

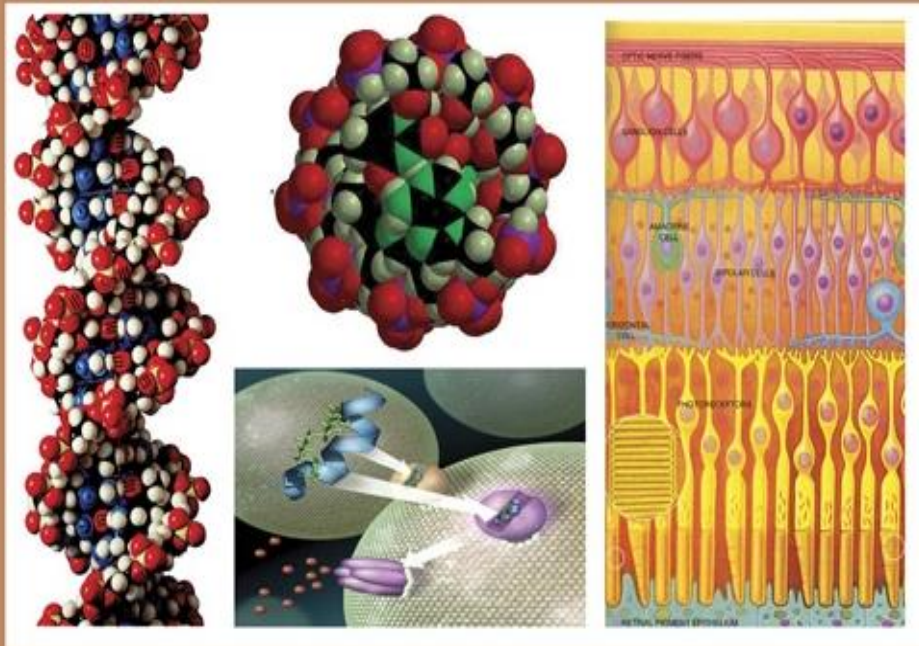


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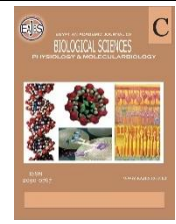
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Evaluation Effect of the Essential Oil of Green Anis (*Pimpinella Anisum*) on The Liver Function of Young Rats Following Exposure to Mercury Chloride

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ABSTRACT

Green anis is a plant known since antiquity with its different therapeutic effects such as; antifungal, antimicrobial, antiviral, analgesic, antioxidant and hepatoprotective effects. However, mercury is a trace element metal that is very common in the environment and has adverse effects on human health. The liver is considered to be the seat of metabolism and the elimination of drugs and exogenous substances from the body.

This work consists on the one hand evaluating the hepatotoxicity induced in young Wistar rats during the period of development, following exposure to a dose of 100mg/L of mercury chloride. On the other hand, we treated young rats with the essential oil of green anis. On the other hand, we measured the biomarkers of the hepatic function such: ALT, ASAT and ALP followed by a histological study. the results demonstrate a significant increase of these biomarkers in intoxicated animals ASAT, ALAT, ALP ($p < 0.01$), ($P < 0.05$), ($p < 0.01$) respectively and an alteration of the liver architecture. Nevertheless, we recorded an improvement in these parameters after the treatment with anis oil for 21 days.

In conclusion, exposure to mercury chloride during the development period induced disruption of liver metabolism by changing serum transaminases levels and liver histology. This could be ameliorated by treatment with the essential oil of *Pimpinella anisum*.

INTRODUCTION

The liver is the target organ responsible for the metabolism of xenobiotics and is therefore vulnerable to damage from chemicals. Liver damage is associated with various xenobiotics, including drugs and industrial chemicals. For this reason, many hepatoprotective agents are being studied to protect the liver from toxic attacks (Jamshidzadeh *et al.*, 2015).

Pimpinella anisum (Anised) is a plant that belongs to the Apiaceae family. It is native to the Mediterranean regions, while it is widely cultivated in India, Pakistan and Iran (Mushtaq *et al.*, 2019). Aniseed contains 1.5 to 5% essential oil, which has several effects namely; digestive, antiparasitic, antibacterial, antifungal, antipyretic, muscle relaxant, convulsive and hepatoprotective effect (Shojaii and Abdollahi Fard, 2012; Jamshidzadeh *et al.*, 2015; Bekara *et al.*, 2016).

