Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 11 (3): 100-108, 2012
ISSN 1682-3974 / DOI: 10.3923/ajps.2012.100.108

Evaluation of the Biological Effects for Adding Cinnamon Volatile Oil and TBHQ as Antioxidant on Rats' Lipid Profiles

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Abstract: Cardiovascular disease is one of the main health problems. Long term consumption of fried products increase the incidence of heart disease, synthetic antioxidants (TBHQ) were used to reduce oil oxidation but they have dangerous side effects. The current trend is to use plant volatile oils as a natural alternative that were found to have favourable taste and antioxidants. The effects of cinnamon volatile oil as a natural antioxidants were studied and also the major components of the volatile oil were identified using GC-MS. During the 45 days experimental period, rats were divided into 4 groups, fed a diet containing 15% oil that was either fresh oil (G1) or heated oil (G2) or heated oil mixed with 0.2% (TBHQ) (G3) and also cinnamon oil (G4). The parameters examined for studying the health effects of the cinnamon oil were, lipid profiles include assessing of total lipids, total cholesterol (TC), Triglycerides, HDL, LDL, VLDL and Atherogenic index. Also serum parameters uric acid, creatinine, bilirubin and glucose were measured. The results showed that the total lipid for (G2) was 117.47%, 113.6% for G3 and 105.1% for (G4) compared to (G1). Same pattern of effect for Triglyceride, (TC) reduced for (G4) to 91.14% comparing to (G1), HDL were insignificantly decreased in (G2, G3, G4) (20.63, 21.22, 23.03 mg dL^{-1}) comparing to (G1) (26 mg dL^{-1}). Adding cinnamon oil (G4) reduced LDL to 86.55% comparing to (G1). Also Atherogenic index was significantly decreased from 3.34 to 1.92 for G2 and G4 respectively. The effects of cinnamon oil on serum parameters found to be positive due to the detected improvements in (G4) compared to (G2). The GC-MS analysis of the cinnamon oil indicated that cinnamaldehyde is main compound in concentration of 64.21%. Adding cinnamon oil can improve lipid profiles which release the incidence of atherosclerosis.

Key words: Cinnamon volatile oil, synthetic antioxidant, TBHQ, lipid profiles, atherogenic index, atherosclerosis

INTRODUCTION

During the past two decades many scientific studies including animal models, epidemiological observations and clinical trials have been conducted to address the effects of fried oils on health and chronic disease.

Free radical reactions occur in human body and food systems. Oils are subject to chemical reactions (oxidation, hydrolysis and polymerization) which can occur particularly during deep fat frying. The extent of these reactions which may be reflected by a decrease in iodine value of the fat and an increase in free fatty acids, depends on the frying conditions (principally the temperature, aeration and duration) (Pokorny *et al*., 2001). Excessive production of these reactive substances can occur, due to oxidative stress caused by the imbalance of bodily previous bodily antioxidant defence system and free radical formation and also oxidised fat and oils enhance the production of these substances. These reactive species can react with biomolecules, causing the development of chronic diseases such as cancers and those that involve the cardiovascular systems (Halliwell, 1989).

Antioxidants have been found to offer protection against these diseases. Dietary previous antioxidants can augment cellular defences and help to prevent oxidative damage to cellular components (Halliwell, 1989). And also antioxidants have been used in food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. These components in food are readily oxidized by molecular oxygen and are major cause of oxidative deterioration, nutritional losses, of flavour

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development and discoloration like cottonseed oil (O'Brien, 2002). Which directly related to the oil's unsaturation degree, the breakdown products formed after oxidation process such as peroxides, aldehydes and ketones shorten the shelf life of oils and turn products unacceptable for consumption (Akgul, 1989).

On the other hand the addition of synthetic previous antioxidants, such as propyl gallate, tertiary butyl hydroquinone butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone has been widely used industrially to control lipid oxidation in foods. However, the use of these synthetic previous antioxidants has been questioned due to their potential health risks and toxicity (King and McCay, 1983; Lam *et al*., 1980).

