https://doi.org/10.48047/AFJBS.6.15.2024.13097-13110

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Xylene-Induced Hepatic and Thyroid Dysfunction in Wistar Rats: Protective Effects of Aqueous Extract from Ghars Date Seeds (*Phoenix dactylifera* L.)

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Volume 6, Issue 15, Oct 2024

Received: 15 Aug 2024

Accepted: 25 Sep 2024

Published: 21 Oct 2024

doi: 10.48047/AFJBS.6.15.2024.13097-13110

Abstract :

The aim of this work is to study the toxic effect of xylene and validate the protective effect of an aqueous extract of date seeds "*Phoenix dactylifera L*" on the thyroid gland and liver in *Wistar Albino* rats. The study was conducted in the laboratory on 15 rats, which were divided into three groups: the first group was considered the control, the second group received 1.2 ml/kg/day of xylene daily for 25 days, and the third group received 1.2 ml/kg/day of xylene and was treated with 300 mg/kg/day of Ghars date pit extract for 25 days. The results obtained in the present study show that treatment with xylene induces xylene overload, a decrease in FT4 and FT3 hormones with an increase in TSH and hyperglycemia, as well as an increase in bilirubin, and the activity of transaminases (TGO,TGP), and alkaline phosphatase, indicating that xylene induces liver function disturbances. Moreover, the histological study results show the presence of inflammation in the liver and hepatic necrosis induced by oxidative stress. However, oral administration of the date pit extract from cultivar caused a decrease in the toxic effect of xylene.

Keywords: xylene, date seeds extract, Ghars variety, liver, hormones, *Wistar Albino* rats.

INTRODUCTION

In the Sahara, there is a wide diversity of plants and trees that have adapted to the desert climate to survive in these extreme conditions. Among these plants, the most well-known in the oasis environment is the "date palm" (Baliga et al., 2010). The cultivation of date palms (Phoenix dactylifera L.) is considered one of the most important crops in arid and semi-arid regions. It plays a significant role in the economic and social life of the populations in these areas (Djerbi, 1994). Dates are produced in 30 countries, but the majority of production is concentrated in Egypt, which accounts for 21% of global production, followed by Iran with 15%, Saudi Arabia with 15%, Iraq with 9%, Pakistan with 7%, and Algeria, which ranks fourth in the world with 12% of global production (FAO, 2015). Dates, the fruits of the date palm, are an essential part of the dietary habits of the Saharan population. They are increasingly gaining attention among consumers, dietitians, and nutritionists (Ben abbes, 2011). Algeria is one of the largest date-producing countries, with over one million tons produced in 2017 according to the FAO (FAOSTAT). There are currently more than 2,000 different cultivars worldwide (Al-Hooti et al., 2002). By-products of the date palm (trunk, leaves, pedicels, etc.) are widely utilized by the inhabitants of the Sahara, with date pits being particularly valued on a large scale (Djerbi, 1994). Numerous research studies have focused on the valorization of date seeds, turning them into acetic acid (Abou Zaid, 1983), activated charcoal (Bouchemal et al., 2008), livestock feed (Abdelbasset, 2012), and date pit-based cosmetic creams (Lecheb, 2008). The primary objective of our work is to study the biological and hormonal effects of Ghars variety date seeds extract on *Wistar albino* rats exposed to xylene-induced toxicity.

Materials and Methods

Materials

Chemicals

All chemicals used in the biochemical tests were of analytical grade. The chemicals required for all biochemical assays were obtained from Sigma Chemicals Co. (USA), except for xylene, which was sourced from Sigma-Aldrich Chemical Co. (USA).

Plant collection and preparation of date seeds extract

The date seeds used in this study originated froma from ITDAS (Technical Institute of Saharan Agricultural Development) located in Ain Ben Naoui, Biskra, Algeria, one of the

most productive date-growing areas in the country. The seeds analyzed were from the Ghars variety. The harvest was carried out in October 2017. The pits were air-dried and then finely ground to obtain a fine powder, stored away from light. To ensure a good comparison between the effects of xylene and the date pits, a dose of 100 mg of Ghars variety powder was dissolved in 10 ml of boiling filtered water and left for 30 minutes. The mixture was then filtered using Whatman filter paper No 1.

