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Effect of (+)-Usnic Acid as a Fat Burner on the Rat Hepatocyte; Correlated Histological and Biochemical *in vivo* Study

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Abstract: Liver injury from dietary supplement mimicking other liver diseases is increasingly recognized. Usnic acid has been marketed as weight-loss aid many years ago in spite its chronic or subchronic effects on animal were not studied. To assess the effect of Usnic acid on the structure of the hepatocytes of male rats and correlate this effect to those changes detected if any in the biochemical study. Forty adult male rats were divided into four groups ten animals each (n = 10); control received standard diet, G1 received 1% carboxymethyl cellulose water solution, G2 received 100 mg usnic acid kg^{-1} and G3 received 300 mg usnic acid/ kg, 5 days for 7 weeks using gastric gavages. Serum glucose, liver functions, lipid profile, lipase, leptin and Insulin were estimated. Liver was processed for electron microscope studies and results were analyzed using SPSS. The liver index was increased significantly in high-dose Usnic acid compared to the control. Hepatocytes showed an increase in lipid droplets, swollen mitochondria, fragmented rough endoplasmic reticulum cisterns, abundant smooth endoplasmic reticulum and focal damage of hepatocyte membranes near bile canaliculi, all these changes were dose dependent. There was significant increase in total protein, albumin and total bilirubin in group received low-dose of Usnic acid. Glucose, magnesium, total protein, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and total bilirubin were significantly increased in group received Usnic acid at high-dose. Serum cholesterol and high density lipoprotein were significantly increased in all treated groups while triglycerides were slightly increased.

Key words: Biochemical, hepatocytes, histological, *in vitro*, rat, usnic acid

INTRODUCTION

Usnic Acid (UA) is a complex polycyclic chemical compound produced naturally as a secondary metabolite by certain lichen species; it was first isolated by Knop in 1844 (Frankos, 2005). Usnea species (Usneaceae) is the main source but it is found in other genera of lichens (Ingolfsdottir, 2002).

The prevalence of obesity is increasing globally, particularly among children and adolescents and nearly half a billion of the world's population (estimated to be 6.5 billions) is now considered to be overweight or obese (Rossner *et al*., 2000; Hameed *et al*., 2002; El-Helaly *et al*., 2009; Suleiman *et al*., 2009; Veghari, 2011). Prevention of obesity should be the primary target but it is also important to develop strategies to treat those already affected with obesity (Rossner *et al*., 2000). Obese patients and overweight individuals who cannot achieve sufficient weight loss through lifestyle and behavioral modifications are urged to use one of several anti-obesity agents (including drugs, nutritional supplements and herbal dietary supplements) to control their body weight. In recent years, UA and its salt form, sodium usniate have been marketed in the US as an ingredient in dietary supplement products (mostly with claims as weight-loss aids, though some as antimicrobial agents) (Frankos, 2005). The actual mechanism of UA as a weight loss agent is still unclear however, it was reported that it acts through raising the body's metabolic rate (Durazo *et al*., 2004). Ingolfsdottir (2002) reported that UA has been promoted for use in weight reduction because of its thermogenic effect.

Liver injury from dietary supplement mimicking other liver diseases is increasingly recognized. Significant liver injury was reported after intake of herbalife and hydroxycut products, tea extracts from *Camellia sinensis*, products containing Usnic acid and high contents of

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Vitamin A, anabolic steroids and others. No uniform pattern of hepatotoxicity has been identified and severity may range from asymptomatic elevations of serum liver enzymes to hepatic failure and death (Stickel *et al*., 2011). Gunawan and Kaplowitz (2004) reported that herbal and natural supplements containing UA like lipokinetix and kambala tea were causing hepatotoxicity with increasing frequency as patients turn more and more to alternative medicine. Yellapu in March, 2011 added a recent case to the increasing number of reports of hepatotoxicity associated with dietary supplements containing usnic acid (Yellapu *et al*., 2011). All these and much more other studies depended on the biochemical tests to documents the hepatotoxic effect of UA. Scarce studies done to investigate the effect of US on the structure of the liver cell but unfortunately they relayed only on routine histological examination and some of them tend did so, *in vitro*. Chronic or subchronic effects of UA in animal studies were also not available in the literature.

Because of this, the this study was conducted to assess the effect of UA on the structure of the hepatocytes of male rats and correlate this effect to those changes detected in the biochemical study in addition to investigate for any possible potential adverse effect of UA at two different doses for long periods (7 weeks). Researchers also aimed at investigating the effect of UA on the fat cells of the peripheral adipose tissue using morphometric study.

MATERIALS AND METHODS

Air-dried pieces (2500 g) of *Usnea articulata* were used for extraction and isolation of UA. *Usnea articulata* (Fig. 1A) was collected from Asir region of Saudi Arabia as it was growing on juniper trees (*Juniperus procera*) in Al-Sawdah. It was identified by Abu-Zinada *et al*. (1986). Usnic acid was isolated in the form of long yellowish crystalline prisms, purified by recrystallization in ethanol, weighed and kept in stoppered vials at room temperature. It was dissolved in 1% Carboxymethyl Cellulose (CMC) water solution (low viscosity vehicle) according to Odabasoglu *et al*. (2006).

Determination of UA in rat plasma by High-Performance Liquid Chromatography (HPLC) was carried out according to Venkataramana and Krishna (1992) with some modifications. All solvents were of HPLC grade, Merck, Darmstadt, Germany. All other materials were of analytical grade. (+) Usnic acid used for this purpose was purchased from Aldrich (Germany).