In recent years the safety of synthetic food additives, including the possible toxicity of these chemicals used as antioxidants has received increasing attention. So, there is need for other components to act as antioxidants and to render food products safer for human (Kahl and Kappus, 1993). Akgul (1989) and Ozcan and Akgu (1995), reported that BHT causes liver expansion. Also, it is well known that these compounds cause unpleasant taste and smells as they easily vaporise and degrade. Yet these antioxidants suffer from the drawback that they are volatile and easily decompose at high temperatures. Additionally, it is still unclear whether continues consumption can lead to health risk (Martinez-Tome *et al*., 2001).

To prevent these harmful effects of synthetic antioxidants, the use of antioxidants which are found in foods and various natural materials is recommended (Ozcan and Akgu, 1995).

Today's antioxidants from natural sources have received much attention and efforts, which have been put in to identify compounds that can act as suitable previous antioxidants to replace synthetic ones. In addition, these naturally occurring previous antioxidants can be formulated as functional foods and that can help to prevent oxidative damage from occurring in the body (Pratt, 1992).

Thus there is an increasing interest in herbs and spices as sources of natural antioxidants (Baratta *et al*., 1998). Especially worthy of note are spices and herbs used for many years to enhance the sensory features of food (Adams *et al*., 1997).

The recent investigation of cinnamon has reported that cinnamon extract ions improve the insulin receptors function (Broadhurst *et al*., 2000; Jarvill-Taylor *et al*., 2001). Moreover the application in medicine, due to the strongly aromatic, sweet and warm odour, cinnamon is among the earliest known spices used by humans (Lee and Balick, 2005).

The volatile oil was screened for its potential as an antioxidant by using *in vitro* models, such as the β-carotene-linoleate and phosphomolybdenum complex method. The volatile oil showed 55.94 and 66.9% antioxidant activity at 100 and 200 ppm concentration, respectively. Also, the volatile oil showed good antioxidant capacity, using the formation of the phosphomolybdenum complex (Jayaprakasha *et al*., 2003).

Wang and Yang (2009) reported that the *trans*-Cinnamaldehyde was 16.25 in cinnamon essential oil of *Cinnamomum zeylanicum* leaf species and also *trans*-Cinnamaldehyde was detected to exist in all the species tested as an important volatile component. Singh *et al*. (2007) found that the major components were (*E*)-cinnamaldehyde (49.9%), along with several other components. Ozcan and Arslan (2011) mentioned that cinnamon oil was the most effective on retarding lipid oxidation of crude oils. Many studies have investigated the cytotoxicity of cinnamon oil and cinnamaldehyde, (King *et al*., 2007) showed that Cinnamaldehyde are antimutagenic against spontaneous mutations in mammalian (human) cells. These data lead to propose that CIN may induce DNA damage that elicits recombination DNA repair which reduces spontaneous mutations. Cinnamaldehyde has been known to have various biological activities including anti-inflammatory and anti-bacterial properties. It was observed that cinnamaldehyde suppressed lipopolysaccharide (LPS)-induced NFκB activation (Reddy *et al*., 2004). Ka *et al*. (2003) reported that cinnamaldehyde induces the ROS-mediated mitochondrial permeability transition and resultant cytochrome c release. This was the first report on the mechanism of the anticancer effect of cinnamaldehyde. The presence of trans cinnamic aldehyde in the essential oil of *C. zeylanicum* might have been responsible for the observed cytotoxicity. In study about the cytotoxic for activity of the essential oil from *C. zeylanicum* was evaluated in both cancer and normal cell line by MTT assay based on cell viability. 5RP7 (H-ras active-rat fibroblasts) and F2408 (normal rat fibroblasts) cells were exposed to the oil and then the viability of the cells was measured and expressed in terms of relative absorbance of oil-treated cells, in comparison to control cells (Unlu *et al*., 2010).

Therefore, the principal goal of this research was to study the effects of using cinnamon volatile oil and TBHQ synthetic antioxidant with fried oil on lipid profiles and haematological parameters in rats to get the possibility for using cinnamon oil as natural antioxidants with the fried oils.