In addition, industrialization in developing countries has led to an increase in metal contamination. Heavy metals are not biodegradable and produce unwanted effects even at low doses (Mumtaz *et al.*, 2019). Mercury (Hg) is an industrial pollutant. It induces serious alterations in human and animal tissues. Different forms of mercury compounds cause toxicity in various organs following accidental and/or occupational exposures (Hosseini *et al.*, 2018). The liver is one of the vital and sensitive organs typically exposed to mercury toxicity (Mumtaz *et al.*, 2019).

The objective of this study is to assess on the one hand the hepatotoxic effect of mercury on developing Wistar rats and on the other hand the hepatoprotective effect of the essential oil of green anise.

MATERIALS AND METHODS

Identification of The Components of The Essential Oil by Gas Chromatography Coupled to Mass Spectrometry:

The constituents of the essential oil were identified by Gas Chromatography Coupled To Mass Spectrometry (GC/MS). Analysis was performed by Varian Saturn 2200 gas chromatography coupled to a Varian 2100 T mass spectrometer, using Saturn Varian software (Version 6, 9, 1). The capillary column was apolar with a length of 30 m, a diameter of 0.25mm, and a thickness of 0.25 μ m. The flow rate of the carrier gas (helium) was 1 ml/min. 1 μ l of essential oil was injected, using split mode (1/20). The temperature of the column was maintained at 50°C for 5 min, then increased by 5°C/min to

300°C (5 min). The detector was flame ionization (FID). The ionization energy was 70 electron volts (eV).

Animals and Treatment:

Experiments were carried out on Wistar rats (obtained from the Department of Biology, Faculty of Sciences, University of Saida) weighing 210 \pm 50 g. The animals were housed with free access to water and food in an animal room, with a 12/12-hour light/dark cycle, at 22 \pm 2°C. They were mated one week after their arrival (three females and one male per cage). After one week of cohabitation with males, females were divided into 02 groups

Group 1: Pregnant females received drinking distilled water.

Group 2: Pregnant females received 100 mg/L of HgCl₂ in distilled water (Chehimi *et al.*, 2012).

Experimental Design:

At birth, pups issued from intoxicated females continued to receive HgCl₂ in drinking water until weaning, while the control rat received only distilled water. In order to test the capacity of anise essential oil (AEO) to attenuate Hg hepatotoxicity which alteration of biochemical parameters and liver function, drug therapy was administered, beginning 24 hours after weaning. At weaning, we got 03 new groups (n = 7) as follows:

Group C: Control rats (issued from control females) received distilled water only.

Group Hg: received an oral solution of HgCl₂.

Group Hg-AEO: intoxicated rats (issued from intoxicated females) that received an intraperitoneal injection with 0,25 ml/kg of body weight AEO daily for 21 days (Asadollahpor *et al.*, 2017). (AEO was injected pure).

The number of animals that suffered was minimized in accordance with the guidelines of the European Council Directive (86/609/EEC).

Determination of Liver Parameters:

The serum samples were used to measure the activity of alanine

aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP) according to the methods of Reitman and Frankle (1957).

Histological Study:

Liver samples were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Paraffin sections (5 µm thick) were prepared, routinely stained with hematoxylin and eosin dyes (Suvarna *et al.*, 2013), and then examined microscopically.

Statistical Analysis:

Results were expressed as mean ± standard error of the mean (SEM). Data were analysed by the two-way analyses of variance (ANOVAs) and Sigmastat software. When a significant difference was found, the Student-Newman-Keuls post-hoc test was conducted. For all analyses, a difference was considered significant at $p \leq 0.05$.

RESULTS

Result Of Gs/Ms:

Gas chromatographic analysis allowed us to identify 12 components for the essential oil of green anise with anethol as the major component.