Forty adult male Sprague-Dawley (SD) rats obtained from the Animal House Unit in King Fahed Medical Research Center (KFMRC) were used in this study. The

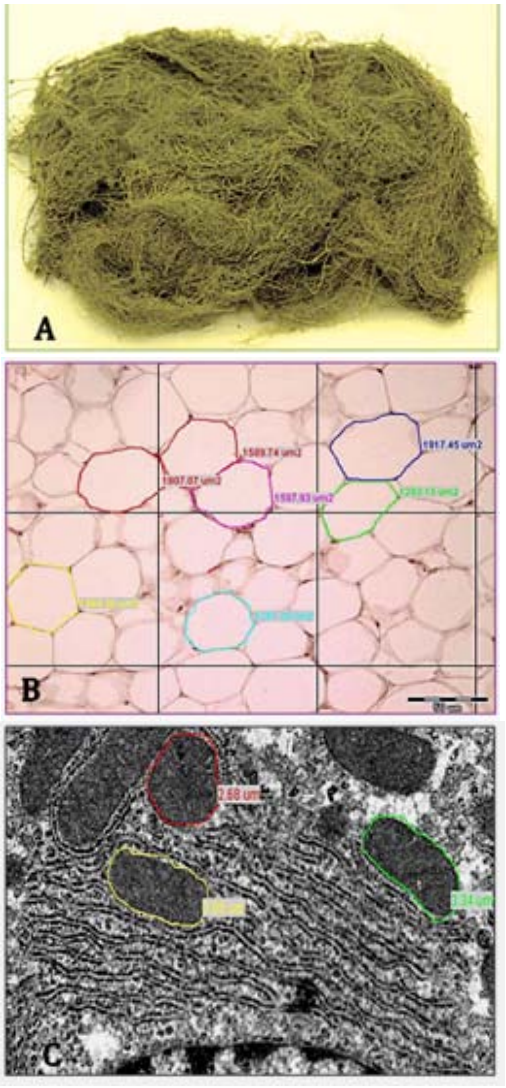

Fig. 1: A) Photograph showing *Usnea articulata* which collected from Al-Sawdah mountain, the Asir region in Saudi Arabia; B) Showing the method of morphometric measurement of adipocytes outlines (area) using software Image-Pro plus Version 6 analyzer and C) Showing the method of morphometric measurement of mitochondria of hepatocytes

animals were housed individually in plastic cages at 20°C and 60% humidity with 12 h dark-light cycle and maintained on a commercial pellet *ad libitum*. All animals received care according to methods approved under institutional guidelines for the care and use of laboratory animals in KFMRC.

The animals were divided into four groups; G0 (n = 10) served as control group and received a standard

laboratory diet, G1 (n = 10) received 1 mL of 1% CMC water solution, G2 (n = 10) received low dose of UA (100 mg kg^{-1} suspended in 1% CMC) and G3 that received high dose of UA (300 mg kg^{-1} suspended in 1% CMC) in an equivalent volume. The above doses were given 5 days $week^{-1}$ for 7 weeks using gastric gavages (feeding tube). Body weight and length (using a portable electronic digital scale) were recorded weekly. Body Mass Index (BMI) was estimated for all animal groups by dividing the body weight (g) by the square of the height in centimeters (Brandt *et al*., 2002).

At the end of experiment, rats of all groups were anesthetized with ether. Blood was collected after overnight fasting between 7:00 and 9:00 am via orbital puncture (Waynforth, 1980) centrifuged and stored at -70°C for biochemical studies. Levels of glucose, magnesium, liver functions, lipid profile and serum lipase were estimated in blood serum. All parameters were determined by enzymatic methods on an automated chemical analyzer (Dimension Rxl Clinical Chemistry System, USA) using automated kits. All the reagents were obtained from Dade Behring Inc. USA. A Leptin ELISA kit (Cat. # EZRL-83K) was purchased from Linco Research, Inc. USA for detection of leptin. An Ultrasensitive Insulin ELISA kit (Cat. # EIA-2943) was purchased from DRG International Inc., USA for detection of insulin concentration.

All animals were sacrificed by cervical dislocation, dissected and the perirenal adipose tissues and livers were removed and weighed. Liver and perirenal adipose tissues indices [(liver or adipose tissue weight/body weight) ×100] were calculated (Zeng *et al*., 2008). According to Ross *et al*. (1989) tissues were fixed in 10% neutral buffered formalin and further processed for light microscopic study. Paraffin sections (5 μm thick) were stained with Hematoxylin and Eosin (H and E) and examined by Olympus BX-51 light microscopy (Japan).

For electron microscope studies, liver slices (1 mm^3) were fixed in Trump's fixative from 0.5-1 h, rinsed twice in distilled water for 15 min each and post-fixed in osmium tetroxide from 0.5-1 h before further processing to semithin (0.5-1 μm) and ultrathin (700-900 E) sections. Semithin sections were stained with toluidine blue. Ultrathin sections were stained by uranyl acetate and lead citrate according to Ross *et al*. (1989). Examination and photography were done by Philip CM 100 electron microscopy (Netherlands).

