Original Article

Protective role of ginger and curcumin against some toxicological effects induced by thermoxidized frying cotton oil

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Abstract

In Egypt, the bad economic situation in many homes often demand that oil previously fried is reused and this may constitute health risk to consumers. The aim of the present work was to investigate the protective role of ginger and curcumin powders against some toxicological effects of thermoxidized frying cotton oil (OFO). Thirty five male albino rats were divided into seven groups: negative control, ginger-treated group (1 g/100 g in diet), curcumin treated group (0.2 g/100 g diet), fresh cotton oil treated group (15 mL/kg orally), OFO – treated group (15 mL/kg orally), OFO (15 mL/kg orally) + ginger (1 g/100 g in diet) – treated group and OFO (15 mL/kg orally) + curcumin (0.2% in diet) – treated group. After 28 days of experiment, the results indicated that OFO treated group showed significant ($p \leq 0.05$) increase in both liver enzymes (AST and ALT) and glucose levels. Significant increase in the frequencies of chromosomal aberrations in somatic and germ cells were encountered. Histopathological changes in liver in form of fatty changes and central vein congestion were observed. The addition of ginger or curcumin to diet of OFO treated group produced improvement in the liver function, decrease in the glucose level, increase in the level of total antioxidants, reduction in the frequencies of chromosomal aberrations and improvement of the hepatic pathological changes. In conclusion, ginger and curcumin can protect against toxicity of frying oil, but, curcumin needs further investigation to find the effective and safe dose

Key words: thermoxidized frying cotton oil, ginger, curcumin, biochemical changes, chromosomal aberrations, histopathological changes

Função protetora de gengibre e curcumina contra alguns efeitos toxicológicos induzidos por óleo de algodão frito termoxidado

Resumo

No Egito, a má situação econômica em muitos lares, geralmente exige que o óleo previamente frito seja utilizado novamente o que pode constituir um risco para a saúde dos consumidores. O objetivo do presente trabalho foi investigar o papel protetor de gengibre e curcumina contra alguns efeitos toxicológicos do óleo de algodão frito termoxidado (OFO). Trinta e cinco ratos albinos machos foram divididos em sete grupos: controle negativo, grupo tratado com gengibre (1 g/100 g de dieta), grupo tratado com curcumina (0,2 g/100 g de dieta), grupo tratado com óleo de algodão fresco (15 mL/kg por via oral), OFO grupo tratado (15 mL/kg por via oral), OFO (15 mL/kg por via oral) + gengibre (1 g/100 g de dieta) e OFO (15 mL/kg por via oral) + curcumina (0,2 % na dieta). Após 28 dias de experimento, os resultados indicaram que o grupo tratado com OFO apresentou significativo aumento ($p \leq 0,05$) de ambas as enzimas do fígado (AST e ALT) e níveis de glicose. Aumento significativo nas freqüências de aberrações cromossômicas em células somáticas e germinativas foi observado. Alterações histopatológicas no fígado em forma de modificações de gordura e congestão venosa central foram identificadas. O grupo tratado com adição de gengibre ou curcumina à dieta de OFO produziu melhoria na função hepática, diminuição do nível de glicose, aumento do nível de antioxidantes totais, redução da freqüência de aberrações cromossômicas e melhoria das alterações hepáticas patológicas. Em conclusão, gengibre e curcumina podem proteger contra a toxicidade do óleo de fritura, mas, a curcumina necessita de mais pesquisas para encontrar a dose eficaz e segura para seu uso.

Palavras-chave: óleo de algodão frito termoxidado, gengibre, curcumina, alterações bioquímicas, aberrações cromossômicas, alterações histopatológicas

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Introduction

As social economic and cultural conditions have changed over the twentieth century, significant alteration occurred in eating habits, including eating outside home and especially in fast food restaurants, which lead to increased consumption of oils used in deep fat frying (Weisburger, 2002).The quality and oxidative level of frying oil may not be well controlled, especially in developing countries (Chao et al., 2007). Frying oil, used continuously or repeatedly at a high temperature is subjective to a series of degradation reactions, thermal oxidation and hydrolysis due to presence of moisture in food and formation of a variety of decomposition compounds (Basuny, 2004). The intake of thermally altered oils and fats represents a source of substances potentially toxic in a diet. The consumption of heated or oxidized oils involves possible health risks such as arteriosclerosis predisposition, mutagenic or carcinogenic actions (Kubow, 1990).

