Global Advanced Research Journal of Medicine and Medical Sciences (ISSN: 2315-5159) Vol. 2(4) pp. 090-099, April, 2013
Available online http://garj.org/garjmms/index.htm

Full Length Research Paper

Histological Study on the Possible Protective Effect of Nigella Sativa Oil on Experimentally Induced Hepatotoxicity in Albino Rats Treated with Sodium Valproate

Nadia M Al-Rouby[1]* and Shefaa M Gawish[2]

[1]Histology Department, Faculty of Medicine, Cairo University, Egypt
[2]Anatomy department, Dubai Medical College, Dubai, United Arab Emirates

Accepted 21 April, 2013

Sodium valproate (depakine, Valproic acid, VPA), a frequently used drug for the treatment of epilepsy, has been used worldwide. However, VPA therapy is responsible for a number of fatal cases of hepatic failure. The mechanism by which VPA causes hepatotoxicity is uncertain. It might be due to failure of antioxidant scavengers. This study was conducted to investigate the possible protective effect of Nigella sativa (NS) oil as an antioxidant in preventing this hepatotoxicity. Thirty two albino rats were divided into four equal groups; control group, NS oil treated group, VPA treated group and VPA+NS oil treated group. At the end of the experiment, blood samples were taken for estimation of liver enzymes (alkaline phosphatase, ALT and AST). The liver of all animals was excised and processed for both light and electron microscopic studies. VPA administration in a toxic dose caused significant increase in liver enzymes, microvesicular steatosis and congestion of blood sinusoids. Hepatocytes showed apparent markedly damaged mitochondria, increased lysosomes and dilatation of sER. Rats received VPA and NS oil showed some improvement of these changes. VPA is a hepatotoxic drug and its hepatotoxicity could be minimized by supplementation of NS oil.

Keywords: Valproic acid, liver, Nigella sativa, albino rats.

INTRODUCTION

Sodium Valproate, an eight carbon branched chain fatty acid, is a broad spectrum anti-epileptic drug that is now used commonly for several other neurological and psychiatric indications such as bipolar psychiatric disorders and migraine. VPA is usually well tolerated. Despite its medical effectiveness, serious complications including hepatotoxicity and hyperammonemic encephalopathy may occur. Although these complications are rare, they are fatal and may be irreversible (Lheureux and Hanston, 2009). Hepatotoxicity caused by VPA is enhanced by the presence of risk factors that include young age, polypharmacy, high VPA serum level, neurological diseases, male gender, concomitant use of hepatic cytochrome P450 inducers and HIV infected men (Kondo et al., 1992; Klee et al., 2000).

Cumulative evidences showed that there were increased levels of free radicals following treatment with

*Corresponding Author E-mail: nadiaalroby@yahoo.com; Phone: 00971503617646; Fax: 00971-4-2646130

VPA. A possible VPA biotransformation and/or alterations in natural antioxidants might contribute to the VPA-associated complications (Klee et al., 2000 and Bykov et al., 2004). However, the main cause of VPA hepatotoxicity was shown to be due to failure of the free radical scavenger system (Barthel, 1998; Hamza and Amin, 2007).

Antioxidants are, however, the primary candidate to counter such toxic effects. Many plant extracts have been shown to protect against chemical –induced toxicity. Nigella sativa (NS) commonly known as black seeds are used in herbal medicine all over the world for treatment of many diseases like asthma, diarrhea, dyslipidaemia. Much of the biological activity of these seeds has been due to thymoquinone, the major component of NS oil. The pharmacological actions of oil and thymoquinone have been reported to induce protection against nephrotoxicity and hepatotoxicity induced by either diseases or chemicals (Ali and Blunden, 2003; Sharma et al., 2009).

This work was conducted to: evaluate the hepatic structural alterations induced by toxic dose of VPA and to monitor the protective effect of NS oil on these changes.

MATERIAL AND METHODS

Chemicals

Nigella sativa (NS) oil was purchased from a local herbal store. It was given in a dose of 0.2ml/kg body weight (Kanter et al., 2009), once a day orally using a feeding tube.

Sodium valproate was purchased from Sigma (Louis, St). It was presented as vials of 400 mg/4 ml (Sanofi-Synthelabo, France). It was dissolved in saline. The dose used was 500 mg/kg body weight. The drug was given by intraperitoneal (IP) injection, daily for seven consecutive days (Nishimura et al., 2000).

Animals

Thirty two adult male albino rats (150-200 g) were provided by the Animal House of Kaser Al Aini Medical College, Cairo University. Rats were fed on standard pellet diet and tap water ad libitum. They were kept as 5 rats /cage with wood chips bedding, twelve hours light/dark cycle and room temperature. This study was approved by the Animal ethics committee of Faculty of Medicine, Cairo University, Egypt.

Method

The rats were equally divided into four groups as follows:

Group I: (Control group): The rats received an equivalent volume of saline intra-peritoneal (IP) for seven consecutive days.

Group II: (NS group) the rats received NS oil for seven consecutive days. The oil was given using a feeding tube. It was given in a dose of 0.2ml/kg body weight (Kanter et al., 2009).

Group III (Intoxication group): Rats of this group were injected daily with IP dose of VPA (500 mg/kg body weight) for seven days. This dose was reported to be toxic (Nishimura et al., 2000).

Group IV (protective group): The rats received NS oil for three days prior to VPA injection. Then they administered NS oil along with VPA for another seven consecutive days.

At the end of the experiment, the rats were subjected to light anesthesia. Retro-orbital blood samples were taken from all groups for estimation of liver enzymes (alkaline phosphatase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Estimation of liver enzymes was done in biochemical lab. of Faculty of Medicine, Cairo University, Egypt.