Determination of total polyphenol content

Determining the total polyphenol content in a sample typically involves using spectrophotometric methods, with the Folin-Ciocalteu reagent being the most common approach.

Procedure:

A- Preparation of Gallic Acid Standard Curve:

- Prepare a series of gallic acid standard solutions with known concentrations (e.g., 10, 20, 30, 40, and 50 mg/L).
- Mix each standard with the Folin-Ciocalteu reagent and sodium carbonate solution according to the protocol (typically 1 mL of Folin-Ciocalteu reagent and 2 mL of sodium carbonate solution).
- Allow the reaction to take place for about 30 minutes at room temperature in the dark.
- Measure the absorbance of each standard solution at 765 nm using a spectrophotometer.
- Plot the absorbance values against the concentrations to create a standard calibration curve.

B- Sample Preparation:

- Dilute your sample to ensure the polyphenol content falls within the range of the standard curve.
- Mix the diluted sample with the Folin-Ciocalteu reagent and sodium carbonate solution as per the protocol.
- Incubate the mixture for 30 minutes in the dark at room temperature.

C- Measurement:

- Measure the absorbance of your sample at 765 nm.
- Use the calibration curve from the gallic acid standards to determine the concentration of polyphenols in the sample.

D- Calculation of Total Polyphenol Content:

- Express the results as milligrams of gallic acid equivalents (GAE) per liter (mg GAE/L) or per gram (mg GAE/g) of the sample.
- To calculate the total polyphenol content, use the formula:

$$\text{Total polyphenol content (mg GAE/g)} = \frac{C \times V}{m}$$

Where :

C = concentration of polyphenols obtained from the calibration curve (mg/L)

V = volume of the sample (L)

m = mass of the sample (g)

This method is widely used due to its simplicity and efficiency in quantifying the total polyphenol content in different types of samples (Singleton et *al*., 1999).

Antioxidant assay

DPPH free radical scavenging assay

The assay to evaluate radical scavenging activity was performed using a DPPH solution prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. For the test, 25 µL of each sample extract or a standard solution of ascorbic acid was mixed with 975 µL of the DPPH solution. The mixture was thoroughly stirred and incubated in the dark at room temperature for 30 minutes. Following incubation, the absorbance was measured at 515 nm using a spectrophotometer.

The percentage of DPPH scavenging activity was calculated using the formula:

$$\%\ \text{DPPH scavenging activity} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

The IC_{50} values, indicating the concentration of the extract required to neutralize 50% of the DPPH radicals, were determined by plotting the inhibition percentages against the concentrations of the samples. (Schaich et *al*.,2015).

FRAP assay

The ferric reducing antioxidant power (FRAP) assay was used to determine the ferric reducing capacity. To prepare the TPTZ solution, 0.312 g of 2,4,6-Tripyridyl-s-Triazine (TPTZ) was dissolved in 100 mL of 40 mM HCl. The ferric chloride ($FeCl_3$) solution was made by dissolving 0.54 g of $FeCl_3$ in 100 mL of distilled water, followed by heating the mixture at 30°C for 10 minutes. This preparation can be performed using test tubes.

For the assay, 0.15 mL of either the extract or the standard was mixed with 2.85 mL of the FRAP working solution at room temperature in the absence of light. The absorbance was then measured at 595 nm.