Morphometric studies were done using the Software Image-Pro plus Version 6 analyzer. Three slides from 3 different animals in each group (5-6 fields/slide) were scanned in each sample. Adipocytes number and area from H and E-stained paraffin sections (Fig. 1B) as well the number of hepatocyte mitochondria and their circumference from electron micrographs (Fig. 1C) were measured. The scale bars of all photographs were standardized depending on actual magnification.

Statistical analysis of the data was performed using the Statistical Package for Social Science (SPSS 12 for Windows) program. The data are expressed as means±Standard Error (SE). Comparison of variables between groups was performed using one-way Analysis of Variance (ANOVA) and Student's t-test as appropriate. The Least Significance Difference test (LSD) was employed to compare means for pairs of groups. All analyses with a $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Effect of UA on body weight: In the 1st week of the experiment, there was an insignificant increase in percentage body weight gain between control (10.73±0.67%) and group received CMC (11.42±0.95%) while there was an insignificant decrease in percentage body weight gain (11.26±0.97) in the high-dose UA relative to the group received CMC. However, from the 2nd week to the end of the experiment, there were insignificant decreases in percentage body weight gain in all groups receiving UA compared with the group received CMC, $p>0.05$ (Table 1).

Effect of UA on BMI: The final BMI (at 7 week from the beginning of the experiment) was found to be insignificantly decreased ($p>0.05$) in high-dose (0.1726±0.01). compared to the BMI change in the group received CMC (0.1865±0.003). The BMI gain showed significant decrease in high-dose UA while no change was observed in low-dose UA compared to the control (Table 2).

Effect of UA on liver and perirenal adipose tissue indices: Usnic acid was found to have no effect on either liver or perirenal adipose tissue indices in low-dose UA compared to the group received CMC. In contrast, the liver index

Table 1: Effect of UA on body weight gain

	Body weight gain (%) (Mean±SE)						
UA dose (mg kg^{-1})	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Control	10.73±0.67	25.60±1.78	43.12±6.10	45.1±5.100	57.10±2.12	62.00±7.100	71.00±5.90
1% CMC	11.42±0.95	26.04±1.84	44.34±5.70	49.61±3.17	58.67±3.60	64.82±4.250	70.24±4.50
100 mg UA kg^{-1}	11.57±0.73	25.41±2.09	37.11±3.07	46.38±3.65	53.20±4.77	59.90±5.291	65.17±5.58
300 mg UA kg^{-1}	11.26±0.97	24.62±1.27	35.68±1.28	44.55±2.36	50.00±2.07	53.58±2.620	60.98±2.84

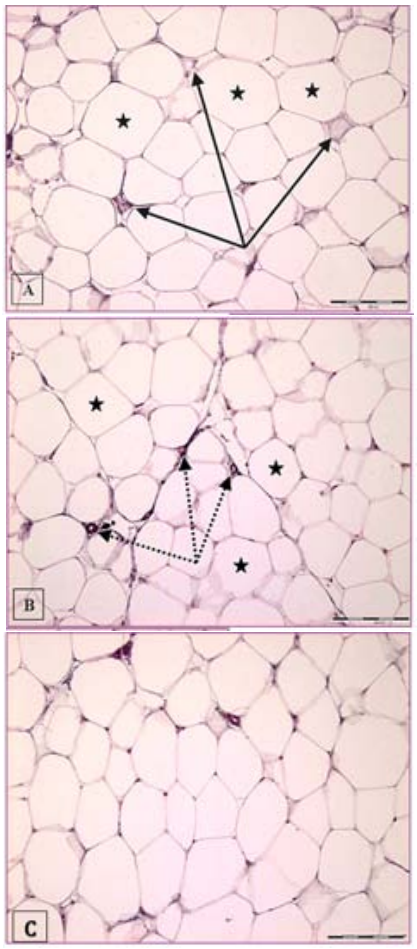

Fig. 2: A, B) Light photographs showing adipocytes from different regions of perirenal adipose tissue of control animal showing adipocytes clusters (stars) separated by thin connective tissue septa (black arrows) and thin walled capillaries (dashed arrows) and C) Adipocytes of rat perirenal adipose tissue after administration of CMC showing normally shaped adipocytes as those of the control (H and E X200)

Table 2: Effect of UA on BMI of rats[1]

UA dose (mg kg^{-1})	Initial BMI (g cm^{-2})	Final BMI (g cm^{-2})	BMI gain (%)
Control	0.1600±0.002	0.1860±0.003	19.01±4.21
1% CMC	0.1564±0.001	0.1895±0.003	21.19±2.06
100 mg UA kg^{-1}	0.1562±0.003	0.1897±0.006	21.41±2.33
300 mg UA kg^{-1}	0.1562±0.004	0.1726±0.010	10.33±5.34*

[1]BMI = Body Mass Index; p*<0.05; Mean±SE shows ±values

Table 3: Effect of UA on liver and perirenal adipose tissue indices[1]

UA dose (mg kg^{-1})	Liver index (%)	Perirenal adipose tissue index (%)
Control	3.00±0.06	1.11±0.12
1% CMC	3.03±0.08	1.18±0.21
100 mg UA kg^{-1}	2.92±0.09	1.29±0.23
300 mg UA kg^{-1}	3.39±0.10*	0.87±0.15

