The Effect of Consumption some Instant Coffee Drinks on Carcinoembryonic Antigen and Sex Hormones of the Experimental Rats

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تأثير استهلاك بعض مشروبات القهوة سريعة التحضير على المستضد السرطانى الجنينى والهرمونات الجنسية لفئران التجارب

ملخص البحث

القهوة سريعة التحضير (نسكافيه) والمبيضات الخالية من منتجات الألبان (كوفي ميت) من المشروبات الشائعة في جميع أنحاء العالم. أجريت هذه الدراسة لمعرفة تأثير تناول بعض مشروبات القهوة سريعة التحضير على المستضد السرطاني المضغي (CEA) والتستوستيرون الكلي(T.T) لفئران التجاربتم تحليل عينات من أنواع مختلفة من القهوة سريعة الذوبان لتحديد المواد الفعالة فيها. تمت دراسة تأثير القهوة سريعة الذوبان على (CEA) و (T.T) لجرذان التجارب. أظهرت النتائج أن التحليل الكيميائي لمستوى الكافيين والكلوروجينيك ومضادات الأكسدة سجلت مستوى أقل في نوع ميكس (3 × 1) عن النوع الكلاسيكي. بينما أظهرت مادة الأكريلاميد نسبة مرتفعة في النوع (3 × 1). تسبب الاستهلاك في زيادة معنوية (P < 0.05) في مستويات الجلوكوز و (TC) و (TG) في مجموعات المزيج (1x3) ، عكس مجموعات النوع الكلاسيك. كما تمت زيادة (CEA) في كلا النوعين من القهوة مقارنةً بالمجموعة الضابطة. بينما انخفض (T.T) معنويا عند أعلى تركيز للنوع (1x3). تم الكشف عن التشريح المرضي والفجوات الكبدية مع الخلايا الالتهابية واحتقان وتوسع الأوعية الدموية. يمكن الاستنتاج أن القهوة لها تأثير بائس على المستهلك. هناك حاجة إلى مزيد من الدراسات بشكل مكثف حول تأثير إضافات القهوة على الحالة الصحية.

الكلمات الرئيسية: القهوة الفورية، التركيب الكيميائي، المستضد السرطاني المضغي، الهرمونات الجنسية.

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Abstract:

Instant coffee (Nescafé) and non-dairy creamer (Coffee- Mate) are commonly consumed beverages all over the world. This study was conducted to investigate the effect of consumption some instant coffee drinks on carcinoembryonic antigen (CEA) and total testosterone (T.T) of the experimental rats. Samples of different types of instant coffee were analyzed to identify their active substances. Effect of instant coffee on (CEA), (T.T) of experimental rats were studied. Results showed that chemical analysis of caffeine level, chlorogenic, and antioxidant values recorded lower level in Mix (3×1) type than classic type of coffee. While, acrylamide showed the high percentage in Mix (3×1) type. Consumption induced significant increase (P<0.05) in. Glucose, (TC) and (TG) levels in mix 3x1 groups, opposite to the classic groups. (CEA) was also increased in both types of coffee in comparison with control. While, (T.T) were significantly decreased at the highest concentration of mix 3x1. Histopathological, hepatic vacuolation with inflammatory cells, besides congestion and dilatation of blood vessels were detected. It could be concluded that coffee supplied miserable effect on consumer. Further studies are intensively needed on effect of coffee additions on health status.

Keyword: Instant Coffee, Chemical composition, Carcinoembryonic antigen, Sex Hormones.