- **For light microscopy**

The liver of all groups was excised. Small pieces of the peripheral parts of the liver were fixed in 10% formol saline for 24 hours (Baran et al., 2004) then processed and paraffin blocks were prepared. Sections of five micrometer thickness were cut and stained using H & E and PAS stains.

The area % that represented the percentage of the PAS positive areas and which were masked by a binary color to the area of the standard measuring frame for all groups was done using the image analyzing system.

- **For transmission electron microscopy (TEM)**

Pieces of liver tissue were rinsed in phosphate buffer (PH 7.4) then fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide and embedded in Epon. Semithin sections of 1 micrometer thick were cut on an LKB ultra microtome and stained with toluidine blue. Ultrathin sections were stained with lead citrate and uranyl acetate (Kimura et al., 1989).

Statistical analysis

Morphometric data were statistically analyzed on IBM / PC using SPSS / PC program (version 13). This was performed using the mean and standard deviation (SD). Comparison of significance between each group and the control was made using student “t” test. Results were considered significant when probability (p) ≤ 0.05.

Table 1. The mean values of alkaline phosphatase (U/L), ALT (U/L) and AST (U/L) ±SD of different groups

Item	Control group	NS treated group	VPA intoxication group	VPA +NS treated group
alkaline phosphatase:				
Mean ±SD	52.5±5.68	54.7±6.83	274±18.76	55±8.49
versus control:				
p value		0.28181	* 0.0001	0.5996
ALT : Mean ±SD	36.5±6.86	37.3±6.12	252.5±5.86	39±8.37
versus control:				
p value		0.4144	* 0.0001	0.5839
AST: Mean ±SD	101.5±5.36	102.3±5.85	400.5±16.83	106±4.05
versus control:				
p value		0.4011	* 0.0001	0.1318

* Significant P value.

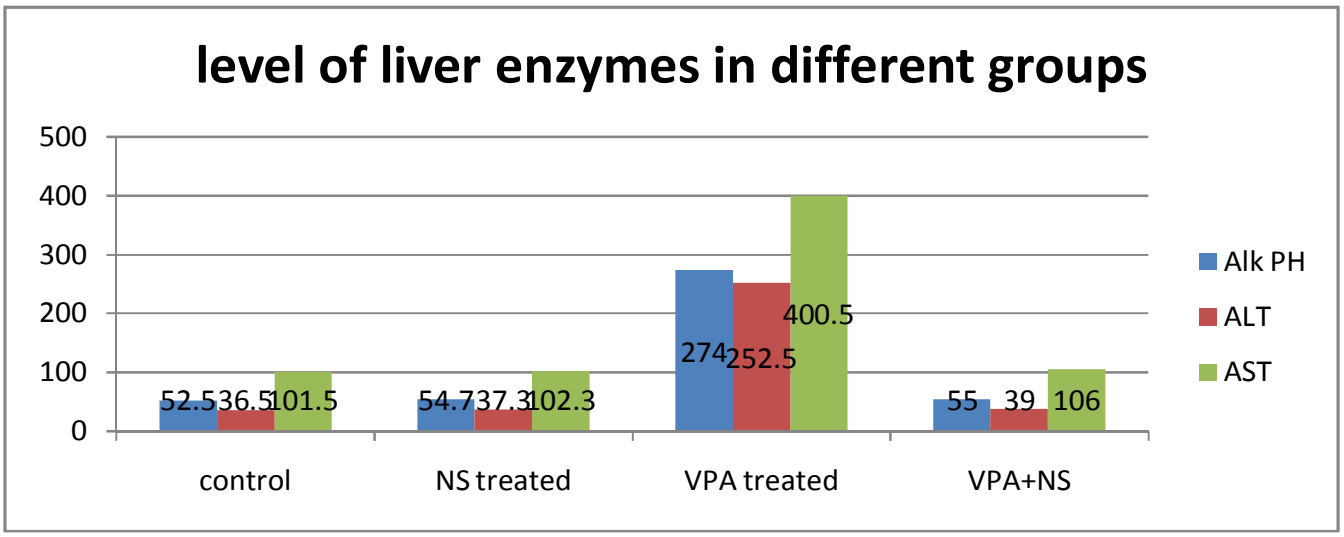

Chart 1. The mean values of alkaline phosphatase (U/L), ALT (U/L) and AST (U/L) in the different groups

RESULTS

Liver enzymes

From table 1 and chart 1, it was clear that administration of toxic dose of VPA caused statistically significant increase in liver enzymes (alkaline phosphatase, ALT and AST) in comparison with the control ($P < 0.05$). These elevated liver enzymes returned back to more or less the control level in the group received both VPA and NS oil.

Histological results

H and E

The liver of **control** rats showed normal histological architecture. The hepatic lobules showed central veins from which the hepatocytes radiated in the form of cords. These cords were separated by blood sinusoids, (plate 1; A). The liver of rats received **NS oil** revealed more or less normal hepatic architecture (plate 1; B). The liver of **VPA intoxication** group (group III) showed focal areas of mononuclear cellular infiltrations. The hepatocytes of the affected area appeared as cords without clear cell boundaries (remark cell lines), (plate 1; C). Many hepatocytes revealed small well circumscribed vacuoles (microvesicular steatosis). Von Kupffer cells appeared swollen and hypertrophied, (plate 1; D). Hepatocytes of some rats showed strong acidophilic cytoplasm and small, dark stained nuclei. There were dilated and congested blood sinusoids and central veins (plate 1; E). All the previous changes were focally distributed. Administration of **NS oil in association with VPA** in the

Table 2. The mean values of area percent of PAS of different groups

Item	Control group	NS treated group	VPA intoxication group	VPA +NS group
Mean ±SD	34.8±3.75	34.6±2.73	20.7±6.00	29.3 ±6.64
Versus control:				
P value		0.88	* 0.0001	0.035

* Significant P value.