[1] *p<0.05; Mean±SE shows ±values

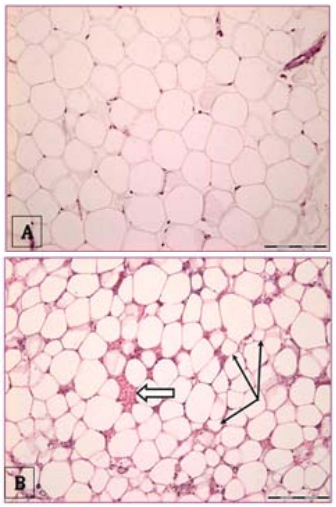

Fig. 3: A) Adipocytes of rat perirenal adipose tissue after administration of UA (100 mg kg^{-1}). The cells look smaller than in the control group. There are no signs of cell necrosis, fibrosis or vascular changes and B) Adipocytes after administration of UA (300 mg kg^{-1}) showing decrease in cell size of most adipocytes (black arrows). Notice the marked capillary congestion and associated intercellular hemorrhage (white arrow) (H and E X200)

was increased significantly in high-dose UA compared to the group received CMC ($p<0.05$) and the perirenal adipose tissue index was insignificantly decreased (Table 3).

Effect of UA on the structure of perirenal adipose tissue: The perirenal fat mass, of the control group was found to consist mainly of white-type adipocytes clustered in lobules and lobes delineated by thin connective tissue septa. Thin walled blood capillaries were seen among the adipocytes (Fig. 2A and B). Adipocytes of perirenal adipose tissue of rats of G1 that received 1% CMC did not differ from those of the control (Fig. 2C).

After administration of low dose UA (100 mg kg^{-1}) for 7 weeks adipocytes looked smaller than in the control. No signs of cell necrosis, fibrosis or vascular changes were noted (Fig. 3A). On the other hand, a decrease in cell size of most adipocytes was observed following administration of high dose of UA (300 mg kg^{-1}) for 7 weeks. Marked capillary congestion and associated intercellular hemorrhage were also observed (Fig. 3B). Adipocytes at the low dose of UA showed a slight decrease in cell area with concomitant increase in cell number per field (40,000 μm^2) as confirmed by statistical morphometric study ($p>0.05$). In the high dose UA group, the adipocyte area was insignificantly decreased while the cell number showed an insignificant increase ($p>0.05$) (Table 1).

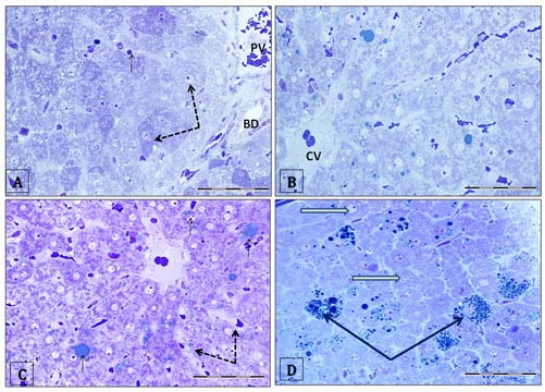

Fig. 4: A) Light photographs of liver of control animal showing light and dark hepatocytes (interrupted arrow) that appear intact with few lipid droplets (arrow). Portal Vein (PV) and Bile Duct (BD) can be seen; B) Liver of rats after administration of CMC appears like the control ones. The Central Vein (CV); C) Liver of rats after administration of UA (100 mg kg^{-1}) showing many hepatocytes with many lipid droples (arrow). Blood sinusoids appear slightly dilated (interrupted arrow) and D) Liver of rats after administration of UA (300 mg kg^{-1}) showing many hepatocytes with numerous accumulated lipid droplets and dense particles (black arrows). Most hepatocytes possess central vesicular nuclei (white arrows) (Toluidine blue X400)

Table 4: Effect of UA on adipocytes (number and area/40.000 μm^2) of rat perirenal adipose tissue

Groups	Adipocyte number	Adipocyte area (μm^2)
Control	46.00±3.30	1180.18±75.010
1% CMC	48.00±3.27	1186.11±76.980
100 mg UA kg^{-1}	51.5±3.890	1136.97±89.300
300 mg UA kg^{-1}	61.94±6.33	1015.81±101.58

Mean±SE shows the ±values

Table 5: Effect of UA on mitochondria (number and circumference/10,000 μm^2) of adult male rat hepatocytes

Groups	Mitochondrial number	Mitochondrial circumference (μm)
Control	27.95±2.08	3.34±0.16
1% CMC	28.15±2.87	3.54±0.14
100 mg UA kg^{-1}	26.71±1.62	3.79±0.12
300 mg UA kg^{-1}	24.70±1.83	3.58±0.14

Mean±SE shows the ±values

Effect of UA on the structure of hepatocytes: Semithin section of liver of both control and experimental animals that received 1% CMC showed normal, intact hepatocytes (Fig. 4A and B). Hepatocytes of the animals received low dose of UA accumulated many lipid droplets while those of the animals received high dose of UA accumulated numerous lipid droplets (Fig. 4C and D) (Table 4 and 5). Examination of liver of control rats using

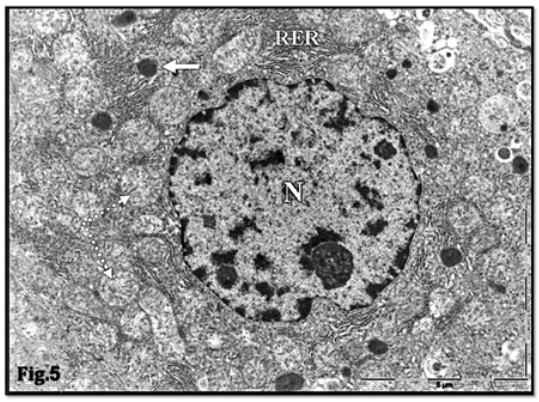

Fig. 5: Transmission Electron Micrograph (TEM) of hepatocyte of control rat showing central Nucleus (N) with small amount of peripheral chromatin and prominent nucleolus. The cytoplasm contains numerous mitochondria with well defined cristae (dotted arrows), a few dense bodies (white arrow) and Rough Endoplasmic Reticulum (RER). X5800