Introduction:

Coffee is one of the most popular beverages, with an estimated four hundred billion cups being consumed each and every year **(Spence and Carvalho 2020).** It considered a major source for sugar-sweetened beverage (SSBs) all over the world. One possible reason for this increased demand might be the increased availability of coffee at beverage shops; some shops also sell coffee, all at a price affordable to teens **(Shih et al., 2019).**

Instant coffee is the extract of coffee beans, derived from brewed coffee beans that enable people to quickly prepare hot coffee by adding hot water or milk to the powder or crystals and stirring **(Camargo 1999).** It is prepared in a wide variety of formats. Cowbell coffee and Nescafe breakfast 3in1 are leading brands of coffee beverages available to consumers **(Choi and Curhan, 2007).**

Coffee is a source of antioxidants including caffeine, chlorogenic acid and trigonelline. it also contains carbohydrates, amino acids, fats, organic acids, caffeol (volatile aroma) and minerals phenolic compounds and diterpenes. Results of studies suggest that drinking coffee has beneficial effects on health, in particular, if consumed regularly **(Higdon and Frei 2006; Martini et al., 2016).**

Caffeine in green liberica coffee beans is 0.173%, while the liberica coffee roasted at 230˚C is 0.126% and chlorogenic acid not found after roasting at 230˚C, where other component like trigonelline and nicotinic acid were appeared after roasting process **(Perdanaet al., 2018).**

coffee mix and the addition of flavoring substances, such as sugar and cream, to coffee resulted in significant alterations in the serum level of HDL-C, TG in subjects who consumed more than 1 cup/d of coffee **(Kim et al., 2014)**,on the contrary important improvements in the lipid profile of the hypercholesterolemic group, who significantly reduced serum levels of TC, LDL-C and TG approximately was found after consuming three cups per day of the soluble roasted coffee during 8 weeks after the coffee intervention **(Martínez et al., 2019)**

Previous studies proved the influence of coffee as liver protector **(Muriel and Arauz, 2010).** While, other studies have reported that consumption of more than three cups of coffee daily was inversely related to incidence of non-alcoholic fatty liver disease, fibrosis/cirrhosis **(Leung et al., 2011)**. Dose- and time-related manner

and that these effects could occur after only a brief exposure **(Bae et al., 2016).** Caffeine enhanced the effect of methomyl leading to deleterious significant changes in carcinoembryonic antigen (CEA) level was observed by the use of caffeine. Histological studies showed that caffeine treated rats showed serious damage and changes in brain cells structure. Treatment with caffeine exerted pronounced drastically changes in the brain constituents of rats **(El-Mahdy *et al.,* 2004).**

Aim of study:

Millions of Egyptians consumed the powdered nondairy creamer and coffee beverage, at least once a day so the present study was constructed to evaluate the effect of consumption of coffee drink on Carcinoembryonic antigen and sex hormones of the experimental rats.

Materials and methods:
Methods:
I- Chemical composition analysis:

Different types of instant coffee were analyzed for the chemical composition including caffeine, chlorogenic, acrylamide and antioxidant. Acrylamide was analyzed using GC/MS-MS analysis. While, antioxidant analyzed using was assessed by the discoloration ethanolic solution of DPPH radical 0.2 aromatic in ethanol according to **Elslimani *et al.* (2013)**.

II- Experimental design:
Experimental animals:

The current study was carried on forty-two (42) adult male albino rats within 170 g as average body weight. The rats were well checked prior the experiment for any infections. All animals were permitted to acclimatize for 1 week in plastic cages (6 animals/ cage) inside a well-ventilated room prior to the experiment fed a diet of standard commercial pellets, and given water ad libitum. The experiment was performed inside Laboratory Animal House, Faculty of Science, Qena Governorate, Egypt.

Group (1): It served as control negative, in which received standard diet and distilled water for 7 weeks.

Group (2): The rats received classic coffee (Cl 1) at dose 0.06 g daily for 7 weeks.

Group (3): The rats received classic coffee (Cl 2) at dose 0.12 g daily for 7 weeks.

Group (4): The rats received classic coffee (Cl 3) at dose 0.18 g daily for 7 weeks.

Group (5): The rats received instant coffee 3x1 (3x1, 1) at dose 0.6 g daily for 7 weeks.

Group (6): The rats received instant coffee of 3x1 (3x1, 2) at dose 1.2 g daily for 7 weeks.

Group (7): The rats received instant coffee of 3x1 (3x1, 3) at dose 1.8 g daily for 7 weeks.