Lipid oxidation is one of the major deteriorative reactions in frying oils and fried foods (Jaswir et al., 2000). Therefore, thermally oxidized oil has many risky properties related to gross symptoms and toxic responses at the biomembrane level (Totani et al., 2006). General effects reported rang from loss of appetite, poor food efficiency, reduced gut absorption enlarged liver, kidney and lung, fatty hepatic necrosis, haemolysis, calciferous myocardial lesions and even death (Oyewole & Olayinka, 2007).

Considerable attention has been given to the application of natural antioxidants in food, because of their potential nutritional and therapeutic effects (Halliwell et al., 1995). The role of ginger (*Zingiber officinal* roscoe) as antioxidants has been comprehended (Borek, 2001). These spices contain substances that retard the rate of oxidation by scavenging free radicals or controlling the break down of peroxides into stable substances that do not promote further oxidation (Oyewole & Olayinka, 2007).

The rhizome of ginger is widely consumed as a common spice throughout the world and is used in traditional oriental medicine. Its major pungent constituent, [6] - Gingerol (1-[4' – hydroxyl – 3' - methoxyphenyl] -5- hydroxy 3- decanone) is reported to have a variety of interesting biochemical activities (Lee & Surth, 1998). Ginger has been used to treat a number of diseased conditions, including headache, cold, arthritis, postoperative nausea and vomiting, motion sickness and to reduce symptoms in patients with nausea of pregnancy (Grant & Lutz, 2002).

Curcumin is a widely used spice and coloring agent in food extracted from the rhizome of turmeric (*Curcuma longa* Linn) (Motterlini et al., 2000). Curcumin has a B-diketone compound which contains two ferulic acid molecules linked via a methylene bridge at the carbon atoms of carboxyl group (Sharma, 1976). Curcumin exhibits anti-inflammatory and antiviral effects and it is also considered as a potent scavenger of reactive oxygen and nitrogen (Joe et al., 2004).

So the aim of the present study was to investigate the role of ginger and curcumin powders in protection against some toxicological effects of consuming frying cotton oil. The potential toxicological effects were studied through biochemical, cytogenetical and histopathological investigations.

Material and Methods

Material

Unpackaged cottonseed oil was used in this experiment because it is less expensive and more available to consumers, especially in rural areas in Egypt. The oil, potatoes, ginger and curcumin powders were obtained from local market in Shebin Elkom, Menofiya governorate, Egypt.

Preparation of oxidized frying oil (OFO)

A volume of 600 mL of cottonseed oil was used to prepare 500 g of fried potatoes *per* day. One batch of 500 g of raw potatoes was fried every day during seven consecutive days. The initial frying temperature was around 180 °C and at the end of each frying the oil was filtered. No replenishment of oil was considered and oil was taken out at the end of the experiment.

Animals

Thirty five male albino rats (Wistar strain) weighting approximately 120 to 150 g were purchased from National Research Center Animal House Colony, Giza, Egypt. They were maintained on the experimental diets and water *ad libitum* at the animal house of Department of Forensic Medicine and Toxicology, Faculty of Medicine for Girls, Al Azhar University. After an acclimatization period of one week, the animals were divided into seven groups. Each group contained five animals that were treated separately in stainless steel cages at a controlled temperature (23 ± 1 °C) and artificially illuminated (12 hours dark/light cycle).