To prepare the sodium acetate buffer, 2.46 g of sodium acetate was combined with 3.6 mL of acetic acid in an Erlenmeyer flask, and distilled water was added to bring the total volume to 100 mL. The pH was adjusted to 3.6 using acetic acid. The FRAP working solution was then prepared by mixing 10 parts of the 300 mM sodium acetate buffer (pH 3.6), 1 part of the TPTZ solution, and 1 part of the 20 mM ferric chloride solution (Oyaizu et al., 1986).

the experimental animals used in the study

Female *Wistar albino* rats (weighing 274.34 ± 50 g) were obtained from the Pasteur Institute in Algiers, Algeria. The animals were acclimated to laboratory conditions for two weeks before the experiments and were housed in large, clean polypropylene cages with unrestricted access to food and water. The rats were maintained under standardized conditions, including a temperature of 24 ± 3°C and a 12-hour light/dark cycle.

Expérimental design

After a 15-day adaptation period, the female rats were divided into three (03) groups, kept under the same conditions, with six rats in each group (n=06), as follows:

- Group 1: Healthy control (Control): The control rats received a standard diet for 25 days.

- Group 2: This group received 1.2 mL of xylene in water daily from 8:00 AM to 4:00 PM, with only water provided for the remainder of the day.

- Group 3: This group received a daily dose of xylene (1.2 mL) from 8:00 AM to 4:00 PM, and for the rest of the day, they were given extracts from Ghars variety seeds at a dose of 100 mg/kg/day in water."

Determination of serum biochemical and hormonal parameters

The automatic analyzer (COBAS INTEGR® 400 plus, Maurimedis) was used to determine AST, ALT, ALP, Total bilirubin, and glucose levels, while the hormone levels (TSH, FT4, and FT3) were measured using the DiaSorin® system.

Histopathological Evaluation

Freshly harvested liver tissues were cut into 2 mm pieces and placed in plastic cassettes, then preserved in 10% formalin for 24 hours. The tissues were processed using an automated tissue processor to produce paraffin-embedded sections, which were subsequently sliced into 2 µm sections using a LEICA® TP1020 rotary microtome. The tissue sections were then stained with hematoxylin and eosin (H&E) for examination, and the structural changes were observed under a ZEISS® Primo Star light microscope.

Statistical Analysis

The results of the biochemical parameters were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using the t-test, with a significance threshold set at $p < 0.05$. All calculations were performed using Excel (2010) and Minitab (version 18) software.

Results and Discussion

Total polyphenol content

the total polyphenol content obtained from the crude extract of date seeds (Ghars), calculated using the equation y=0.0007x-0.0036 with a correlation coefficient of R^2=0.99, derived from a calibration curve based on gallic acid as the standard, which was determined to be **0,129 ± 0,00473** mg/mL.

Antioxidant assay

DPPH free radical scavenging assay

The DPPH free radical scavenging method evaluates the ability of a compound or plant extract to act as an antioxidant. When plant extracts that can donate a hydrogen atom are mixed with the deep violet-colored DPPH solution, they reduce the radical, causing the violet color to fade. Table I presents the results of the antioxidant activity.

Table I : Radical scavenging potential of date seeds Ghars as determined by DPPH assay

IC50 mg/mL	Ghars seeds extract
	0.301±0.001

FRAP Assay

The FRAP assay showed a value of 134.5 mg EAA/g of extract for the Ghars seed extracts. This value is the highest among the antioxidant activity tests conducted.

Effect of date seed extracts on serum liver enzymes

Table II , Our results show a significant increase in the activity of AST, ALT, and alkaline phosphatase (ALP) in the xylene-only group compared to the control. Furthermore, the results indicate that treatment with the Ghars cultivar causes a significant decrease in AST and ALT activity, and a highly significant decrease in alkaline phosphatase activity compared to the xylene-contaminated group.

Table II : Serum liver enzyme activity in control , xyléne and Ghars date seeds treatment.

Treatment	**AST (**IU/L)	**ALT (**IU/L)	**ALP (**IU/L)
Control	111 ± 0.1	49 ± 0.1	120 ± 0.1
Xyléne	129 ± 0.1	64 ± 0.1	221 ± 0.1
Xyléne + Ghars date seeds	115 ± 0.1	47 ± 0.1	140 ± 0.1

Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP).