III- Sampling:

a- Blood and organs samples:

Blood samples were collected for biochemical analysis according to **Schermer (1967)**. Tissues specimens from liver were collected from sacrificed animals in all groups, and then fixed in 10% neutral buffered formalin for histopathological examinations.

b- Biochemical analysis:

Biochemical analysis had estimated by spectrophotometer using standard test kits.

a. Determination of serum glucose using enzymatic colorimetric method assessed according to **Trinder (1969)**.

b. Determination of serum total cholesterol (TC) according to **Richmond (1973)** using spectrophotometer.

c. Determination of serum triglycerides (TG) according to **Fossati and Prencipe (1982)** using spectrophotometer.

d- Determination of serum low density lipid- Cholesterol (LDL) described by **Lee and Nieman (1996).**

e- Determination and calculation of CEA and T.T were measured using technique known enzyme-linked immunosoebent assays (ELIZA) in the Laboratory using SpectraMax340 according to **Patrono and Peskar (1987)**.

IV- Histopathological examination:

Fixed liver specimens in formalin, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax according to **William and Linda (2000)**. Sections about 5µm thickness were prepared and stained with a standard stain of Harries hematoxylin and eosin (H & E.) for the histopathological examinations.

V - Statistical analysis:

One-way analysis of variance (ANOVA) performed to compare between control and other treated groups, followed by post-hoc analysis (Dunnett's test) using SPSS (Statistical Package for Social Sciences) according to **Version, S. P. S. S. (2018)** The data were expressed in form of Mean ± Standard Deviation. The difference was significant at $P< (0.05)$.

Results and Discussion:
1- Chemical analysis:

Data in table (1) illustrate the level of caffeine, chlorogenic, and antioxidant content of instant coffee classic and (Mix (3×1) Nescafé, as shown in table (1) caffeine level, chlorogenic, and antioxidant values recorded lower level in Mix (3×1) type than classic type of coffee. While, acrylamide and fat levels showed the high percentage in Mix (3×1) type than in classic type. The level of caffeine level of classic and mix (3×1) were 36.4 and 2.92 mg/g, respectively. Similar finding was obtained by **(Rezk et al., 2018)**. Chlorogenic acid content was varied greatly in the previous samples 2036.23 and 45.46 µg/g for classic and mix (3×1), respectively. Different finding was obtained by **(Mills et al., 2013). Yusianto (2014)** illustrated that this change may occur because the chemical components of the coffee change if roasted for long period such as chlorogenic acid in coffee beans are 8% and reduced to 4.5% during roasting process, it turned into caffeic acid and quinic acid. Acrylamide content was 4.48 µg/g in classic type, such result was similar to **(Mojska, and Gielecinska, 2013) result** that recorded (358 µg/kg) but different from the type coffee Mix (3×1).

In the study of **Gebeyehu and Bikila (2015)** ferric reducing power assay was used to measure the total antioxidant power of water-soluble components of coffee and is expressed as ascorbic acid equivalent antioxidant capacity in milligram per gram of the dried coffee samples. The ferric reducing power values of the extracts were 9.532, 9.159, 8.955, 6.751(ml/g) for Wembera, Burie, Goncha and Zegie coffees, respectively.

The fat contents of instant coffee ranged from .001% in Classic to 22.34% in 3×1 the fat contents in the samples varied significantly (p<0.05) due to the roasting conditions **Endeshaw and Belay (2020)**. This was in agreement with the findings of **Liu and Kitts (2011)** that

the fat content of coffee beans increases with roasting temperature due to the degradation of carbohydrates and the evaporation of volatile chemicals. In addition, the oil also released to the outer surface of the bean during roasting. and it may be due to coffee additives such as non-dairy creamers.

