Tissue and Cell. 2014; 46(1): 78-85.
22. Rahhal B, Jaradat N, Shraim WB, Zyoud A, Hattab S. The diuretic activity of *Ephedra alata* and *Plumbago europaea* in mice using an aqueous extract. Moroccan Journal of Chemistry. 2018; 6(4) :6-4.
23. Dahiru D, Onubiyi JA, Umaru HA. Phytochemical screening and antiulcerogenic effect of *Moringa oleifera* aqueous leaf extract. African Journal of Traditional, Complementary and Alternative Medicines. 2006; 3(3): 70-75.
24. Tawfek N, Amin H, Abdalla A, Fargali S. Adverse effects of some food additives in adult male albino rats. Current Science International. 2015; 4(4): 525-537.
25. Jahromi HK, Jahromi ZK, Davami MH, Ramazani A, Afzali M, Saleh S, Kherameh ZK. The effect of hydro-alcoholic extract of *Ephedra pachyclada* on serum concentrations of neuropeptide Y and ghrelin hormones and body weight in male rats. Int. J. Pharm. Res. Allied Sci. 2016; 5(1): 135-139.
26. Fawcett J, Scott J. A rapid and precise method for the determination of urea. Journal of clinical pathology. 1960; 13(2): 156-159.
27. Seeling HP, Wust H. Colorimetric method for determination of creatinine. Arztl. Lab. 1969; 15(34).
28. Walker HK, Hall WD, Hurst JW. Clinical methods: the history, physical, and laboratory examinations. 1990.
29. Tietz NW. Clinical guide to laboratory tests. In Clinical guide to laboratory tests 1995 (pp. 1096-1096).
30. Tietz NW. Fundamentals of Clinical Chemistry, Philadelphia, W. B. Saunders Co. 1976.
31. Skeggs LT, Hochstrasser HC. Thiocyanate (colorimetric) method of chloride estimation. Clin. Chem., (1964); 10: 918-936.
32. Kregiel D. Health safety of soft drinks: contents, containers, and microorganisms. BioMed research international. 2015; 2015(1): 128697.
33. Abdel-Kader MS, Kassem FF, Abdallah RM. Two alkaloids from *Ephedra aphylla* growing in Egypt. Natural Product Sciences. 2003; 9(2): 52-55.
34. Huang J, Giannasi DE, Price RA. Phylogenetic relationships in Ephedra (Ephedraceae) inferred from chloroplast and nuclear DNA sequences. Molecular Phylogenetics and Evolution. 2005; 35(1): 48-59.
35. Hegazi GA, El-Lamey TM. Callus induction and extraction of ephedrine from *Ephedra alata* Decne. cultures. American-Eurasian Journal of Agricultural & Environmental Sciences, 2011; 11: 19-25.

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