Experimental design

The animals were divided into seven groups namely: Group 1, animals were orally administered by distilled water and considered as control. Group 2, animals were fed on diet containing 1g/100g ginger powder (Ahmed & Sharma, 1997). Group 3, animals were fed on diet containing 0.2g/100g curcumin powder (Asai & Miyazawa, 2001). Group 4, animals were administered orally with daily dose of fresh cotton oil (15 mL/kg body weight) (Sandal & Kalia, 2000). Group 5, animals were administered orally with daily dose of oxidized frying cotton oil (15mL/kg) (Sandal & Kalia, 2000). Group 6, animals were administered orally with daily dose of oxidized frying cottonseed oil (15mL/kg) and fed diet containing 1g/100g ginger. Group 7, animals were administered orally with daily dose of oxidized

frying cotton oil (15mL/kg) and fed diet containing 0.2g/100g curcumin. Animals were treated daily for 28 days.

At the end of the experiment, all animals were fasted for 12 hours and then blood samples were collected from venous plexus under diethyl ether anesthesia. Blood samples were left to clot and sera separated using cooling centrifugation and stored at -20 °C until used to biochemical analysis. Femora and testis were used for chromosomal aberrations analysis in somatic and germ cells and liver was used in the histopathological analysis.

Biochemical analysis

Liver enzymes: Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were studied according to Young (1995).

Glucose level analysis was carried according to Trinder (1979).

Total Antioxidants were measured using antioxidant assay kit, Cayman chemical company. The assay relies on the ability of antioxidants to inhibit the oxidation of ABTS` (2, 2` – Azino di – [3- ethylbenzthiazoline sulphonate] to ABTS" by metmyoglobin. The amount of ABTS" produced was monitored by reading the absorbance at 750 nm or 405 nm. The capacity of the antioxidant to prevent ABTS` oxidation is compared with that of Trolox, water – soluble tocopherol analogue and is quantified as millimolar Trolox equivalents (Kampa et al., 2002).

Chromosome aberration analysis

At the end of the treatment, animals of all treated groups were injected intraperitoneally with colchicine to arrest cell division at metaphase. Three hours after injection, animals were sacrificed for preparation of the chromosomes of bone-marrow cells and spermatocytes. Chromosomes of bone-marrow cells were prepared following the Yosida & Amano (1965) methodology and the chromosomes of germ cells were prepared according to Brewen & Preston (1987) method. The glass slides were stained with 10% Giemsa stain and 100 good metaphase spreads of each cell types for all animals were examined microscopically to analyze the different types of chromosomal aberrations.

Histopathological examination

The liver of all animals were examined grossly. Subsequently they were fixed in 10% phosphate buffered formalin, embedded in paraffin and sections of 5 µm – thickness were stained with haematoxylin and eosin.

Statistical Analysis

Statistical analyses were performed with SPSS software. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for multiple comparisons between pairs (Snedecor & Cochran, 1968). Results are reported as mean values ± S.D. and differences were considered significant when (P≤0.05).

Results and Discussion

The effect of ginger and curcumin on the levels of serum AST, ALT, glucose and total antioxidant induced by thermoxidized frying cottonseed oil in rats are summarized in table 1. The current study revealed that OFO increase the level of serum AST and ALT significantly (p≤ 0.05) (249.20 ± 2.94 and 160.00 ± 3.51 IU/L, respectively) in comparison to control (36.20 ± 3.89 and 36.30 ± 4.81 IU/L, respectively). In the current study the animals treated with ginger or curcumin plus OFO showed improvement in the liver function enzymes ALT (81.80 ± 2.04, 131.40 ± 2.07) and AST (91.40 ± 2.07 IU/L, 124.6 ± 4.5 IU/L) respectively (table 1). These results were in agreement with Oyewole & Olayinka (2007) that reported that garlic and onion reduced the increase that induced by heated oil in ALT and AST levels in rats.

Table 1. Effect of ginger and curcumin on levels of serum AST, ALT, glucose and total antioxidants in thermoxidized frying oil treated rats.