MATERIALS AND METHODS

Materials: Fried Cottonseed oil without any antioxidant additive agents was obtained from The National Foundation for plant extracts (Alexandria). Synthetic antioxidants, Tertiary Butyl Hydro Quinone (TBHQ) was obtained from The Extraction Oils Co. Alexandria. Cinnamon essential oil was and other chemicals used in chemical analysis were obtained from *Sigma-Aldrich Co.* Analysis of essential oils using GC-MS was carried out at, the Central Laboratory, Faculty of Pharmacy. Chemicals kits of Cormay Co. were used in blood analysis which were done at the Central laboratory of the Faculty of Pharmacy.

Methods

Determination of essential oils major constituents by gas chromatography-mass spectrometry (GC-MS): GC-MS analysis of the essential oils was performed using a Shimadzu 2010 gas chromatograph equipped with a Shimadzu QP 2010 mass spectrometer in the electron impact mode (70 eV) according to (Wu *et al*., 2008). The analysis was conducted using Rtx®-5 capillary column (cross bond 5% diphenyl- 95% dimethyl polysiloxane 30 m, 0.25 mm I.D. film thickness 0.25 μm) under the following conditions: Injector, ion source and interface temperatures were set at 220, 200, 250°C, respectively. The oven temperature was held at 50°C for 3 min and then programmed to 240°C at a rate of 3°C min^{-1}. Helium was the carrier gas, at a flow rate 1 mL min^{-1} and split injection with split ratio 1:100. Diluted samples (1/100 in acetone, v/v) of 1.0 μL were injected manually. The components were identified based on the comparison of their retention times and mass spectra with those of standard authentic samples.

Frying conditions: Fresh potato slices (500 g/once), shelled before frying and cut into sticks. The temperature was adjusted on 177°C the batch of potato slices was fried every 30 min, during 2 h day^{-1} for 3 days (6 h frying) (Daniel *et al*., 2005).

Experimental design: Male healthy albino rats (6 rat/group), seven weeks old weighing about (80-85 g). They were kept in the animal kedges. The rats were housed in a well aerated steel mesh cages for two weeks before the beginning of the experiments. During this adaptation period, the rats were fed on the basal diet. It consisted of defatted powder milk 33%, lactose 5%, cellulose 3%, cottonseed oil 10%, salt mixture 4%, vitamins mixture 1% and cornstarch 44% (El Shafei, 2001).

After feeding on the basal diet for two weeks (*ad libitum*), the rats were divided into four groups (6 rats in each) and were fed for seven weeks (42 days), the basal diet with cottonseed oil treated differently for each group. Group 1 (G1) was given fresh cottonseed oil (Negative control). For the remaining groups, the cottonseed oil was used for frying potato for one hour. The oil was then cooled at room temperature and this treated oil or fried oil was used as follows: Group (G2) was given the basal diet with the fried cottonseed oil (positive control). Group (G3) was given the basal diet and the treated oil with added 200 mg kg^{-1} TBHQ before frying. Group (G4) cinnamon oil was added at 0.2% with heated oil:

- G1: Control Fresh oil
- G2: fried oil
- G3: fried oil+TBHQ
- G4: fried oil+Cinnamon oil

The diets and water were supplied (*ad libitum*). At the end of the experimental period, the rats were killed by decapitation. The blood samples were collected in tubes and centrifuged at 3000 rpm 30 min to obtain the serum by using micropipette and then was frozen at −20°C for further analysis. The blood samples with heparin subjected to serum for estimation of: uric acid (kidney function), creatinine (kidney function), total bilirubin (liver function), glucose, total protein, total lipids, total cholesterol, triglyceride, HDL (High Density Lipoproteins), LDL (Low Density Lipoproteins), VLDL calculated, atherogenic factors (calculated), the procedure of each test was done according to the protocol on the kits.

Statistical analysis: The data were analysed by one-way ANOVA, CoStat computerized programme, where appropriate, treatment means were compared using the Duncan's multiple range test. p-values<0.05 were considered as showing a significant difference between treatment means.

RESULTS AND DISCUSSION

GC-MS analysis: Analysis of cinnamon oil revealed that cinnamaldehyde is the major constituent of the oil with a percentage of 65.21%.