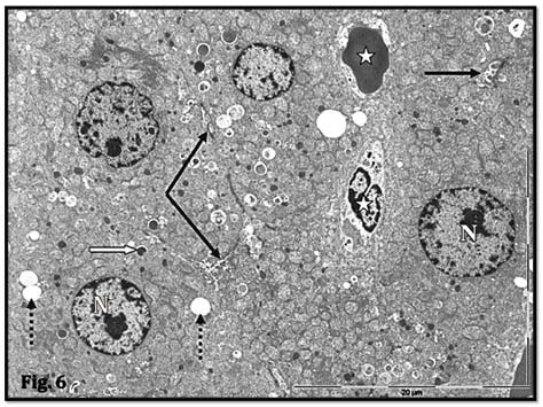

Fig. 6: TEM of rat hepatocytes from group received 1% CMC. They appear like those received 1% saline. It show the relationship of hepatocyte cords to hepatic sinusoids containing blood cells (stars). Bile canaliculi between adjacent cells can also be observed (black arrows). Hepatocytes show vesicular Nuclei (N), numerous mitochondria, a few lipid droplets (dashed arrows) and lysosomal structures (white arrow). X1950

EM showed intact hepatocytes. They were polyhedral in shape with one or two rounded vesicular nuclei. The cytoplasm showed rounded and elongated mitochondria with moderately electron-dense matrix and intact cristae as well as Rough Endoplasmic Reticulum (RER) with intact clusters (Fig. 5). Hepatocytes of rats received 1% CMC appeared like those of the control (Fig. 6-8). Administration of UA resulted in ultrastructural changes

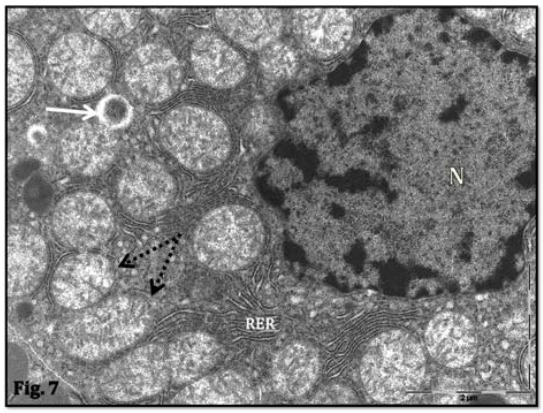

Fig. 7: TEM of hepatocyte from group received CMC showing central Nucleus (N) with small amount of peripheral chromatin. The cytoplasm contains numerous mitochondria with well defined cristae (dotted arrows), a few dense bodies (white arrow) and Rough Endoplasmic Reticulum (RER). X10000

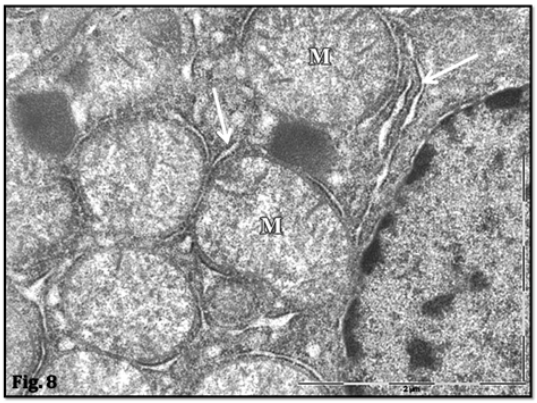

Fig. 8: TEM of rat hepatocyte from group received CMC showing numerous Mitochondria (M) with well-defined cristae and some RER cisterns (white arrows). X19000

in the rat hepatocytes. In the low-dose group, the hepatocytes showed an increase in lipid droplets and focal damage of hepatocyte membranes near bile canaliculi (Fig. 9).

Most hepatocytes showed swollen mitochondria with electron lucent matrix and preserved cristae. Fragmentation of RER cisterns was also observed (Fig. 10 and 11).

In the high-dose group, the hepatocytes showed marked changes. There was focal loss of membrane integrity near bile canaliculi or at the sinusoidal surface in some hepatocytes (Fig. 12). Higher magnifications of hepatocytes showed swollen mitochondria with electron-

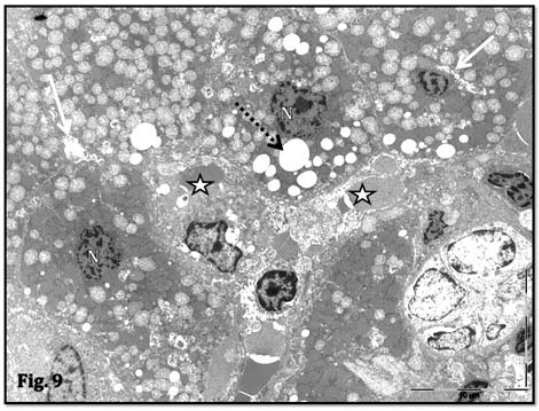

Fig. 9: TEM of rat hepatocytes from UA low-dose group (100 mg kg^{-1}) showing some hepatocytes near blood sinusoids (star). Hepatocytes show irregular Nuclei (N), numerous mitochondria, numerus lipid droplets (dashed arrows). Slight damage of hepatocyte membranes near bile canaliculi is noticed (white arrows). X1950