Table (1): The chemical composition of caffeine, chlorogenic, acrylamide antioxidant and fats presented in some instant coffee drinks

Parameters	Classic		Mix (3×1)		LSD at 0.05
	g	Sachets (1.8 g)	g	Sachets (18 g)	
Caffeine	36.4 [a]±.096 mg/g	65.52 [a]± 0.753 mg/sachet	2.92 [b]±.03 mg/g	52.56[b]± 0.806 mg/sachet	1.77
Chlorogenic	2036.23 [a]±6.91 μg/g	3665.23[a]±114.5 μg/sachet	45.46 [b]±.97 μg/g	818.28[b]±8.93 μg/sachet	184.263
Antioxidants	9.58 [a]±.12 mmTE/g	17.25[a] ± 0.108 mmTE/sachet	.339 [b]±.003 mmTE/g	6.11[b]± 0.036 mmTE/sachet	0.182
Acrylamide	4.48 [b]±.04 μg/g	8.08[b]±0.005 μg/sachet	7.32 a±.04 μg/g	131.76[a]±2.1 μg/sachet	3.369
Fats	0.0001 [b]±0 g/100g	0.00018[b] ±0.00002 g/sachet	22.38 [a]±.58 g/100g	4.03[a]± 0.07 g/sachet	0.113

(TE) / sachet = mmol Trolox equivalents / sachet

2- Biochemical analysis:
Effect of coffee drinks on glucose level of experimental rats:

As shown in table 2, Fig. (1), glucose level of rats that consumed 3x1 (1), (2) & (3) exhibited significant increase (P<0.05) than other groups. On the contrary, there was significant decrease (P<0.05) in glucose level among rats that consumed classic coffee. Such result was compatible with result (**El-Moneim et al., 2009، Urzúa et al., 2012**) who reported that classic coffee had significantly reduced fasting serum glucose levels, coffee prevented increase in glucose level due to its main component, caffeine,which inhibits adenosine receptors that stimulates hepatic glucose production through the activation of A2B adenosine receptors. Caffeine might also have stimulated glucose transport through activation of cyclic AMP-dependent protein kinase α1.

As cleared in table 2, it was found that the level of chlorogenic acid in the classic is higher than the type 3x1 with a large percentage,

which may explain the lack of glucose in the blood, consistent with (**Akash et al., 2014; Ludwig et al., 2014; Buscemi et al., 2016; Tajik et al., 2017**) phenolic compound CGA has been shown to reduce blood glucose concentrations in animal experiments and increased sensitivity to insulin, and slowed the appearance of glucose in circulation after glucose load,also may prevent diabetes, and to inhibit alpha-amylase and alpha-glucosidase activity, two key enzymes responsible for digestion of dietary carbohydrates, resulting in a reduction of intestinal absorption of glucose.

The contrary, glucose level was increased in Mix 3x1 coffee is similar with (**Agardh et al., 2004)** that confirm Milk, cream and/or sugar was added to the coffee would most likely bias the relative risks towards an increased risk of diabetes.

Study (**Van Dam and Feskens, 2003**) confirmed that coffee with a full-fat cream and sugar in daily cup such a range of glucose burdens contribute to varying extents of hyperglycemia and affect progression to type 2 diabetes mellitus and cell failure, possibly due to glucotoxicity and could negate any beneficial effects on glucose metabolism that might be conferred by its contents

It was concluded that coffee and Nescafe consumption increased the blood glucose levels, such result was consistent with (**Lane 2011; Shi et al., 2016)** its which indicated that coffee and Nescafe significantly increased the blood glucose levels in diabetic male rats with the control.

Effect of instant coffee drinks on Total cholesterol (TC), Triglyceride (TG) and low-density lipid (LDL) level of experimental rats:

From data listed in table (2), Fig. (1) TC, TG and LDL levels were significantly increased (P<0.05) among rats groups that consumed 3x1 (2) & (3), On the contrary, there was significant decrease (P<0.05) level among all rats consumed classic coffee. That result was consistent with (**Martínez et al., 2019)** who found low significantly reduced their serum levels of TC, LDL and TG approximately after consuming three cups per day of the soluble roasted coffee. It may be due to presence of polyphenols and chlorogenic acid in classic coffee reduce TC levels (**Murase et al., 2011 and Meguro et al., 2013**). However, little has been reported about the mechanism of action of these substances: it has been reported only that CGA inhibits cholesterol biosynthesis (**Cho et al., 2010 and Karthikesan et al., 2010)**. Moreover, phenolic acids of

coffee enhance cholesterol efflux and decrease its blood concentrations. In addition, roasted coffee contains quinides that possesses favorable effects on serum cholesterol and blood lipids **(Shearer et al., 2003).**