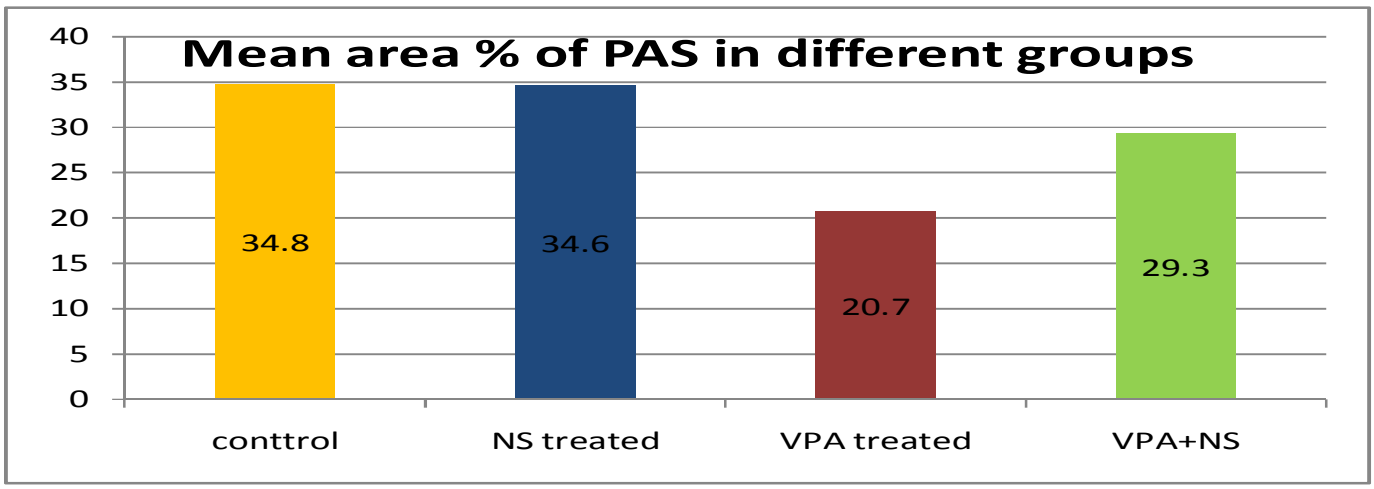

Chart 2. Mean area % of PAS in the different groups

protective group (group IV) reduced most of the previous changes, (plate 1; F).

PAS staining

Liver cells of **control** rat revealed normal distribution of glycogen granules. The peripheral hepatocytes in zone I showed intense (++++) positive PAS reaction while central cells in zone III revealed moderate (++) reaction. There was strong (+++) reaction in the wall of the central veins (plate 2; A). In rats treated with **NS oil** the glycogen distribution appeared more or less homogenous (plate 2; B). The liver of **the intoxication** group showed marked decrease in glycogen content of hepatocytes. The distribution of glycogen granules was heterogeneous (plate 2; C)**.** Rats of the **protective** group showed that most of hepatocytes had strong (+++) positive PAS reaction and few cells showed mild (+) reaction (plate 2; D).

Table 2 and chart 2 showed that administration of toxic dose of VPA caused statistically significant decrease in the area % of positive PAS in comparison with the control ($P < 0.05$). This decrease returned back to more or less the control level in the protective group.

Electron microscopic results

The liver cells of **control** rats showed euchromatic nuclei, mitochondria with apparent cristae, RER, SER and glycogen granules (plate 3; A). Hepatocytes of rats of the group treated with **NS oil** revealed more or less the same ultrastructure as the control group (plate 3; B). Rats treated with **VPA** (group III) showed markedly damaged mitochondria as most of the mitochondria revealed loss of their cristae (plate 3; C). There was apparent increase in darkly stained lysosomes, sER appeared dilated and proliferate. The nuclei of some hepatocytes appeared small, darkly stained, irregular or indented with margination of hetrochromatin (plate 2; D). There was widening of the nuclear space, (plate 2; E). Liver cells of rats from the protective group had more or less normal euchromatic nuclei. The mitochondria were apparently less damaged but there was still some dilatation of sER (plate 2; F).