Biological evaluation

Lipid profiles: Biological evaluation of feeding the experimental rats for seven weeks, diets containing fried oil with added cinnamon volatile oil. Measurements of

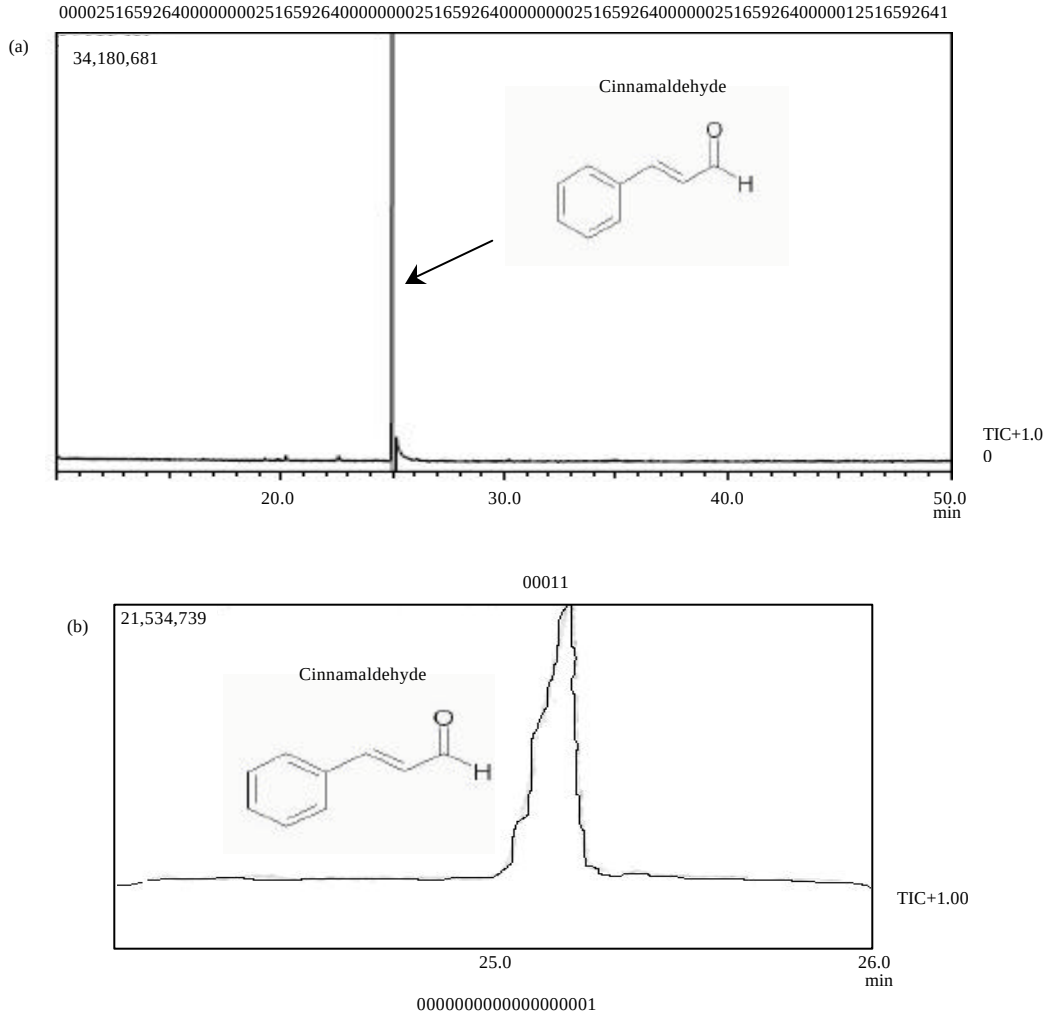

Fig. 1(a-b): (a) GC-MS analysis of cinnamon oil, (b) GC-MS analysis of main compound of cinnamon oil (cinnamaldehyde)

evaluations included serum lipids profile (Fig. 1-3) and haematological parameters (Fig. 3).

The highest total lipid contents; 551.06 and 533.03, mg dL^{-1} were found in G2 fried oil and G3 fried oil with TBHQ, respectively, representing significantly increased percentages of 17.74 and 13.6% compared with G1 control group (469.07 mg dL^{-1}). Moreover, total lipid contents in G4 cinnamon oil were the lowest (493.26 mg dL^{-1}) comprising only 5.1% as compared to G1 (Fig. 3).