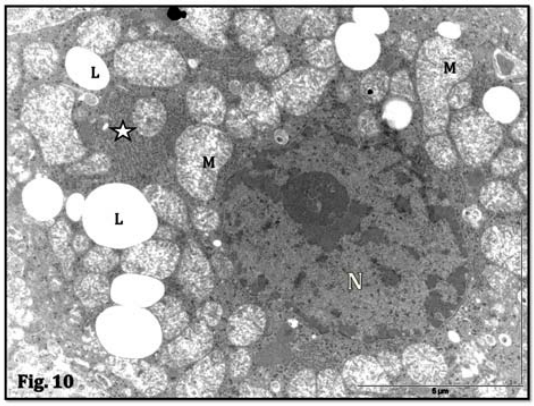

Fig. 10: TEM of hepatocyte from UA low-dose group (100 mg kg^{-1}) showing an increase in cytoplasmic and Nuclear (N) density and fragmented RER (star). The Mitochondria (M) appear swollen and cristae are still preserved. The cytoplasm shows lipid droplets of various sizes (L). X5800

dense matrix and no apparent cristae. More fragmented RER and abundant Smooth Endoplasmic Reticulum (SER) were also observed in most hepatocytes (Fig. 13-15). Administration of UA resulted in an insignificant decrease ($p>0.05$) in mitochondria number/10^4 μm^2 compared to the group received CMC and an insignificant increase ($p>0.05$) in mitochondrial circumference in all group received UA for 7 weeks (Table 2).

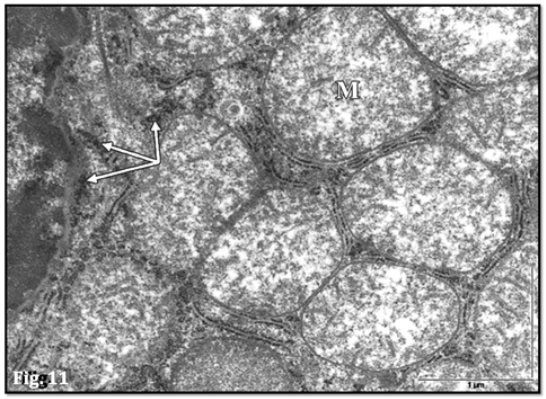

Fig. 11: TEM of rat hepatocyte from low-dose UA-treated group (100 mg kg^{-1}). Notice the swollen Mitochondria (M) with electron-lucent matrix and cristae. Also, notice the fragmented RER (white arrows). X19000

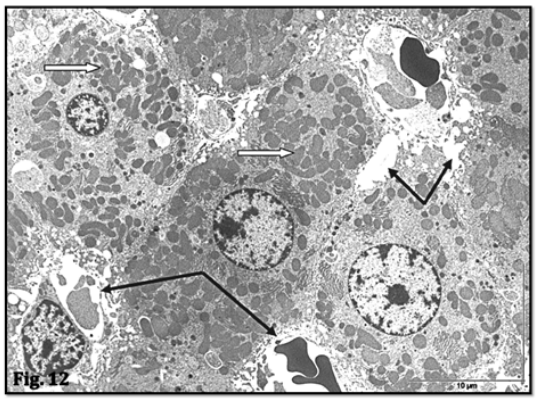

Fig. 12: TEM of rat hepatocytes from UA high-dose group (300 mg kg^{-1}) showing focal damage to hepatocyte membranes near blood sinusoids (black arrows). Notice the increase in density of the mitochondrial matrix in some hepatocytes (white arrows). X1950

Effect of UA on rat serum composition: There were significant increases in TP, ALB and TBIL ($p<0.05$) in low-dose UA group compared to the control. However, there were insignificant differences in the other liver function tests ($p>0.05$). In animals receiving UA at high-dose, GLU, MG, TP, ALT and TBIL were increased significantly whereas AST was decreased ($p<0.05$). The other liver function tests showed insignificant differences ($p>0.05$) (Table 6).

Effect of UA on rat serum lipids: Serum CHOL and HDL were increased significantly in all groups received UA ($p<0.05$) while the TG level was insignificantly increased ($p>0.05$). Serum LDL level showed an insignificant increase in low-dose UA group ($p>0.05$) and significantly ($p<0.05$) increased in high dose UA group compared to the group received CMC (Table 7).