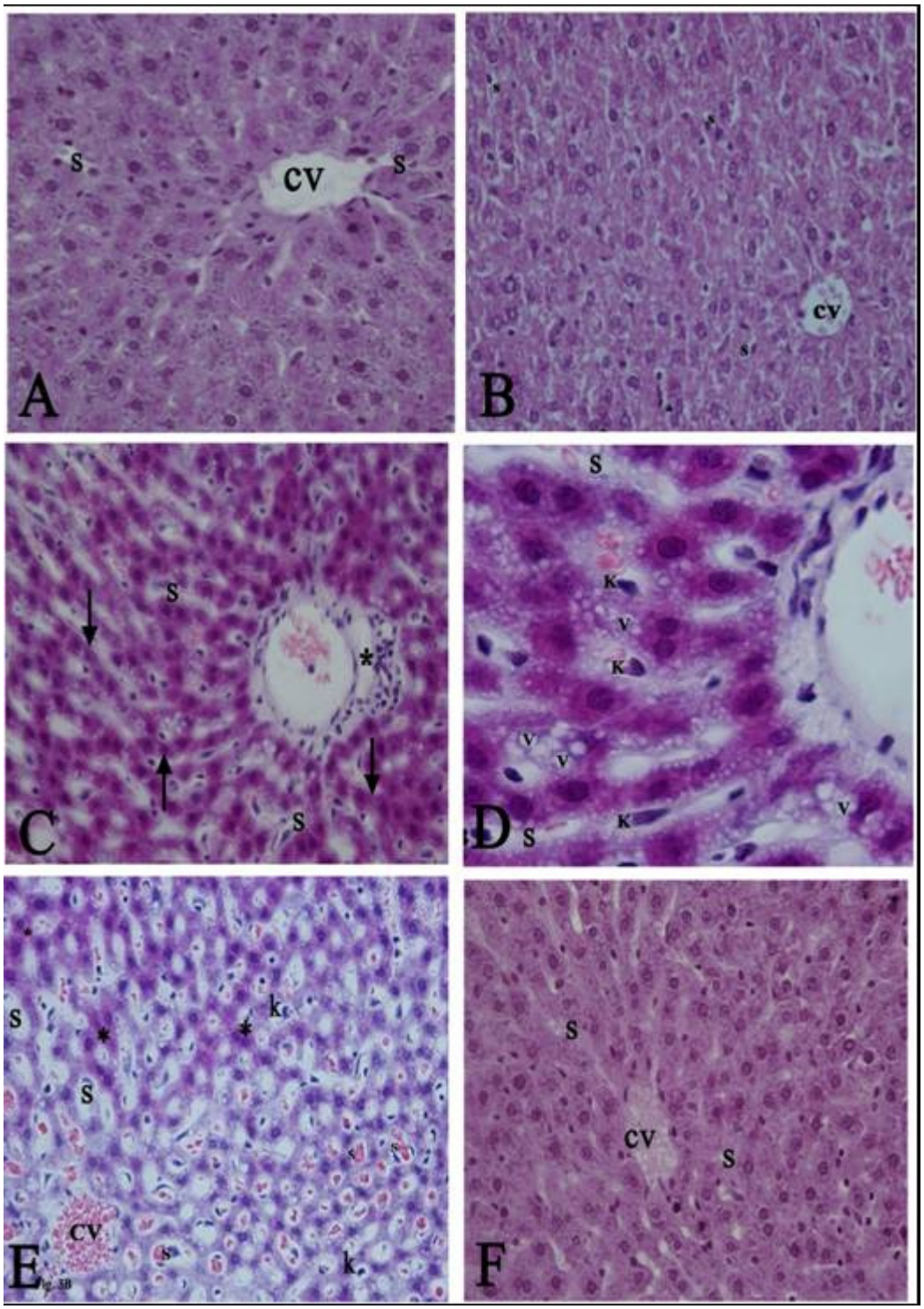

Plate 1. H & E stained liver sections of; A): control rat showing the central vein (CV) from which cords of hepatocytes radiate. In between cords, there are blood sinusoids (S), (x400). B): a rat from the 2nd group showing more or less normal architecture, (x400). From C to E) rats from VPA intoxication group; C): reveals that the blood sinusoids (S) appear dilated. The hepatocytes appeared as cords without clear cell boundaries (remark cell lines), (arrows). There is focal mononuclear cellular infiltration (*), (x400). D): Many hepatocytes revealed small well circumscribed vacuoles (V). Von Kupffer cells (k) appeared hypertrophied, (x1000). F): Some hepatocytes appear more acidophilic (*) than others. Von Kupffer cells (k) are seen. Notice the congestion and dilatation of blood sinusoids and central vein, (x400). F): a rat from the protective group showing more or less normal blood sinusoids (S) around the central vein (CV), (x400).