Effect of date seed extracts on serum biochemical parameters

In table III for the blood glucose , the results show a significant increase in the toxic group compared to the control. Treatment with the Ghars cultivar in xylene-intoxicated rats induced a highly significant decrease in blood glucose levels. The total bilirubin concentration significantly increased in the toxic group compared to the control, and treatment with the Ghars cultivar in xylene-intoxicated rats led to a highly significant decrease in total bilirubin concentration.

Table III : Serum biochemical parameters in control, xyléne and Ghars date seeds treatment.

Treatment	Total Bilirubin (mg/L)	Glucose(g/L)
Control	2.4 ± 0.01	1.28 ± 0.001
Xyléne	3.5 ± 0.01	1.40 ± 0.001
Xyléne + Ghars date seeds	2.55 ± 0.01	1.15 ± 0.001

Effect of date seed extracts on serum thyroid hormones

The table IV , The results show a significant decrease in FT3 and a highly significant decrease in FT4 in the xylene-contaminated group compared to the control group. On the other hand, after treatment with the Ghars cultivar, the results show a significant increase in FT3 and a highly significant increase in FT4. The results also reveal a significant increase in TSH in the xylene-contaminated group compared to the control group, while treatment with the Ghars cultivar led to a highly significant decrease in TSH levels.

Table IV : Serum thyroid hormones in control, xyléne and Ghars date seeds treatment.

Treatment	**TSH (**µIU/L)	**FT4 (**pmol/L)	**FT3 (**pmol/L)
Control	0.006 ± 0.0001	22.19 ± 0.01	6.00 ± 0.01
Xyléne	0.016 ± 0.0001	19.00 ± 0.01	4.75 ± 0.01
Xyléne + Ghars date seeds	0.0065 ± 0.0001	21.87 ± 0.01	5.80 ± 0.01

thyroid-stimulating hormone (TSH), free thyroxine (FT4), and free triiodothyronine (FT3).

Histopathological Evaluation

Examination of liver sections from the control group revealed a normal histological structure of classic hepatic lobules with central veins and blood sinusoids (Figure 1). In contrast, significant hepatocyte necrosis was observed in the liver sections of rats treated with xylene alone.

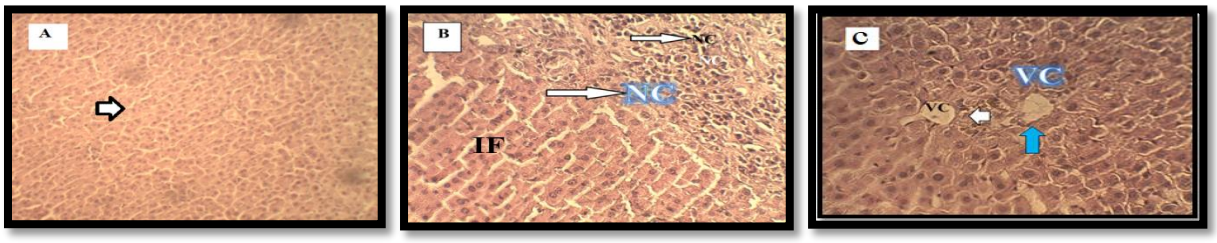

Figure 1

Effects of Phoenix dactylifera seeds on xylene-induced histopathological changes in Wistar rats**:**
A: Control group. B: Animals treated with xylene. C: Animals treated with xylene and Ghars date seeds.

DISCUSSION

Xylene exposure can occur through inhalation, ingestion, or contact with the eyes or skin. It is primarily metabolized in the liver by the oxidation of a methyl group and conjugation with glycine to produce methylhippuric acid, which is excreted in the urine (Sedivec V, Flek J 1976). Xylene has both acute and chronic health effects. The type and severity of the effects depend on several factors, including the amount of the chemical you are exposed to and the duration of exposure (Ogata M, Tomokuni K, Takatsuka Y 1970).