Table 1: Components identified by GC/MS

Identified compounds	Retention time (min)	Concentration (%)
α-pinène	8,11	1,81
Camphène	8,56	0,36
Cis-betaocimène	9,72	0,28
α-phellandréne	10,91	0,22
Limonène	11,68	8,01
3-carène	12,91	0,43
Fenchone	14,22	8,99
α-campholène aldéhyde	16,15	0,47
γ-Elémène	18,1	0,11
Estragole	18,81	7,25
Tans-anéthol	20,33	55,44
Cis-Anéthol	21,60	0,18

The transaminase assay revealed a significant elevation in ASAT and ALAT levels in mercury-exposed animals compared to control animals ($p < 0.01$), ($P < 0.05$) respectively. However, serum levels of AST and ALT in mercury-exposed animals treated with AEO showed a significant decrease compared to mercury-exposed animals ($P <$

0.05).

In addition, phosphatase levels showed a significant increase in mercury-intoxicated rats ($p < 0.01$). while the levels of alkaline phosphatase in intoxicated rats treated with essential oil were significantly lower than those of rats exposed to mercury ($p < 0.01$).

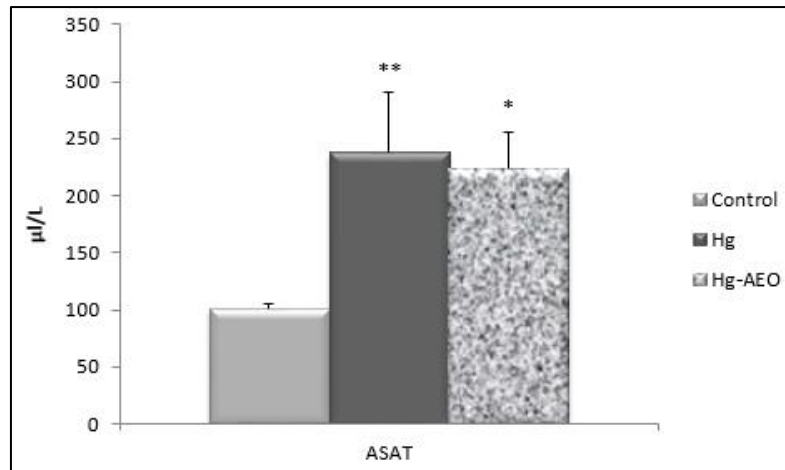


Fig. 1: Comparison of serum ASAT levels of controls, Hg and Hg-AEO. Values are expressed as mean \pm SEM: ****** $P < 0.01$ (Control vs.Hg); ***** $P < 0.05$ (Hg vs. Hg-AEO).

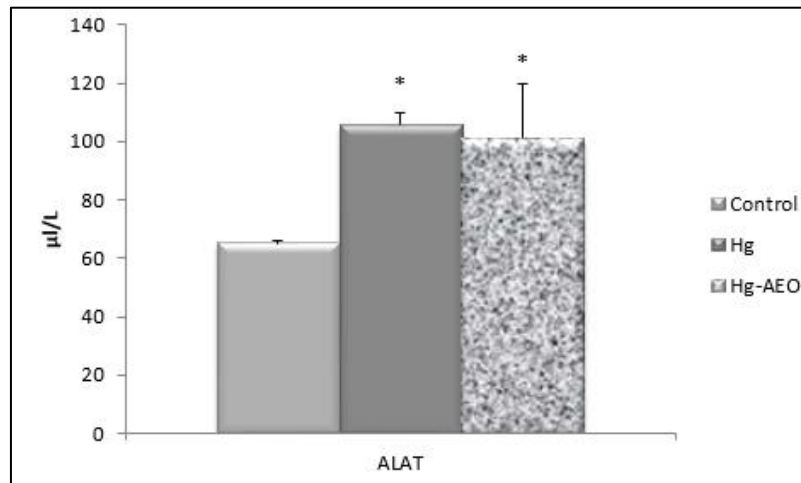


Fig. 2: Comparison of serum ALAT levels of: Controls, Hg and Hg-AEO. Values are expressed as mean \pm SEM: ***** $P < 0.05$ (Control vs. Hg); ***** $P < 0.05$ (Hg vs. Hg-AEO).