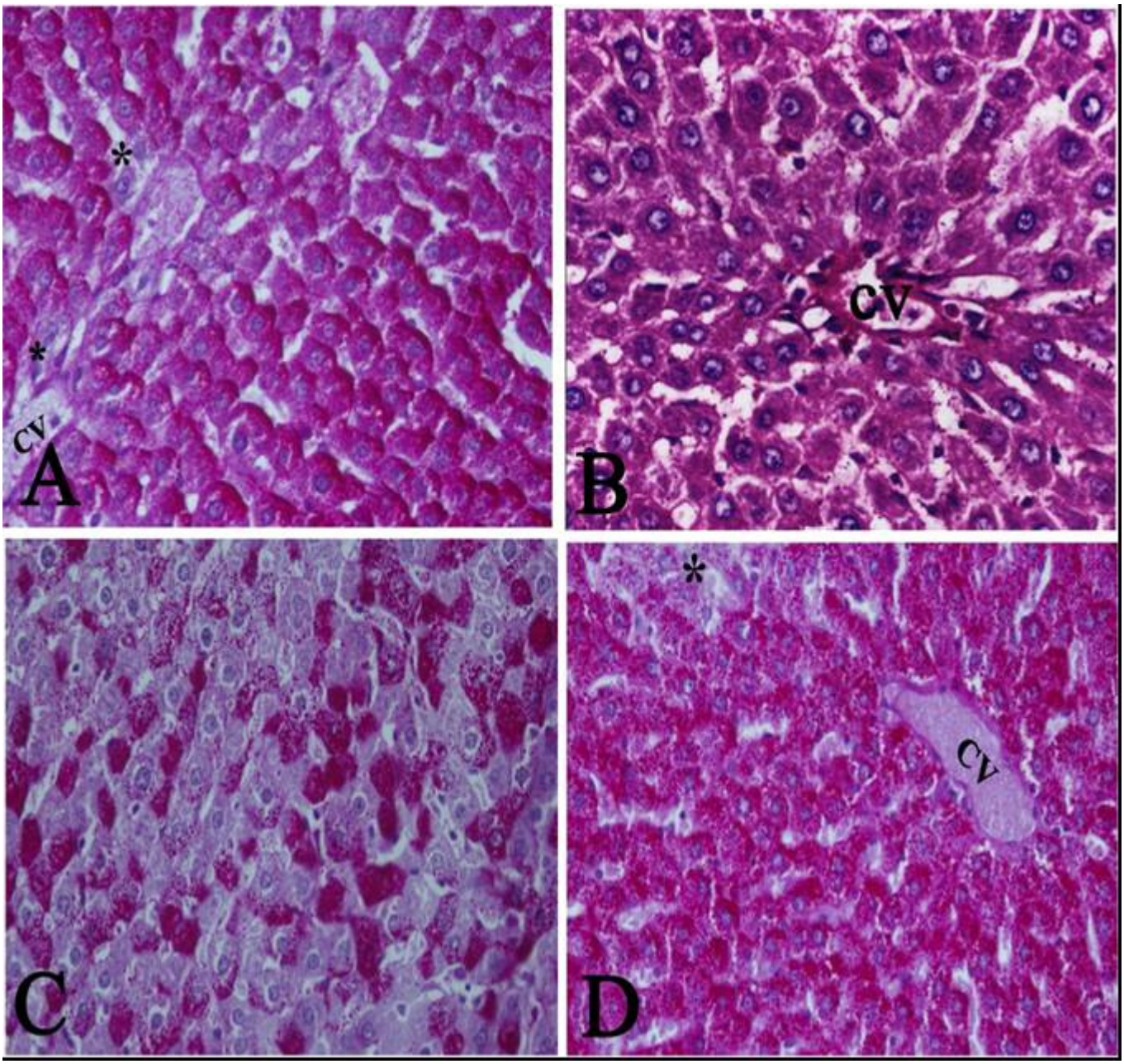

Plate 2. PAS and H stained liver sections of (x400) A): control rat showing that the peripheral hepatocytes have intense positive reaction while the central cells show moderate reaction (*). Notice strong reaction of the wall of central vein (CV).
B): rat administered NS oil only shows homogenous distribution of glycogen granules in the hepatocytes. C): a rat from intoxication group shows marked decrease in PAS reaction with hetrogenous distribution D): a rat from the 4 th group showing patches of mild (*) PAS reaction. Most of hepatocytes and the wall of the central vein showed strong positive reaction.