The thyroid gland produces thyroxine and triiodothyronine, which participate in growth and mental development. They also help regulate metabolism (Di Palma, T., Conti, A., de Cristofaro, T., Scala, S., Nitsch, L., Zannini, M.S., 2011), the chemical process in the body for creating and using energy. The thyroid gland is controlled by the pituitary gland, which produces thyroid-stimulating hormone (TSH). The TSH is released by the pituitary in response to the levels of thyroxine and triiodothyronine in the blood (Di Palma, T., Filippone, M.G., Pierantoni, G.M., Fusco, A., Soddu, S., Zannini, M., 2013).

The objective of our study is to evaluate the toxic effect of xylene on the thyroid gland and liver, and to validate the antidote effect of the date seed extract in Wistar albino rats. DPPH is

a stable free radical that accepts an electron or proton to give a stable molecule. It is widely used in screening for free radical scavenging activity (Pokorny et al., 2001) and is often employed for rapid results to screen molecules with antioxidant activities in plant extracts (Yi-Zhong C et al., 2006). In our results, vitamin C exhibited a much stronger anti-radical power compared to our extracts. However, according to Hatzidimitriou et al. (2007), natural antioxidants often have lower antioxidant activity than synthetic antioxidants. Our results also show that the date seed extract of the Ghars cultivar has four times lower activity than ascorbic acid.

Bilirubin is a yellow pigment that results from the breakdown of hemoglobin. It is found primarily in bile and in small amounts in the blood (Haleng et al., 2007). Bilirubin is produced by the spleen and bone marrow cells and is transported through the blood to the liver, where it is transformed into bile pigments that are reabsorbed or eliminated in the stool (and partially excreted in urine). The increase in bilirubin in the toxic group could be explained by liver dysfunction and suspected gallbladder issues, indicating a problem in the excretion stage of bilirubin into bile (Vaishnav et al., 2015). Additionally, our results show that the date seed extract from the Ghars cultivar plays a role in increasing bile excretion of bilirubin, consequently reducing serum bilirubin levels (Weber, L et al., 2003).

Xylene administration induces several metabolic changes. The results show a highly significant increase in serum glucose levels in rats exposed to xylene. These variations are related to the disruption of the hypothalamic-pituitary-adrenal axis, reflecting hyperglycemia associated with activation of the sympathetic nervous system. The reaction in the active limbic system causes the hypothalamus to produce corticotropin-releasing hormone (CRH), which stimulates the pituitary to release ACTH (adrenocorticotropic hormone), an activator of the adrenal glands to produce and secrete cortisol in the blood (Pourramzanzidesaraei et al., 2013). Cortisol has many actions (Jacotot & Campillo, 2003), including leading to large catabolism of liver glycogen (Kumar & Rajini, 2009; Ksheerasagar & Kaliwal, 2006; Yousef et al., 2006). On the other hand, our results show that the date seed extract plays a role in reducing blood glucose levels. These results are in agreement with the study by Dalia H et al. (2014), which indicates that TGO, TGP, and PAL activity significantly increases in the xylene-treated group compared to the control group. These enzymes are normally contained within liver cells. In the case of liver damage, liver cells release these enzymes into the bloodstream (Singh et al., 1998, Ozturk et al., 2009).

Our data revealed that treatment with the date seed extract from the Ghars cultivar significantly decreased the elevation of liver function parameters induced by xylene (TGO, TGP, and PAL) in the serum. The reduction of these parameters to near-normal levels is a sign of stabilization of plasma membranes and liver tissue repair. According to the study by Tassaneeyakul et al. (1996), the anti-lipid peroxidation effect of the date seed extract prevented the harmful effects of free radicals generated by xylene, leading to healing of liver parenchyma and hepatocyte regeneration. Additionally, the improvement in liver integrity in the groups treated with the date seed extract was clearly evident.