Mix coffee intake induced a signi- cent increase in TC and TG levels of experimental rats. The increase level of cholesterol and triglyceride seen. Similar finding was obtained by **(Kim et al., 2017; Kim et al., 2013; Grosso et al., 2017; Du et al., 2020).** As cleared coffee consumption significantly increased TC, TG and LDL. It was found that instant coffee mix intakes were significantly associated with serum triglyceride and LDL level in subjects who consumed more than 1 cup/d of coffee (P<0.05).

Cai et al., (2012) reported that coffee directly increased TC, LDL-cholesterol and triglycerides when consumed boiled or non-filtered. Saturated fat (palm oil), used in filled milk and liquid coffee whiteners increases LDL cholesterol, a major cause of atherosclerosis and CVD, and replacing it with polyunsaturated or monounsaturated fat decreases LDL cholesterol **(Sacks et al., 2017).**

Table (2): The effect of some instant coffee drinks on serum glucose level (mg/dl) and lipid profile (TC, TG and LDL (mg/dl) of albino rats of control group, Classic groups (Cl 1, 2 & 3), and 3x1 groups (3x1 1, 2, & 3). (Mean± SD)

Parameters Groups	Glucose and lipid profile			
	Glucose (mg/dl)	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)
Control	123.4 ± 2.07^b	109.3 ± 3.1^c	144.1 ± 0.97^b	46.8 ± 1.63^b
Cl (1)	116.6 ± 1.2^c	103.0 ± 1.5^b	137.7 ± 2.48^c	45.5 ± 0.89^b
Cl (2)	110.4 ± 1.1^c	96.0 ± 0.97^b	130.6 ± 0.69^c	43.25 ± 0.69^b
Cl (3)	105.4 ± 2.7^c	81.8 ± 0.89^b	122.0 ± 0.83^c	39.7 ± 0.52^c
3x1 (1)	130.6 ± 1.14^a	$113.28\pm1.47_c$	145.4 ± 0.89^b	48.4 ± 0.54^b
3x1 (2)	141.8 ± 2.0^a	132.0 ± 0.96^a	158.6 ± 1.67^a	53.6 ± 0.89^a
3x1 (3)	155.8 ± 1.84^a	147.5 ± 0.97^a	165.6 ± 3.5^a	56.4 ± 1.14^a
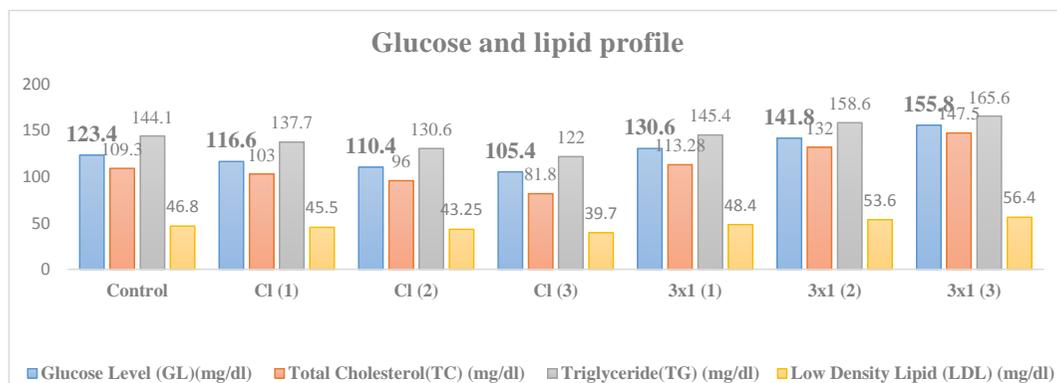
Fig. (1): The effect of some instant coffee drinks on serum glucose level (mg/dl) and lipid profile (TC), TG and LDL (mg/dl) of albino rats of control group, Classic groups (Cl 1, 2 & 3), and 3x1 groups (3x1 1, 2, & 3). (Mean± SD).