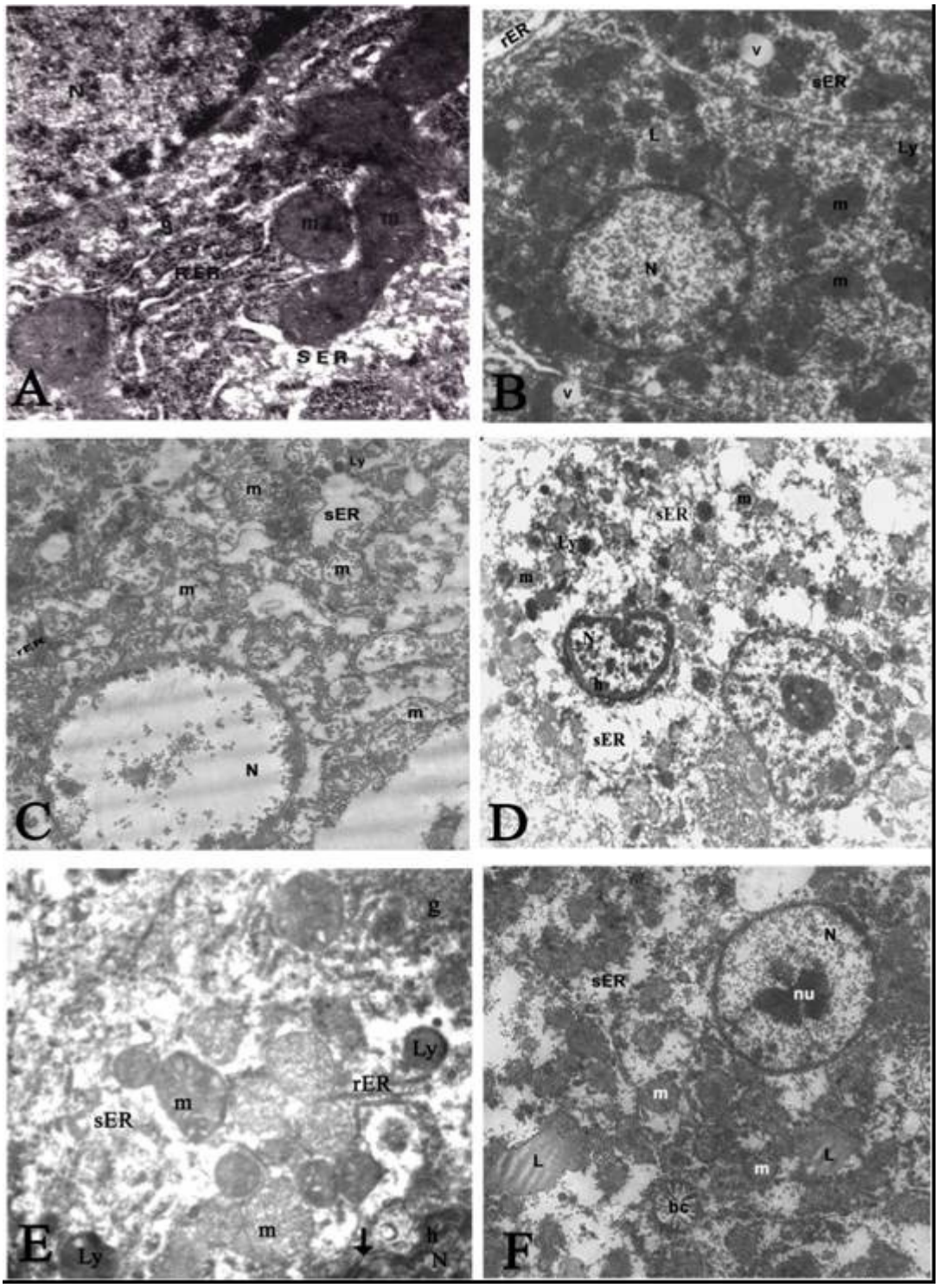

Plate 3. TEM of hepatocyte from A): control reveals part of the nucleus (N), rER, sER and glycogen granules (g). Notice the cristae of the mitochondria (m), (x6000). B): a rat received NS oil only showing pale nucleus (N), mitochondria (m), sER , rER, lysosomes (Ly) and lipid droplets (L) ,(x8000). From C to E are sections of rats from group III, C): showing markedly damaged mitochondria (m) and wide cisternae of sER, lysosomes (Ly) and rER. A pale nucleus (N) is seen, (x8000).D): two adjacent hepatocytes from a rat of group III shows dilated sER, mitochondria (m) and many darkly stained lysosomes (Ly). The nucleus (N) on the left side appears irregular, indented, and small with margination of chromatin (h). The nucleus on the right side appears more or less regular, (x 6000). E): Localized glycogen granules were observed in the cytoplasm. Notice the indentation of the nucleus with widening of nuclear space (arrow), (x6000). F): a rat of the group received VPA and NS oil showing the nucleus (N) with prominent nucleolus (nu), mitochondria (m), bile canaliculus (bc) sER, and lipid droplets (L), (x6000).

DISCUSSION

Sodium valporoate (VPA) is a simple fatty acid largely used as an anticonvulsant. This structure might enable VPA to interact with cell membranes, which may account in part for both its therapeutic and adverse effects. VPA is extensively metabolized by the liver via glucuronic acid conjugation, mitochondrial beta- and cytosolic omega oxidation to produce multiple metabolites. Some of which may be involved in its toxicity. As clinical usage of VPA increased, reports of its hepatotoxicity began to appear. This hepatotoxicity ranged from mild increased aminotransferase enzyme in 15 to 30 percent of patients to liver cell failure and death in some patients. The mechanism by which VPA caused hepatotoxicity was poorly understood (Reynolds et al., 1996; Baran et al., 2004; Lheureux and Hantson, 2009).

This study reinforced the previous results (Kimura et al., 1989; Siemes and Nau, 1991; Roma- Giannikou et al., 1999; Knapp et al., 2008; El-Gharieb et al., 2010). El-Gharieb et al., (2010) reported that administration of VPA caused significant elevation of liver enzymes. The clinical spectrum of VPA –associated hepatotoxicity ranged from slight increase in liver enzymes without clinical manifestations over reversible slight to severe liver dysfunction to fatal liver failure (Siemes and Nau, 1991). The elevation in liver enzymes was referred to liver cell injury (Knapp et al., 2008). On the other hand, Lee et al., 2009 reported that ALT and AST didn't change with oral administration of VPA. This might be due to different route and / or dose of drug administration.

The current study revealed that I.P. administration of toxic dose of VPA for seven consecutive days caused focal areas of liver cell degeneration which were accompanied with occasional hypereosinophilic hepatocytes , mononuclear cellular infiltration, prominent microvesicular steatosis (that referred to a variant form of hepatic fatty infiltration) without specific localization, remark cell lines of hepatocytes and Von Kupffer cells' enlargement. These findings were parallel to the results of others (Roma- Giannikou et al., 1999; Baran et al., 2004; Khan et al., 2005).

As the mechanism by which VPA causes hepatic damage is uncertain, hepatotoxicity was suspected to result from formation of toxic VPA metabolites (Jezequel et al., 1984; Siemes et al., 1993). A possible mechanism of VPA- induced hepatotoxicity was that it caused depression of free radical scavenging enzyme activities (Hamza and Amin, 2007). The cause of liver cell injury was due to decreased plasma and tissue carnitine (Knapp et al., 2008). Another cause for liver cell injury might be due to decreased activity of complex IV of the respiratory chain and /or depletion of hepatic pool of glutathione (Krahenbuhl et al., 1995). One of the first noticeable morphological changes in VPA- treated livers was the accumulation of cytoplasmic fat (Olson et al., 1986). This might be due to not only the accumulation of estrified VPA or its metabolites but also to lipids which were unable to be secreted due to interference with vesicular movement which might lead to fat accumulation. A second study mentioned that VPA interfered with the mitochondrial inner membrane. The loss of that membrane integrity might interfere with the release of calcium from internal stores. This calcium could be a contributor to the secretory problems caused by VPA either directly affecting the movement of the vesicles along microtubules or indirectly via a second messenger system so the cause of lipid accumulation was not only due to over production but also due to inability to be secreted at a rate to match the production (Bellringer et al., 1988**)**. Microvesicular steatosis which characterizes VPA-associated liver injury possibly occurs via interference with the process of fatty acid B-oxidation through reactive intermediates which inhibit key enzymes in B-oxidation cycle or by idiosyncrasy for production of toxic VPA metabolites (Cotariu and Zaidman , 1988; Hautekeete et al., 1990). This microvesicular steatosis was without specific localization which was parallel to the results of Baran et al., (2004). Also, the present study revealed increased acidophilia of some hepatocytes that was most probably due to acidophilic degeneration disclosed by light microscopy. A markedly damaged mitochondria plus marked proliferation and dilatation of sER that was surrounded by layers of rER were characteristic to areas of acidophilic degeneration (Oda et al., 1973).