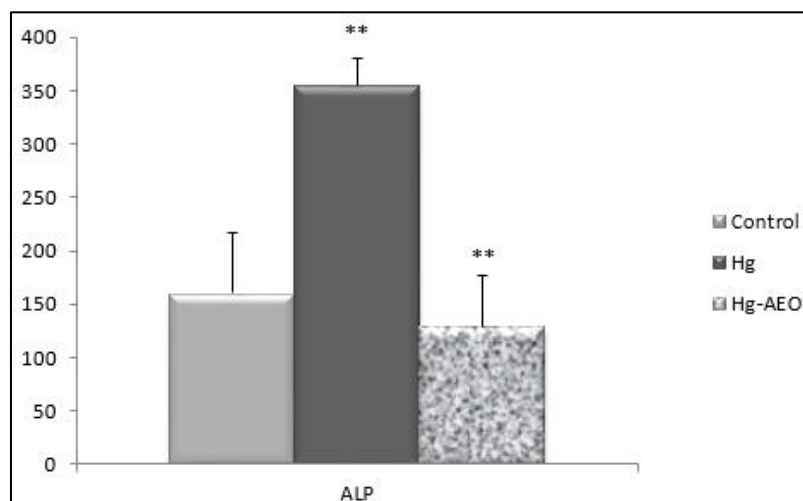


Fig. 3: Comparison of serum ALP levels of Controls, Hg and Hg-AEO. Values are expressed as mean \pm SEM: ****** $P < 0.01$ (Control vs. Hg); ****** $P < 0.01$ (Hg vs. Hg-AEO).

Histology:

A histological study of the liver of young rats intoxicated by mercury chloride showed that its architecture is preserved with severe vascular congestion and hypertrophy

of the hepatocytes. Moreover, the liver of rats exposed to HgCl_2 and treated with AEO showed vacuolar degeneration, the presence of binuclear foci, the presence of Kupffer cells and slight congestion.