Effect of coffee drinks on carcinoembryonic antigen (CEA) and Total testosterone (T.T) level of experimental rats

As noticed from table (3) regarding to results of CEA, rats consumed 3x1 (3) scored the highest significant increase (P<0.05) followed in rats consumed Cl (3), 3x1 (2) groups. While the obtained data of T.T hormone recorded in CI (3) group the highest significant increase (P<0.05). Conversely, rats consumed 3x1 (3) that recorded the lowest.

Table (3) result showed that total testosterone (T.T) level was increased at the highest concentration of classic coffee due to caffeine content. Caffeine has potential to stimulate spermatogenesis. Its consumption may lead to an increase in sex drive and improve male factor fertility **(Onuoha and Edo, 2018).** On the contrary, caffeine treatment for 4 weeks could impair body reproductive organs weight, sperm characteristics, LH/FSH level, and also testicular cyto-architecture (**Oluwole *et al.,* 2016).** As such different comorbidities were associated with TT level decline Obesity, metabolic syndrome, diabetes and dyslipidaemia were identified as risk factors of incident testosterone deficiency TD **Haring et al., (2010).**

Level of carcinoembryonic antigen (CEA) was increased at the highest concentration of mix 3x1 coffee correlated to acrylamide content (table3). It was reported that acrylamide induced a significant increase in the levels of serum CEA **(Hamdy *et al.,* 2017).** Acrylamide is included on the list as a carcinogen is an industrially produced

chemical with known neurotoxic, reproductive toxin and carcinogenic effects. The carcinogenicity associated with acrylamide is mostly attributed to its metabolism by liver. Acrylamide is found in many types of foods, including coffee **(Nuyan, 2008 and Wang *et al.,* 2020)**. It was demonstrated that acrylamide stimulate formation of the toxic compounds and procarcinogens which in turn further potentiates hepatotoxicity, mutagenicity and carcinogenicity on liver and kidney of rabbits **(Nuyan, 2008)**.

Table (3): The effect of some instant coffee drinks on tumour marker and hormones as (CEA and T.T (ng/ml) of albino rats of control group, Classic groups (Cl 1, 2 & 3), and 3x1 groups (3x1 1, 2, & 3)

| Parameters Groups	CEA (ng/ml)	T.T (ng/ml)
Control	1.02 ± 0.179 [c]	2.538 ± 0.079 [b]
Cl (1)	1.02 ± 0.13 [c]	2.776 ± 0.33 [b]
Cl (2)	1.1 ± 0.005 [c]	2.806 ± 0.125 [b]
Cl (3)	1.66 ± 0.055 [b]	3.668± 0.329 [a]
3x1 (1)	1.04 ± 0.055 [c]	2.492 ± 0.12 [b]
3x1 (2)	1.63 ±0.049 [b]	2.288±0.237 [b]
3x1 (3)	2.66 ±0.055 [a]	1.538±0.079 [c]
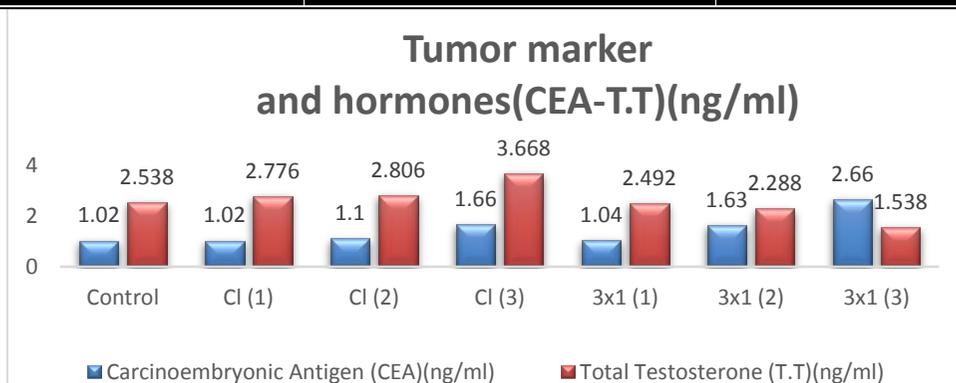
Fig. (2): The effect of some instant coffee drinks on tumour marker and hormones as (CEA and T.T (ng/ml) of albino rats of control group, Classic groups (Cl 1, 2 & 3), and 3x1 groups (3x1 1, 2, & 3).