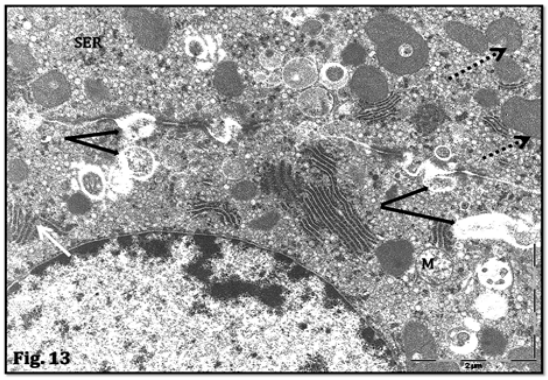

Fig. 13: TEM of rat hepatocytes from UA high-dose group (300 mg kg^{-1}) showing focal loss of membrane integrity near bile canaliculi (black arrows). Fragmented RER (white arrow) and abundant Smooth Endoplasmic Reticulum (SER) are seen. Notice, some mitochondria have electron dense matrix (dotted arrow) while some appear swollen (M) X10000

Table 6: Effect of UA on glucose level and liver functions[3]

UA dose (mg kg^{-1})	Control	Mean±SE (1% CMC)	Mean±SE (100 mg UA kg^{-1})	Mean±SE (300 mg UA kg^{-1})
Serum parameters				
GLU (mmol L^{-1})	6.80±0.270	6.66±0.340	7.40±0.430	9.00±0.600*
Mg (mmol L^{-1})	1.04±0.030	1.01±0.040	1.07±0.040	1.34±0.050*
TP (g L^{-1})	64.20±0.900	62.40±0.650	65.80±0.840*	66.88±3.000*
ALB (g L^{-1})	12.80±0.180	13.80±0.200	14.90±0.230*	14.13±0.640
ALP (µ L^{-1})	129.00±8.790	125.70±7.030	115.10±5.040	117.00±11.16
AST (µ L^{-1})	91.80±3.200	89.30±3.360	86.30±3.100	78.00±1.360*
ALT (µ L^{-1})	55.60±3.010	54.50±2.330	57.50±1.920	74.25±6.070*
GGT (µ L^{-1})	2.11±0.510	2.22±0.280	2.00±0.410	2.71±0.570
TBIL (µmol L^{-1})	2.30±0.150	2.20±0.130	3.00±0.150*	5.00±0.500*
LDH (µ L^{-1})	672.30±53.89	676.90±84.78	599.30±63.27	340.88±39.23*

[3]UA = Usnic Acid; GLU = Glucose; Mg = Magnesium; TP = Total Protein; ALB = Albumin; ALP = Alkaline Phosphatase; AST = Aspartate Aminotransferase; ALT = Alanine aminotransferase; GGT = G-Glutamyl transferase; TBIL = Total Bilirubin; LDH = Lactate Dehydrogenase; ±values are shown as Mean±SD; *$p<0.05$

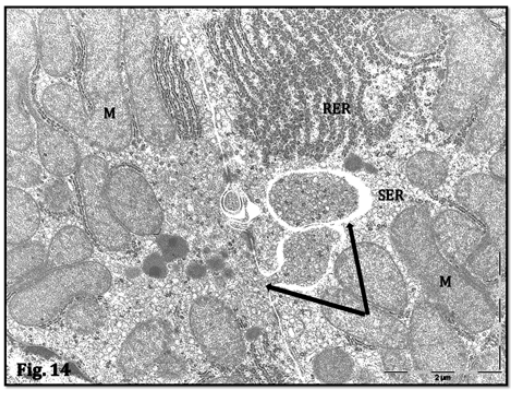

Fig. 14: TEM of rat hepatocytes from UA high-dose group (300 mg kg^{-1}) showing abundant fragmented RER and Smooth Endoplasmic Reticulum (SER) most of mitochondria have electron dense Matrix (M). Notice, focal loss of membrane integrity near bile canaliculi (black arrows) are seen. X10000

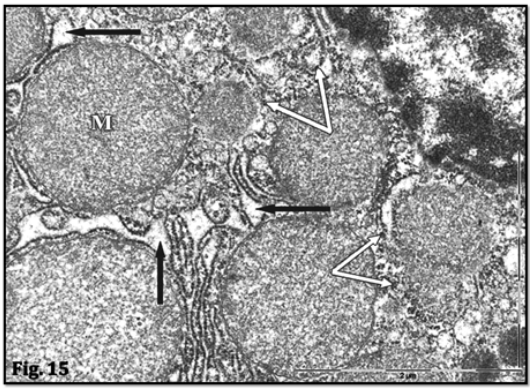

Fig. 15: TEM of rat hepatocyte from high-dose UA-treated group (300 mg kg^{-1}). Notice the swollen Mitochondria (M) with ill-defined cristae. Also, see the dilated (black arrows) or fragmented RER (white arrows). X19000

Effect of UA on serum levels of lipase, leptin and insulin: Table 8 shows an insignificant increase ($p>0.05$) in serum lipase level at low dose of UA whereas it increased significantly ($p<0.05$) in high-dose UA group compared to the group received CMC. Although, the leptin level was decreased in groups received UA, this decrease was insignificant ($p>0.05$) in comparison to the group received CMC (Table 8). The insulin serum level showed insignificant decreases ($p>0.05$) in the experimental groups compared to the control (Table 8).