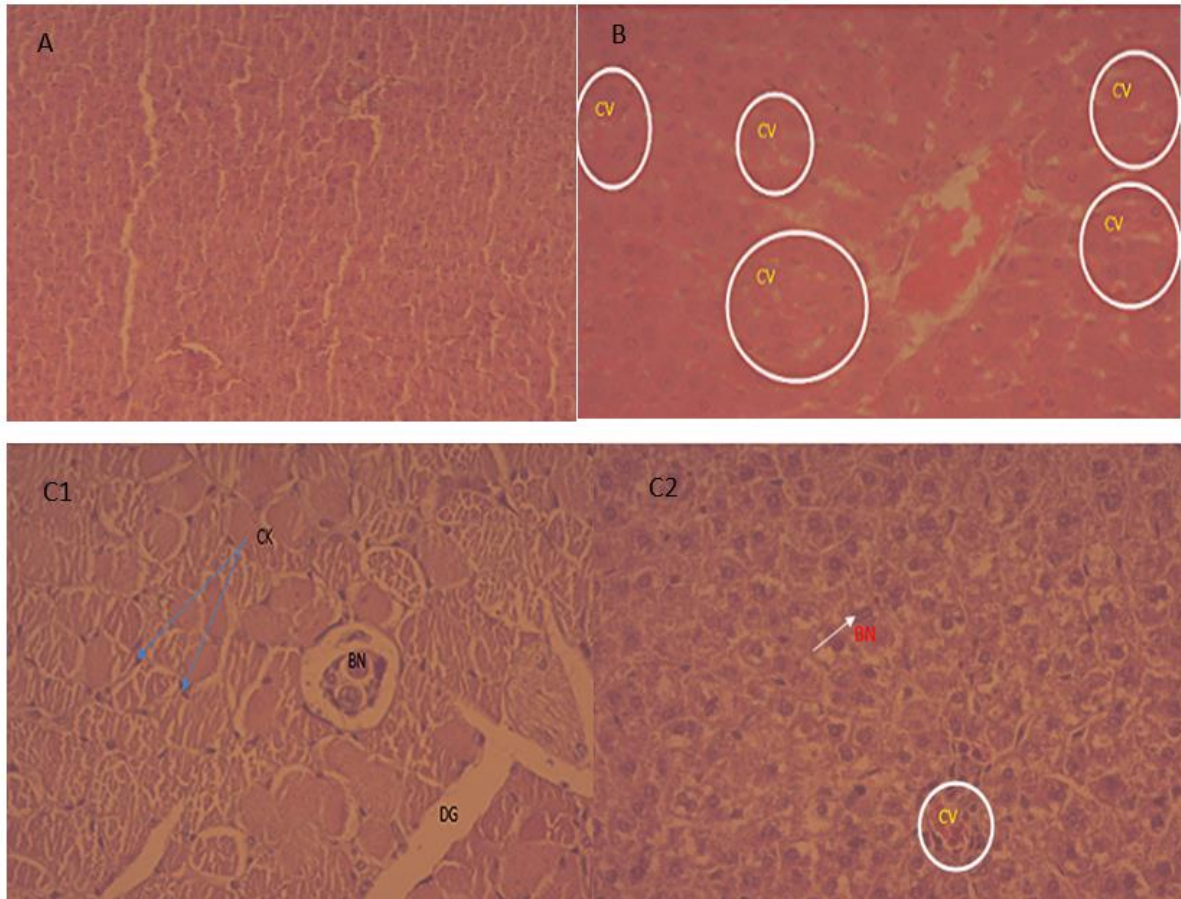


Fig. 4: Histological sections in the liver with Gr \times 40 with A: control group, B: intoxicated group, C(1,2): treated intoxicated group; BN: binuclear cell; DG: degenerescence; Ck: Kuppfer cell; Cv: vascular congestion.

DISCUSSION

The enzymatic activity of transaminases and alkaline phosphatase showed a significant increase in the batch poisoned by mercury compared to that of the control batch, which reflects hepatotoxicity due to liver injury and is explained by the leakage of these enzymes from tissue to plasma due to the alteration of membrane permeability caused by mercury, these results are in agreement with the work of Deepmala *et al.*, (2013) and Bahi and Necib, (2015). Rats exposed to mercuric chloride show elevated levels of ASAT, ALAT and ALP

evidence of its accumulation in the liver (Kumar *et al.*, 2014).

In addition, the elevation of the plasma activity of ASAT at the mitochondrial level of hepatocytes and in the cytoplasm indicates structural damage in the liver thus causing an increase in the permeability of the membranes of the hepatocytes leading to the release of the enzyme in the liver and in the blood. Furthermore, ALAT is found in the cytosol of hepatocytes and its release from damaged cells increases its activity in plasma (Hazelhoff *et al.*, 2018).

Elevation of ASAT indicates

degradation of hepatocytes. Various studies have shown that the latter increases in the circulatory system when hepatocytes or other organs are damaged or necrotic (Nathwani *et al.*, 2005; Mumtaz *et al.*, 2019).

On the other hand, mercury-exposed animals treated with *P.anisum* essential oil have significantly reduced ASAT, ALAT and ALP levels compared to untreated exposed animals. El-Sayed *et al.*, 2015 mentioned a hepatoprotective effect of green anise essential oil against carbon tetrachloride-induced liver cirrhosis based on transaminase assay. Anise essential oil was able to restore serum levels of ASAT, ALAT, and ALP that were elevated following tetrachloride carbon exposure (El Sayed *et al.*, 2015). The decrease in serum AST, ALT and ALP levels suggest that *P.anisum* extract could prevent liver cell damage (Cengiz *et al.*, 2008).

Regarding the liver of young rats exposed to HgCl₂, the architecture is preserved with severe vascular congestion and hypertrophy of hepatocytes. These results corroborate with the work of Hosseini *et al.*, 2018 which confirms the presence at the hepatic level of inflammatory cell infiltration and inflammation of the central vein following exposure to a dose of 5 mg/kg of HgCl₂ of which the latter increases the production of numerous ROS, such as superoxide and H₂O₂, which cause lipid peroxidation and subsequently oxidative tissue damage. Furthermore, the administration of HgCl₂ for 21 days intraperitoneally led to severe diffuse vacuolar infection, and degeneration of hepatocytes (Abarikwu *et al.*, 2017). Indeed, liver damage due to the introduction of mercury and cadmium is well known by higher intensities of liver enzymes in serum showing the damage caused at the level of liver tissue and the exit of enzymes into the blood (Mumtaz *et al.*, 2019). Similarly, the results of the study conducted by Hazalhoff *et al.*, 2018 show that female rats exhibited higher HgCl₂-induced hepatotoxicity than male rats, as evidenced by greater alterations in plasma markers, liver injury, and histopathology (Hazalhoff *et al.*, 2018). The