3-Histopathological results:

Histological alterations in liver agreed with **Leung *et al*., (2011)** who showed that consumption of more than three cups of coffee per day has been inversely related to the incidence of non-alcoholic fatty liver disease, fibrosis/cirrhosis. Theses results match with data belonging to **El-Ghany *et al,.* (2012)** who evaluated the effects of some instant coffee drinks on rat's liver. Caffeine group showed congestion of central vein. However, liver of rat of cocoa group showed non-significant changes except Kupffer cells activation. Liver

from Nescafe group revealed slight congestion of hepatic sinusoids while liver of rat from coffee group showed slight Kupffer cells activation. Also, **Manne and Saab (2015)** reported that caffeine in coffee induced hepatotoxicity and injuries in the liver tissues. Moreover, **Choi *et al,.* (2018)** noticed liver necrosis surrounding hepatic portal vein characterized by cell death and damage when administrated the 10-week-old male C57BL/6 mice with coffee extracts for 10 days by oral gavage (300 mg/kg.b.wt). Acrylamide (ACR) led to the development of cytoplasmic fatty vacuolation and necrosis of the centrilobular hepatocytes with lymphocytic infiltration **(Sharma *et al.*, 2008)**. ACR inhibited GST, resulting in increased metabolism of glycidamide by the CYP450 pathway
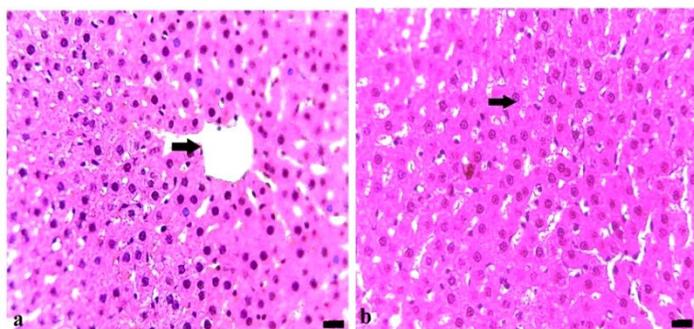
Fig. 3 (a-b): Photomicrograph of liver of the control negative group showed normal criteria, where hepatocytes arranged in hepatic cords and have a distinctive central nucleus with prominent nucleolus. Blood vessels appeared normally comprising normal central vein, blood sinusoid and portal area. (H& E., X 400)
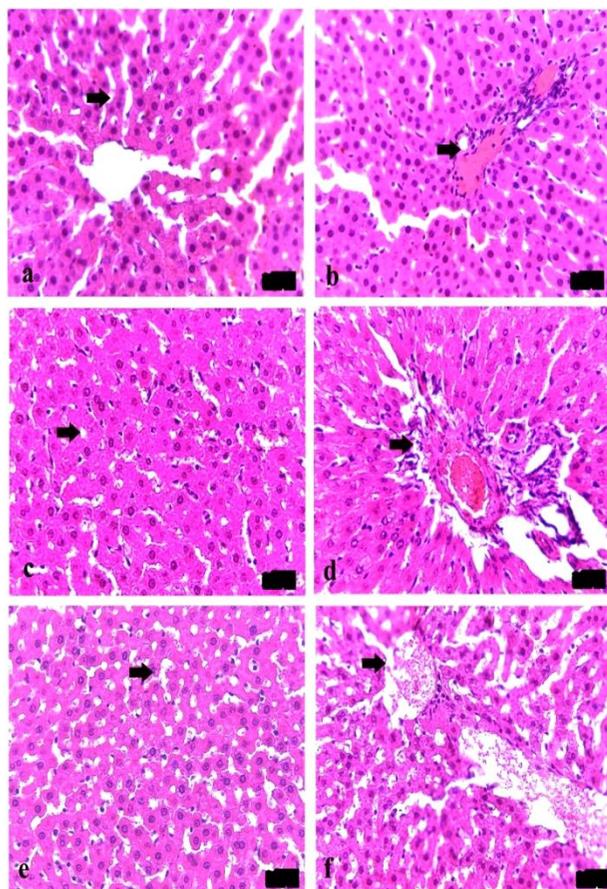
Fig. 4 (a-f): Photomicrograph of liver of classic
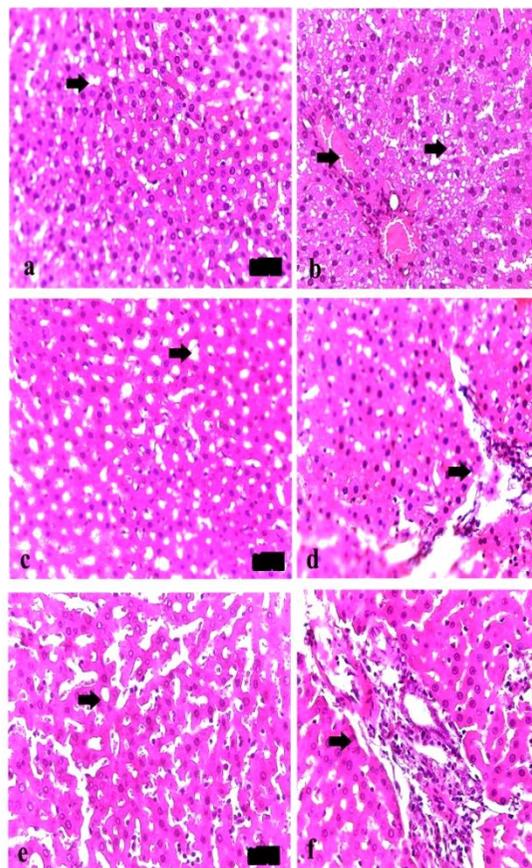
Fig. 5 (a-f): Photomicrograph of liver