Experimental groups		AST IU/L	ALT IU/L	Glucose Mg/dL	Total antioxidant/mM
Control	M±SD	36.20 ± 3.98 a	36.30 ± 4.81 a	55.00 ± 5.00 a	2.36 ± 0.65 a
ginger	M±SD	22.40 ± 2.51 a	21.80 ± 2.00 a	59.00 ± 2.23 a	3.15 ± 0.19 b
Curcumin	M±SD	52.20 ± 2.16 b	60.00 ± 3.53 b	46.00 ± 4.18 a	2.91 ± 0.44 a
Fresh oil	M±SD	40.20 ± 1.64 b	40.00 ± 2.91 b	56.0 ± 6.50 a	2.55 ± 0.57 a
Theroxidized Frying oil	M±SD	249.20 ± 2.49 f	160.00 ± 3.51 f	130.00 ± 3.53 e	2.45 ± 0.63 a
Theroxidized Frying oil + ginger	M±SD	81.80 ± 2.04 d	91.40 ± 2.07 d	73.40 ± 3.20 c	3.49 ± 0.93 b
Theroxidized Frying oil + curcumin	M±SD	131.40 ± 2.07 e	124.60 ± 4.5 e	96.00 ± 3.67 d	2.44 ± 0.65 a

Values with different superscript letters within columns represent significant statistical differences (P≤0.05).

The OFO treated group also showed significant increase (p≤0.05) in glucose level (130.00 ± 3.53 mg/dL) in comparison to control (55.00 ± 5.00 mg/dL). It is known that OFO feeding induced higher oxidative stress (Izaki et al., 1994), which may result in production of free radicals and oxidative damage to many organs such as liver and pancreas (Addis, 1986).The mechanism of hyperglycemia may occur due to the hypo-insulinaemia which is caused by impaired function of pancreatic beta cells or increased liver extraction of insulin from the portal blood (Chao et al., 2007). Meanwhile, feeding on ginger and curcumin induced significant decrease in the glucose level (73.40 ± 3.20 mg/dL) and (96.00 ± 3.67 mg/dL) respectively (Table 1). Ahmed & Sharma (1997) reported that long-term intake of ginger had hypoglycaemic and hypolipidaemic effects. Saxena & Vikram (2004) also observed that curcumin reduced the glucose level in experimental induced-diabetic rats.

In the present study the level of total antioxidants increased significantly (P ≤ 0.05) in rats treated with ginger alone or ginger + OFO (3.15 ± 0.19 mM and 3.49 ± 0.93 mM) compared to control (2.36 ± 0.65 mM), while, curcumin showed insignificant increase in total antioxidants (2.91 ± 0.44 mM) compared to control (2.3 ± 0.65 mM) (Table 1). It was reported that living organisms had developed complex antioxidant systems to counteract reactive oxygen species (ROS) and to reduce their damage (Kampa et al., 2002).

These antioxidant systems include enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), macro molecules such as albumin, ceruloplasmin and ferretin and an array of small molecules including ascorbic acid, tocopheral, B-carotene, reduced glutathione, uric acid and bilirubin. Endogenous and food derived antioxidants represents the total antioxidant activity of the system (Halliwell, 1996). The cooperation among different antioxidants provides greater protection against attack by ROS, than any single compound alone. Thus the overall antioxidant capacity may provide more relevant biological information compared to that obtained by the measurement of individual component, as it considers the cumulative effect of all antioxidants (Koracevic et al., 2001). The significant increase of total antioxidants in the present study in ginger treated groups coincides with Ajith et al. (2006) who found that treatment with ginger extract prevented lipid peroxidation by enhancing SOD, CAT and GPX activities, while the free radical trapping capacity of curcumin resides mainly in its phenolic contents beside a B-diketone moiety (Masuda et al., 1999). These compounds possibly prevent the ROS from acting on DNA (Srinivas et al., 1992) suggesting a possible role of curcumin as a chain breaking antioxidant against lipid peroxidation (Rajakrishnan et al., 1999).