sex-related differences observed in the liver damage produced by inorganic mercury may be explained by the greater accumulation of this metal in the liver of females. At a sublethal dose of mercuric chloride of 1.23 mg/kg treated rat tissues show liver cell damage and altered metabolic activities through the ASAT and ALAT marker enzyme pathways (Jagadeesan *et al.*, 2014). Following HgCl₂ exposure, the liver tissue was completely damaged, and degenerative and necrotic qualitative changes were observed in almost all structures of the liver tissue (Wadaan, 2009).

However, mercury-exposed animals treated with *P.anisum* essential oil have significantly reduced ASAT, ALAT and ALP levels compared to untreated exposed animals. El-Sayed *et al.*, 2015 mentioned a hepatoprotective effect of green anise essential oil against carbon tetrachloride-induced liver cirrhosis based on transaminase assay. Anise essential oil was able to restore serum levels of ASAT, ALAT, and ALP that were elevated following tetrachloride carbon exposure (El-Sayed *et al.*, 2015). The decrease in serum AST, ALT and ALP levels suggest that *P.anisum* extract could prevent liver cell damage (Cengiz *et al.*, 2008).

On the other hand, the therapy based on *P.anisum* EO reduced the damage of which we noticed vacuolar degeneration, the presence of binuclear foci which explains that the cells regenerate and enter a division phase, the presence of Kupffer cells which are the macrophages of the liver which indicates the presence of immune defense.

Moreover, the therapy with aqueous extract allowed the presence of peripheral lesions, foci of necrosis, normal sinusoidal capillaries and the presence of a significant number of Kupffer cells. Thus, the treatment with *P.anisum* including EO and aqueous extract has an almost similar effect in the face of mercury intoxication. Our results are partially different from those of Asadollahpoor *et al.*, 2017 who found that histopathological examinations also confirmed less vacuolar degeneration and

decreased macrovascular steatohepatitis in tissue sections of animals treated with high doses of *P. anisum* HE and aqueous extract in rats with non-alcoholic fatty liver disease. In addition, the hexanolic extract of anise seeds possesses protective effects in vitro and in vivo against carbon tetrachloride-induced hepatotoxicity, probably due to its antioxidant constituents (Jamshidzadeh *et al.*, 2015). Also, aspartame consumption induced hepatotoxicity, and the use of *P. anisum* EO as an antioxidant decreased the toxicity of aspartame (El Haliem *et al.*, 2011).

CONCLUSION

In conclusion, the results of this study show that exposure of young Wistar rats to mercury chloride during gestation and lactation period induced an increase in biomarkers of liver function as well as liver tissue damage. In addition, treatment with *P. anisum* essential oil reduced the level of these biomarkers and the damage that was caused by mercury.