Several commercial medications such as toothpaste, mouthwash, deodorants, creams, antibiotic ointments and sunscreen products are known to contain

Table 7: Effect of UA on serum lipids[1]

UA dose (mg kg^{-1})	TG	CHOL	LDL	HDL
	----------mmol L^{-1}----------			
Control	0.65±0.11	1.73±0.08	0.30±0.02	0.51±0.02
1% CMC	0.62±0.10	1.76±0.07	0.27±0.02	0.53±0.01
100 mg UA kg^{-1}	0.77±0.12	2.11±0.10*	0.31±0.02	0.64±0.03*
300 mg UA kg^{-1}	0.66±0.07	2.62±0.14*	0.39±0.03*	0.79±0.05*

[1]UA = Usnic Acid; TG = Triglycerides; CHOL = Cholesterol; LDL = Low Density Lipoprotein; HDL = High Density Lipoprotein; *$p<0.05$; ±values are shown as Mean±SD

Table 8: Effect of UA on serum lipase, leptin, Insulin and glutathione[1]

UA dose (mg kg^{-1})	Lipase (μ L^{-1})	Leptin (ng mL^{-1})	Insulin (μg L^{-1})
Control	105.10±3.78	1.72±0.12	0.71±0.10
1% CMC	103.80±3.77	1.78±0.79	0.76±0.12
100 mg UA kg^{-1}	109.00±3.80	1.49±0.61	0.74±0.12
300 mg UA kg^{-1}	129.25±9.43*	0.26±0.13	0.54±0.13

[1]*$p<0.05$; ±values are shown as Mean±SD

UA (Ingolfsdottir, 2002). Recently, UA was reported to be a fat burner and an uncoupler of oxidative phosphorylation and therefore was suggested as a food supplement for weight loss (Abo-Khatwa *et al*., 1996; Hsu *et al*., 2005). Severe hepatotoxicity is associated with the dietary supplements, containing UA, Lipokinetix (norephedrine hydrochloride, sodium usniate, 3, 5-diiodothyronin, yohimbine hydrochloride and caffeine) and UCP-1 (150 mg of usnic acid, 525 mg of L-carnitine and 1050 mg of calcium pyruvate) were reported in human by Favreau *et al*. (2002) and Sanchez *et al*. (2006).

No available studies dealt with the long term, *in vivo* effect of the UA on the histological structure of experimental animal liver and this was what this study aimed at. It also aimed to correlate any detected changes, at the cellular level, to those of the biochemical study.

Most animals (with standard body weight) receiving UA in this study appeared healthy and showed normal activity with no signs of stress at either dose used (100 and 300 mg kg^{-1}). These results did not match with those reported by Soderberg (1953) during their feeding experiments in other animals. Sodium usneate administered i.v. to anaesthetized cats at doses of 10 mg kg^{-1} led to an augmented rate of metabolism with symptoms such as hyperventilation, increased oxygen consumption and rise in body temperature (Soderberg, 1953).

Usnic acid suspension in CMC produced no significant decrease on body weight at either dose. However, significant decrease in BMI was observed at the high dose only. The fact is no data is available about the effect of UA on body weight in animals, despite the potential use of UA in alternative medicine as a commercial health-promoting product. Therefore, the present study provides such data for the first time. The liver index of UA treated rats was increased significantly

while their perirenal adipose tissue index showed an insignificant decrease. These results were observed in particular with the high dose of UA. The increase in liver index could be due either to hepatocyte mitochondrial swelling or to the accumulation of lipid droplets. Kumar *et al.* (2007) described mobilization of stored lipids in cases of ATP deficiency with subsequent deposition within liver parenchyma.

A focal loss of membrane integrity with subsequent leakage of cytoplasmic contents was observed in hepatocytes receiving a high dose of UA and this could explain such significant increase in ALT activity relative to the control. These results were in concordance with Pramyothin *et al.* (2004). They showed that (+) UA at high dose (1 mM) induced loss of cell membrane integrity in isolated rat hepatocytes which was detected by the release of cellular transaminases (AST and ALT) into the culture media. Abundant smooth endoplasmic reticulum was among the ultrastructural finding observed in the groups received UA and this could be attributed to its involvement in the detoxification of the drug.

On the other hand, this study showed no increase in either AST or ALP enzymes which could be attributed to the limited defect observed in membrane integrity in most hepatocytes. Some researchers considered (+) UA a lesser hepatotoxin due to its lesser effect on the transaminase activity and liver cell injury (Pramyothin *et al*., 2004).

Total Bilirubin (TBIL) was significantly increased in animals receiving both low and high doses of UA. The increase in total bilirubin was most probably due to impaired conjugation of bilirubin (Awad, 1997). Altered rough endoplasmic reticulum and mitochondrial, observed on the ultrastucture of the hepatocytes together with lack of ATP resulting from uncoupling of oxidative phosphorylation induced by UA (Abo-Khatwa *et al*., 1996; Pramyothin *et al*., 2004; Han *et al*., 2004) could predispose to such impairment.

High dose of UA significantly increased CHOL, LDL and HDL while TG was altered slightly. Done *et al.* (1969) reported that total serum cholesterol levels may give some information about liver functions. Regards TG, any fat burner agent was reported to be associated with increased TG serum level (Hasegawa *et al*., 2003).

The slight increase in TG was most probably due to the mobilization of stored fat in adipose tissue. This suggestion was confirmed in the present study by both morphological and morphometric studies of perirenal fat mass, adipocytes showed a decrease in area as well as an increase in cell number. It also confirmed by the increased level of serum lipase, together with decreased level of insulin although, it was insignificant in groups received UA detected in this study. Insulin was known to have a lipogenic effect (Kraus and Slentz, 2009) so its decrease might indicate lipolysis or at least degreased lipogenesis.

CONCLUSION

This study shows that animals received a high oral dose of UA showed a slight increase in serum glucose compared to the control in spite that hepatocytes did not show any signs of glycogen depletion. Uncoupling of oxidative phosphorylation with a consequent possible defect in energy transformation pathways could underlie such an increase in glucose level (Harris, 1997).

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