From the results of chromosomal aberrations presented in figure 1 and 2 it has been demonstrated that OFO significantly increased the frequency of total chromosomal aberrations in both bone marrow ($P \leq 0.05$) (36.80 ± 6.37) and spermatocytes (14.2 ± 0.83)

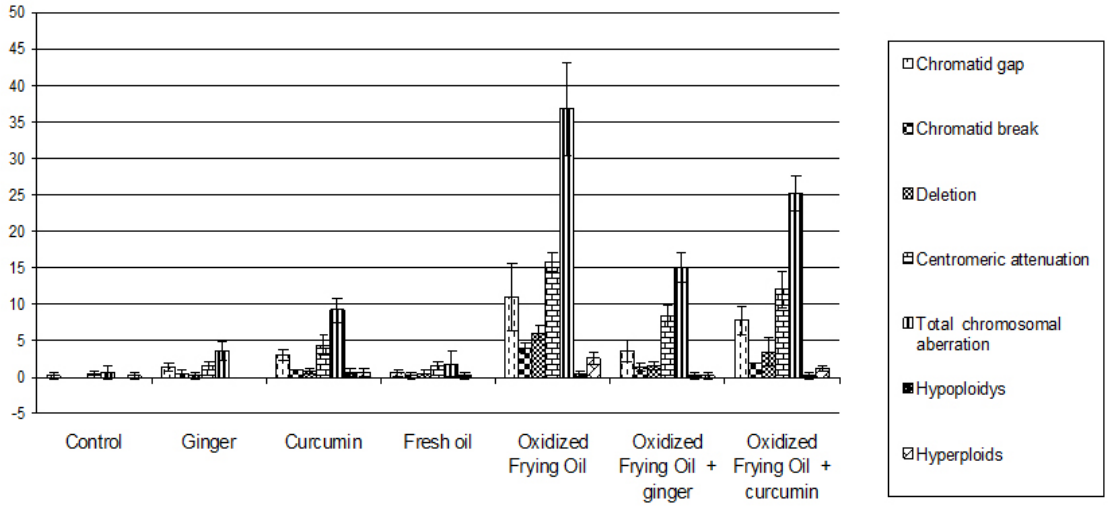

Figure 1. Frequencies of chromosomal aberrations induced in bone marrow cells of all experimental groups