By using PAS reaction, the present study revealed that VPA administration in toxic dose caused a decrease and heterogenous distribution of glycogen content of hepatocytes as confirmed by determination of area percent of PAS. This result was coincided with that reported by others (Baran et al., 2004; Beger et al., 2009). Beger and his colleagues (2009) explained the decreased glycogen content of hepatocytes after treatment with VPA could be due to altering activities of various enzymes included in glycogen synthesis as well as decreased glucose re-absorption and increased disposal via urine. While Granneman et al., (1984) mentioned that the major clearance route of VPA and its toxic metabolites is glucuronidation. Comprise of this metabolic route, directly or indirectly by situations in which glycogen is depleted. Glaumann et al., (1979) proved the uptake and degradation of glycogen by Kupffer cells. This might be a cause of glycogen depletion in liver cells and at the same time a cause for swelling of Kupffer cells. Another cause for Kupffer cell hypertrophy might be lipid deposition in their cytoplasm (Roth et al., 1996).

Ultrastructurally, the present study revealed that administration of VPA caused mitochondrial damage, apparently increased electron dense lysosomes, proliferation and dilatation of sER and lipid accumulation. The nuclei of some hepatocytes were pyknotic (small, irregular and dark) with chromatin margination. The same

findings were reported by other investigators (Bellringer et al., 1988; Kimura et al., 1989; Mesdjian et al., 1996). Mitochondrial damage might be proposed that VPA, by entering the fatty acid pathway, forms abnormal degradation products with subsequent sequestration of CO ASH leading to competition with endogenous substrate, impairment of fatty acid oxidation and structural damage of mitochondria (Lee et al., 2009). The nuclear changes could be degenerative changes. The appearance of many large electron dense lysosomes in the cytoplasm of hepatocytes of rats administered VPA in toxic dose could be explained by the accumulation of damaged mitochondria and so lysosomes were accumulated to perform autophagolysis. Dilatation of sER could be due to retention of unprocessed lipids within their cisternae and in this case sER was hypoactive. Hypertrophy and dilatation of sER was constant finding in lipophilic drugs, VPA is a fatty acid, as they metabolized in the liver (Kawata et al., 1982). Increased sER might be an adaptive response of the liver cell enabling it to metabolize the drug more rapidly than can the liver cell of an untreated rat.

Administration of VPA and NS (protective group) caused some improvement of the histological changes at both light and electron microscopic levels. At the light microscopic level hepatocytes revealed less microvesicular steatosis and increased PAS reaction. Ultrastructurally, hepatocytes revealed less damaged mitochondria, normal euchromatic nuclei and less dilated sER. Hepatocytes didn't return completely to the control pattern this might be due to short duration of NS oil administration.

CONCLUSION

It could be concluded that; 1) hepatotoxicity must be considered as a possible early side effect of VPA treatment and this could be monitored by estimation of liver enzymes 2) Dietary supplement with NS oil represents a potential therapeutic agent in reducing VPA-induced hepatotoxicity. 3) Further controlled, randomized and probably multitrials are required to better delineate the prophylactic role of NS oil and optimal regimen of administration in the management of VPA hepatotoxicity.

REFERENCES

Ali B, Blunden G (2003). Pharmacological and toxicological properties of Nigella sativa. Phytother Res. 17(4):299-305.

Baran O, Yildirim A, Akkus M (2004). The protective role of folic acid and vitamin E against toxical effects of valproic acid on liver tissue during period of gestation. Dicle. Tup. Dergisi. 31(4):17-23.

Barthel G (1998). Hepatotoxicity of Valproate in isolated rat hepatocytes: influence of prooxidative agents and starvation. Doctoral thesis, Date of defense: 2000-12-14, Static URL:http://www.diss.fuberlin.de/diss/receive/FUDISS_thesis_000000000072.

Beger R, Hansen D, Schnackenberg L, Cross B, Fatollahi J ,Lagunero F, Sarnyai Z, Boros L (2009). Single valproic acid treatment inhibits glycogen and RNA ribose turnover while disrupting glucose –derived cholesterol synthesis in liver as revealed by the (U-C(6)-d- glucose tracer in mice. Metabolomics; 5(3):336-345.

Bellringer M, Rahman K, Coleman R (1988). Sodium valproate inhibits the movement of secretory vesicles in rat hepatocytes. Biochem. J. 243(2):513-9.

Bykov I, Mal'tsev A, Gurinovich V, Nefedov L (2004). Biochemical basis of valproic acid toxicity: role of oxidative stress and effects of L-carnitine. Biomed. Khim. 50 (4):384-389.

Cotariu D, Zaidman J (1988). Valproic acid and the liver. Clin. Chem. 34(5):890-7. Clin. Chem. 34(5):890-897.

El-Gharieb M, El-Masry T, Emara A, Hashem M (2010). Potential hepatoprotective effects of vitamin E and Nigella sativa oil on hepatotoxicity induced by chronic exposure to malathion in human and male albino rats. Toxicol. and Environ. Chem. J. 92(2):391-407.