types groups. Liver of classic (1) showed slight dilatation in central vein and blood sinusoids with mild degree of vacuolar degeneration with cytoplasmic vacuolation of the hepatocytes (a), congestion and inflammation in the portal area with inflammatory cells infiltration surrounding the portal wall (b). Liver of classic (2) showed vacuolar degeneration of the hepatocytes (c), moderate degree of portal inflammation and congestion (d). Liver of classic (3) showed extensive signs of cytoplasmic vacuolation (e) and congestion of the blood vessels (f). (H& E., X 400)

of 3x1 types groups. Liver of 3x1 (1) showed noticeable signs of vacuolar degeneration characterized by cytoplasmic vacuolation of the hepatocytes (a), congestion and inflammation in the portal area, besides hepatic necrosis (b). Liver of 3x1 (2) showed cytoplasmic vacuolation of the hepatocytes (c), congestion in the blood vessels comprising portal area (d). Liver of 3x1 (3) showed extensive degree of cytoplasmic vacuolation with highly inflammatory cells infiltration (e) and severe congestion of the blood vessels, besides necrosis of the hepatocytes (f). (H& E., X 400)

Conclusion:

The results of this study proved that types of instant coffee drinks effected dangerously on the healthy status of the tested rats by increasing lipid profile and decreasing the level of male hormones. So, it caused, by long period, many diseases as diabetic, hypertension and cancer.

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