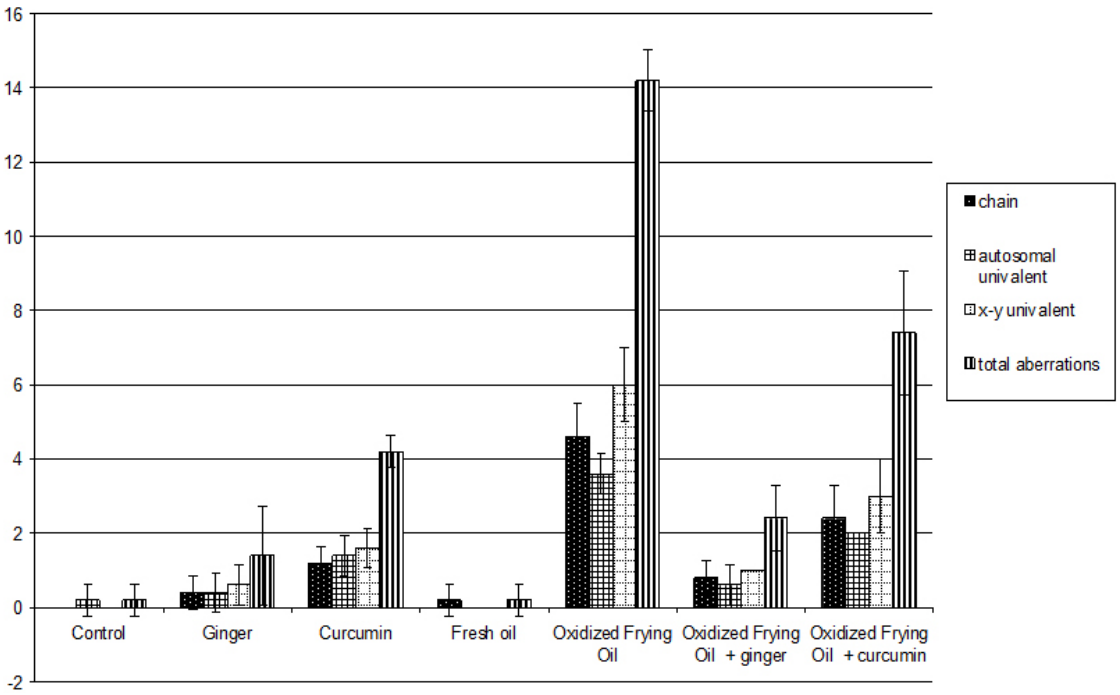

Figure 2. Frequencies of chromosomal aberrations induced in spermatocytes of all experimental groups

compared to control group (0.60 ± 0.84 and 0.20 ± 0.44 respectively). The administration of ginger or curcumin concurrent with thermoxidized frying oil showed significant decrease (P≤ 0.05) in the frequencies of chromosomal aberrations in bone marrow (15.00 ± 2.00 and to 25.2 ± 2.38) and spermatocytes (2.40 ± 0.89 and 7.40 ± 1.67) when compared to OFO treated group for both cell types (36.80 ± 6.37 and 14.20 ± 0.83). No significant difference between control and ginger or fresh oil treated groups were observed, while the curcumin treated group showed significant increase in frequency of total chromosomal aberrations in marrow and spermatocytes (9.20 ±1.64 and 4.20 ± 0.44, respectively) in comparison to control for both cell (0.60 ± 0.84 and 0.20 ± 0.44, respectively). Chromosomal aberrations were structural and numerical; the observed structural chromosomal aberrations in bone marrow cells were chromatid gaps, chromatid breaks, deletions and centromeric attenuation. The observed types of numerical aberrations were hypoploidys and hyperploidys. The observed types of structural aberrations in spermatocyte were chain, x-y univalent and autosomal univalent.

Latchoumycanadane & Mathur (2002) reported that reactive oxygen species (ROS) produced as a result of frying oil consumption considered important agent in cytotoxic effect in spermatozoa and on cellular constituents. ROS also produced permeability changes of testicular membrane (Chance et al., 1979). In some cases complete cessation of spermatogenesis has been determined (Tewfik et al., 1998). Previous literatures reported that highly oxidized oils may produce hydrocarbons that were thought to have carcinogenic effect. The genotoxicity of OFO may be related to acrylamide as it may have potential carcinogenicity and genetic and generative toxicity (FAO/WHO, 1988 and Rosen & Hellenes, 2002). Different reports have shown that curcumin seems to exert antimutagenic potential towards several compounds (Shukla et al., 2003; Polasa et al., 2004; Corona-Rivera et al., 2007 and AlSuhaibani, 2009). Results of the present study showed that both ginger and curcumin induced significant decrease in the frequency of chromosomal aberrations than control. In the present study the antioxidant activity of both ginger and curcumin may be considered the possible mechanism of protection against the chromosomal aberrations induced by OFO. Ginger had no significant increase in the frequency of chromosomal aberration when compared with control. This is in agreement with (Langner et al., 1998) reported that ginger has been listed in "Generally Recognized as Safe" (GRAS) documents of USFDA. Curcumin, on other hand, produced some chromosomal aberration, which is in agreement with Araujo et al. (2001 and Elmakawy & Sharaf (2006) that mentioned that curcumin has been reported to induce a significant increase in the frequency of chromosomal aberrations.