Glaumann H, Fredzell J, Jubner A, Ericsson J (1979). Uptake and degradation of glycogen by Kupffer cells. Exp. and Molecular Pathol.31(1):70-80.

Granneman G, Wang S, Kesterson J, Machinist J (1984). The hepatotoxicity of valproic acid and its metabolites in rats. II. Intermediary and valproic acid metabolism. Hepatol. 4(6):1153-8.

Hamza A, Amin A (2007). Apium graveolens modulates sodium valproate-induced reproductive toxicity in rats. J. Exp. Zool. A Ecol. Genet. Physiol. 1: 307(4) 199-206.

Hautekeete M, Degott C and Benhamou J (1990). Microvesicular steatosis of the liver. Acta. Clin. Belg. 45(5):311-326.

Jezequel A, Bonazzi P, Novelli G, Venturini C, Orlandi F (1984). Early structural and functional changes in the liver of rats treated with a single dose of valproic acid. Hepatol. 4(6):1159-66.

Kanter M, Akpolat M, Aktas C (2009). Protective effects of the volatile oil of Nigella sativa seeds on beta cell damage in streptozotocin-induced diabetic rats: a light and electron microscopy study. J. Mol. Histol. 40(5-6):379-85. doi: 10.1007/s10735-009-9251-0. Epub 2010 Jan 5.

Kawata S, Seki K, Shinji Y, Tarui S, Sugiyama T, Yamano T (1982). Hypertrophic and hypoactive smooth endoplasmic reticulum in hepatocytes uremic patients. A morphometric and biochemical study. Gastroenterol. Jpn. 17(3):192-200.

Khan S, Shakoor K, Jan M, Khattak A, Shah S (2005). Study of histopathologic changes in the liver of albino rats, induced by toxic dose of valproic acid. Gomal. J. Med. Sci. 3(1):15-18.

Kimura A, Yoshida I, Yamashita F, Kuriya N, Yamamoto M, Nagayama K (1989). The occurrence of intramitochondrial Ca2+ granules in valproate-induced liver injury. J. pediatr. Gastroenterol. and Nutr. 8(1):13-18.

Klee S, Johanssen S, Ungemach F (2000). Evidence for a trigger function of valproic acid in xenobiotic-induced hepatotoxicity. Pharmacol. Toxicol. 87 (2):89-95.

Knapp A, Todesco L, Beier K, Terracciano L, Sägesser H, Reichen J, Krähenbühl S (2008). Toxicity of valproic acid in mice with decreased plasma and tissue carnitine stores. J. Pharmacol. Exp. Ther. 324(2):568-75.

Kondo T, Kaneko S, Otani K, Ishida M, Hirano T, Fukushima Y, Muranaka H, Koide N, Yokoyama M (1992). Association between risk factors for valproate hepatotoxicity and altered valproate metabolism. Epilepsia; 33(1):172-7.

Krahenbuhl S, Mang G, Kupferschmid H, Meier PJ and Krause M (1995). Plasma and hepatic carnitine and coenzyme A pool in a patient with fatal valproate induced hepatotoxicity.Gut. 37(1):140-143.

Lee M, Lee Y, Kim B, Shin K, Chung B, Baek D, Jung B (2009). The relationship between glucuronide conjugate levels and hepatotoxicity after oral administration of valproic acid. Arch. Pharm. Res. 32(7):1029-1035.

Lheureux P, Hantson P (2009). Carnitine in the treatment of valproic acid-induced toxicity. Clin. Toxicol. (phila). 47(2):101-111.

Mesdjian E, Zamora A, Montet A, Bonneton J, Guitaoui M, Genton P, Montet J (1996). Ursodeoxycholate improves hepatobiliary dysfunction induced by valproate-carbamazepine treatment in the rat. Life Sci. 59(13):1069-79.

Nishimura T, Sakai M, Yonezawa H. (2000). Effects of valproic acid on fertility and reproductive organs in male rats. J. Toxicol. Sci. 25 (2):85-93.

Oda M, Funatsu K, Okazaki I, Kamegaya K, Tsuchiya M, Sambe K (1973). Morphogenesis of acidophilic bodies in experimental D-galactoisamine induced hepatitis. Gastroenterol. Japonica. 8(1):79.

Olson M, Handler J, Thurman R (1986). Mol. Pharmacol. 30:520.

Reynolds J, Parfitt K, Parsons A, Sweetman S (1996). Folic acid. Martindale: the extra pharmacopoeia 31th ed. London: the royal pharmaceutical society. 1361-1362.

Roma- Giannikou, Syriopoulou V, Kairis M, Pangali A, Sarafidou J, Constanto A (1999). In vivo effect of Sodium valproate on mouse liver. Cell Mol. Life Sci. 56:363-369.

Roth B, Fkelund M, Fan B, Hägerstrand I, Nilsson-Ehle P (1996). Lipid deposition in Kupffer cells after parenteral fat nutrition in rats: a biochemical and ultrastructural study. Intensive Care Med. 22(11):1224-1231.

Sharma N, Ahirwar D, Jhade D, Gupta S (2009). Medical and pharmacological potential of Nigella sativa. A review. Ethnobotanical Rev. 13:946-955.

Siemes H, Nau H (1991). Valproate –associated hepatotoxicity-pathogenesis, clinical aspects, therapy and prevention. Klin Padiatr. 203(6):411.9 (abstract)

Siemes H, Nau H, Schultze K, Wittfoht W, Drews E, Penzien J, Seidel U (1993). Valproate (VPA) metabolites in various clinical conditions of probable VPA- associated hepatotoxicity. Epilepsia 34 (2):332-46.