Furthermore, our study shows that histological examination revealed severe liver damage, including hepatitis and necrosis, in the xylene-treated group (Morley et al., 1970; Klaucke et al., 1982). This increase in enzymes was induced by xylene overload. However, histological examination also showed that the date seed extract reduced the severity of liver damage caused by excessive xylene in the liver tissues of the groups treated with the date seed extract. The extract demonstrated a superior effect in stimulating the enzymatic antioxidant capacity of liver cells (Wiart., 2006), according to studies by Dalia H et al. (2014).

Thyroid hormone synthesis is under the control of TSH, and thyroid hormones play a general role as metabolic accelerators. They are important in lipid and glucose metabolism, food intake, and fat oxidation. Thyroid dysfunction is associated with weight changes (Normand Blanchard H., 2009). Our results indicate a decrease in T4 and T3 levels and an increase in TSH levels in the xylene-treated group compared to the control. This suggests hypothyroidism, a condition in which the thyroid gland produces insufficient hormones, leading to slow metabolism and fat accumulation due to xylene accumulation in the liver.

The groups treated with date seed extracts from Ghars cultivar showed a significant increase in values approaching those of the control group, suggesting that the date seed extract reduced the deficiency in thyroid hormones, benefiting both the thyroid gland and liver. According to Gallois M. (2008), various associations between thyroiditis and liver diseases have been reported. Our findings already indicated that the liver of this group suffered from inflammation and necrosis, suggesting a link between thyroiditis and liver disease.

Histological alterations observed in hepatocytes, such as inflammation and necrosis in the xylene-only group, could be due to free radical generation and lipid peroxidation induced by xylene. In contrast, the general morphology of the liver lesions in rats treated with the date

seed extract from the three cultivars was much improved and appeared normal compared to rats treated with xylene alone.

Conclusion

The study assessed the antitoxic effects of *Phoenix dactylifera L.* Ghars seed extracts in female Wistar albino rats exposed to xylene. The extracts showed significant potential in normalizing serum enzyme levels, biochemical parameters, and thyroid hormone levels. As a result, the raw extract from these date seeds exhibits promising potential in alleviating xylene toxicity and supporting the regulation of metabolic processes and thyroid function.

Conflict of interest

The authors declare no conflict of interest.

References

- Al-Hooti, S. N., Sidhu, J. S., Al-Saqer, J. M., & Al-Othman, A. (2002). Chemical composition and quality of date syrup as affected by pectinase/cellulase enzyme treatment. *Food chemistry*, *79*(2), 215-220.
- Ali, S. E. M., & Abdelaziz, D. H. A. (2014). The protective effect of date seeds on nephrotoxicity induced by carbon tetrachloride in rats. *Int J Pharm Sci Rev Res*, *26*(2), 62-68.
- Baliga M.S., Baliga B. R.V., Kandathil S. M., Bhat H.P., Vayalil P. K.,2011 .- A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.) Food Research International. Food Research International, Volume 44, Issue 7, Pages 1812-1822.
- Ben abbes F. (2011). Etude de quelques propriétés chimiques et biologiques d'extraits de dattes « *Phoenix dactylifera* L. ».Mémoire de Magister en Génie des procédés pharmaceutiques. Universite Ferhat Abbas-Setif: 1-11 p.
- Bouchemal N., Merzougui Z., Addoun F., 2011. - Adsorption en milieux aqueux de deux colorants sur charbons actifs a base de noyaux de datte.Journal de la Société Algérienne de Chimie. 21(1), 1-14
- Di Palma, T., Filippone, M.G., Pierantoni, G.M., Fusco, A., Soddu, S., Zannini, M., 2013. Pax8 has a critical role in epithelial cell survival and proliferation. Cell Death Dis. 4, e729.
- Djerbi M. (1994). Précis de phoeniciculture. Ed FAO, Rome : 23-191 .

Gallois M., L'hypothyroïdie : quand la thyroïde se dérègle ?, thèse de diplôme d'Etat de docteur en pharmacie de Lille 2, 2008.