The biochemical and genotoxic effects of OFO were confirmed by the histopathological examination of liver. Liver histopathological examination of ginger or curcumin treated animals showed normal hepatocytes and central vein (figure 3A), whereas, the livers of fresh cottonseed oil treated animals showed vacuolated hepatocytes or unstained cytoplasm (figure 3B). The animals treated with OFO showed fatty changes in some hepatocytes which had the characteristic signet ring appearance (figure 3C and 3D). Some of the hepatocytes near the fatty cellular infiltration appeared with paler cytoplasm than other hepatocytes. Congestion in the central vein was also seen (figure 3C and 3D). Similar observations were reported by Garibagaoglu et al. (2007) the hepatic injury may be related to ROS which were reported to damage the cellular organelles through peroxidation of phospholipids, protein and nucleotides (Latchoumycandane & Mathur, 2002). Also, in the current study rats treated with fresh oil showed vacuolation of some hepatocytes or unstained cytoplasm. Hamoudah & El Najar (2002) observed the fatty infiltration in fresh oil treated group which was packaged in their study. So the fresh unpackaged oil used in the present study did not differ from the fresh packaged, however, the problem was due to the repeated use of this oil.

Co-administration of ginger or curcumin produced significant improvement of the hepatic injury in the present study (figure 3E and 3F). Meanwhile, in the case of curcumin the central vein was still congested but less than OFO- treated group (figure 3G and 3H). This improvement may be due to antioxidant effect of both ginger and curcumin. Additionally there is growing evidence that the hepatoprotective effect of curcumin takes place directly at the level of hepatocytes by reducing the intercellular levels of cholesterol and cytotoxic bile acids (Sambaiah & Srinivasan, 1989).

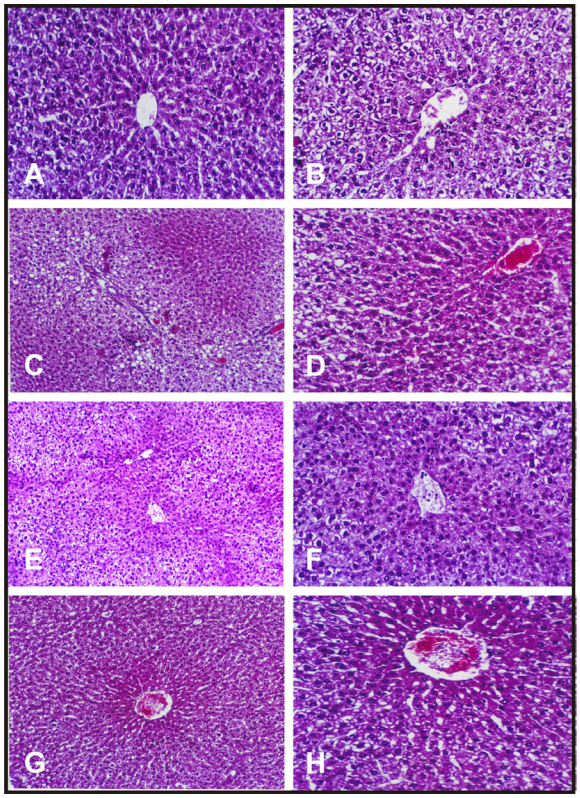

Figure 3. Photomicrographs of rat liver section of (A) normal central vein (short arrow) and the hepatocytes arranged around it in a radiating manner (H&E x 200). (B) Fresh oil treated group showing many of hepatocytes appearing vacuolated or with unstained cytoplasm (arrow), the nucleus still prominent and vesicular (H&E x 200). (C) Oxidized frying oil group showing fatty changes which have the characteristic signet ring appearance (arrow) (H&E x 100). (D) Oxidized frying oil group showing congestion in the central vein (arrow) and fat cells present in between the hepatocytes (H&E x 200). (E) Oxidized frying oil + ginger treated group showing central vein nearly restoring its normal appearance (H&E x 100). (F) Magnification of the previous section (H&E x 200). (G) Oxidized frying oil+ curcumin treated group showing central vein less congested (H&E x 100). (H) Magnification of the previous section showing intracellular vacuolation and deeply stained easinophilic central hepatocytes (H&E x 200).

Conclusion

The present results have indicated that oxidized frying oil induced hepatotoxic and genotoxic effects. Ginger and curcumin had a protective effect against these toxicities through the increasing of the total antioxidants. We recommend by using ginger and curcumin with frying food.

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