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REFERENCES

- Abarikwu, S.O., Benjamin, S., Ebah, S.G., Obilor, G., Agbam, G., (2017): Effet protecteur de l'huile de Moringa oleifera contre l'hépatite et la néphrotoxicité induites par HgCl₂ chez le rat. *Journal de physiologie fondamentale et clinique et pharmacologie*, 28 (4): 337-345.
- Asadollahpoor, A., Abdollahi, M., Rahimi, R., (2017): Pimpinella anisum L. fruit: Chemical composition and effect on rat model of nonalcoholic fatty liver disease. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, 22.
- Bahi, A., Necib, Y., (2015): L'effet protecteur des antioxydants naturels dans l'intoxication du mercure chez le rat Albinos Wistar. Doctoral thesis. Mentouri-Constantine 1 Brothers University.
- Bekara, A., Ait hamadouche, N., Kahloula, K., Sadi, N., Aoues, A.K., (2016): Effect of Pimpinella Anisum On histological and biochemical damage in cerebrum and cerebellum of young rats intoxicated by lead acetate, *Pharmacologie on ligne*, Group 11- 12.
- Cengiz, N., Ozbek, H., Him, A., (2008): Effets hépatoprotecteurs de l'extrait de graine de Pimpinella anisum chez le rat. *Pharmacologyonline*, 3: 870-874.
- Chehimi, L., Roy, V., Jeljeli, M., Sakly, M., (2012): Chronic exposure to mercuric chloride during gestation affects sensorimotor development and later behaviour in rats. *Behavioural brain research*, 234(1) : 43-50.
- Deepmala, J., Deepak, M., Srivastav, S., Sangeeta, S., Kumar, S.A., Kumar, S.S., (2013): The protective effect of combined treatment with dithiothreitol, zinc and selenium protects acute mercury-induced oxidative injury in rats. *Journal of Trace Elements in Medicine and Biology*, 27 (3): 249-256.
- El Haliem, N.G., Mohamed, D.S., (2011) : Effect of aspartame on the histological structure of liver and renal cortex of adult male albino rats and possible protective effect of Pimpinella anisum oil. *Egyptian Journal of Histology*, 34 (4): 715-726.
- El-Sayed, M.G.A., Elkomy, A., Samer, S., ElBanna, A.H., (2015): Hepatoprotective effect of Pimpinella anisum and Foeniculum vulgare against carbon tetrachloride induced fibrosis in rats. *World J Pharm Pharm Sci*, 4(6): 77-88.
- Hazelhoff, M.H., Torres, A.M., (2018):

- Gender differences in mercury-induced hepatotoxicity: Potential mechanisms. *Chemosphere*, 202: 330-338.
- Hosseini, A., Rajabian, A., Fanoudi, S., Farzadnia, M., Boroushaki, M.T., (2018): Protective effect of Rheum turkestanicum root against mercuric chloride-induced hepatorenal toxicity in rats. *Avicenna journal of phytomedicine*, 8(6) : 488.
- Jagadeesan, G., Bharathi, E., (2014): In vivo restoration of the hepatic and nephro-protective potential of hesperidin and ellagic acid against rats poisoned with mercury chloride. *Biomedicine & Aging Pathology*, 4 (3): 219-222.
- Jamshidzadeh, A., Heidari, R., Razmjou, M., Karimi, F., Moein, M.R., Farshad, O., Shayesteh, M.R.H., Akbarizadeh, A.R., (2015): An in vivo and in vitro investigation on hepatoprotective effects of Pimpinella anisum seed essential oil and extracts against carbon tetrachloride-induced toxicity. *Iranian journal of basic medical sciences*, 18(2) : 205.
- Kumar, G., Srivastava, A., Sharma, S.K., Gupta, Y.K., (2014): Safety evaluation of an Ayurvedic formulation based on mercury (Sidh Makardhwaj) on the brain, liver and kidneys in rats. *The Indian Journal of Medical Research*, 139 (4): 610.
- Mumtaz, S., Ali, S., Khan, R., Andleeb, S., Ulhaq, M., Khan, M.A., Shakir, H.A., (2019): The protective role of ascorbic acid in the hepatotoxicity of cadmium and mercury in rabbits. *Environmental Science and Pollution Research*, 26(14) : 14087-14096.
- Mushtaq, A., Anwar, R., Ahmad, M., (2019): Memory enhancing effect of anise (pimpinella anisum) with respect to its antioxidant activity in albino mice. *JAPS: Journal of Animal & Plant Sciences*, 29(2).
- Nathwani, R.A., Pais, S., Reynolds, T.B., Kaplowitz, N., (2005): Serum alanine aminotransferase in skeletal muscle diseases. *Hepatology*, 41(2): 380-382.
- Shojaii ,A., Abdollahi Fard, M., (2012): Review of pharmacological properties and chemical constituents of Pimpinella Anisum. *ISRN pharmaceuticals*, 2012 , vol. 2012
- Suvarna, S.K., Layton, C., Bancroft, J.D., (2013): Bancroft's theory and practice of histological techniques. 7 th Ed. England: Churchill Livingstone. Elsevier.
- Wadaan, M.A., (2009): Effects of mercury exposure on blood chemistry and liver histopathology of male rats. *Journal of Pharmacology and Toxicology*, 4(3): 126-131.