- Haleng, J., Pincemail, J., Defraigne, J. O., Charlier, C., & Chapelle, J. P. (2007). Le stress oxydant. *Revue médicale de Liège*, *62*(10).

- Hatzidimitriou E.F., Nenadis N., Tsimidou M.Z., 2007. Changes in the catechin and epicatechin content of grape seeds on storage under different water activity (aw) conditions. Food Chemistry. 105: 1504-1511.

- Jacotot, B., & Campillo, B. (2003). Nutrition humaine. *(No Title)*.

- Kaliwal, B. B., & Ksheerasagar, R. L. (2006). Histological and biochemical changes in the liver of albino mice on exposure to insecticide, carbosulfan. *Caspian Journal of Environmental Sciences*, *4*(1), 67-76.

- Klaucke DN, Johansen M, Vogt RL. An outbreak of xylene intoxication in a hospital. Am J Ind Med. 1982;3:173-8.

- Lecheb, F., 2010. Extraction et caractérisation phyico-chimique et biologique de la matière grasse du noyau des dattes : essai d'incorporation dans une crème cosmétique de soin. Thèse Magister, Université M'HAMED BOUGARA, Boumerdès. 114 p.

- Morley R, Eccleston DW, Douglas CP, Greville WEJ, Scott DJ, Anderson J. Xylene poisoning: a report on one fatal case and two cases of recovery after prolonged unconsciousness. BMJ. 1970;3:442-3.

- Normand Blanchard, H. (2009). *Prise en charge actuelle de l'hyperthyroïdie en France* (Doctoral dissertation, thèse de diplôme d'Etat de docteur en pharmacie de Lille 2).

- Ogata M, Tomokuni K, Takatsuka Y. Urinary excretion of hippuric acid and m-or pMethylhippuric acid in the urine of persons exposed to vapours of toluene and m-or p-xylene as a test of exposure. Br J Ind Med. 1970;27:43-50.

- Oyaizu, M. (1986). Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese journal of nutrition and dietetics*, *44*(6), 307-315.

- Ozturk, I.C., Ozturk, F., Gul, M ., Ates, B.,& Cetin, A., (2009). Protective effects of ascorbic acid on hepatotoxicity and oxidative stress caused by carbon tetrachloride in the liver of Wistar rats. Cell Biochemistry and Function. 27: 309–315. 91.

- Pokorny J., Yanishlieva N., Gordon M., 2001. Antioxydants in food, Practical applications. Woolhead Publishing Limited. ISBN: 185573-463X.

- Schaich, K. M., Tian, X., & Xie, J. (2015). Reprint of "Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays". *Journal of functional foods*, *18*, 782-796.
- Sedivec V & Flek J (1976) The absorption, metabolism, and excretion of xylenes in man. Int Arch Occup Environ Health, 37: 205-217.
- Singh ,B., Saxena, A.K., Chandan B.K., Anand, K.K., Suri O.P., SuriSatti K.A., & SuriSatti, N.K. (1998). Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia. 69: 134–140.
- Singleton V.L., Orthofer R., Lamuela-Raventós R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. Orlando Academic Press: 152-178.
- Vaishnav, A., Kumari, S., Jain, S., Varma, A., & Choudhary, D. K. (2015). Putative bacterial volatile-mediated growth in soybean (Glycine max L. Merrill) and expression of induced proteins under salt stress. *Journal of Applied Microbiology*, *119*(2), 539-551.
- Weber, L.W.D., Boll, M., Stampfl, A., 2003. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Critical Reviews in Toxicology 33, 105–136.
- Wiart, C., 2006. Anti-inflammatory plants ethnopharmacology of medicinal plants:asia and the pacific. USA: Totowa .

- Yi-Zhong C., Mei S., Jie X., Qiong L., Corke H., 2006. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sciences. 78(25): 2872-2888.
- Yousef, M. I., Awad, T. I., & Mohamed, E. H. (2006). Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. *Toxicology*, *227*(3), 240-247.
- https://www.fao.org/news/archive/newsbydate/2015/fr/