Total cholesterol for G3 (92.91 mg dL^{-1}) and G2 (89.531 mg dL^{-1}) showed an increase of 21.90% and 26.51%, respectively, when compared to G1 which was 73.44 g dL^{-1}. But a decrease of -8.85% was observed in the G4 (66.94 mg dL^{-1}) in which cinnamon oil was added when compared to G1 control group (Fig. 2).

Compared to the G1 control group (Fig. 2), Triglyceride contents (161.99, 159.3 mg dL^{-1}) found in both the fried oil G2 and TBHQ G3 was 10.04 and 8.22% when compared to G1. Whereas a significant decrease of -13.85% was seen in triglycerides contents in the cinnamon oil group G4 (126.8 mg dL^{-1}).

The changes in HDL levels of rats fed on the tested diets, Fig. 3 showed values of 20.63 mg dL^{-1} in G2 fried oil and 21.22 mg dL^{-1} in G3 representing significant decrease of -20.68 and -18.41% compared to G1. There was no significant decrease observed in G4 (23.07, mg dL^{-1}) cinnamon oil group when compared to G1 (26 mg dL^{-1}) control group.

LDL levels were extremely low (18.47 mg dL^{-1}) in G4 compared to those of G1 (21.34 mg dL^{-1}) control group representing significantly decrease percentages of

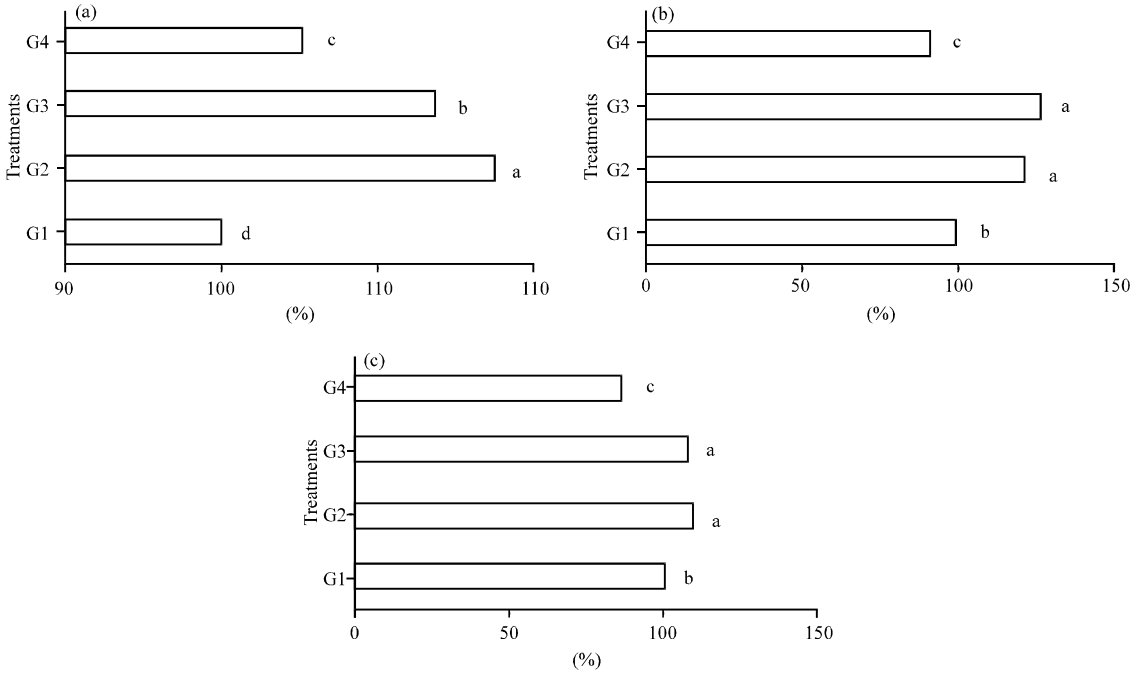

Fig. 2(a-c): Percentage changes of lipid profile (a) serum total lipids (b) total cholesterol and (c) triglycerides of rats fed fried oil with TBHQ or cinnamon volatile oil compared to the control, Letters displayed above the columns of figures are the significance variation among treatments at $p<0.05$

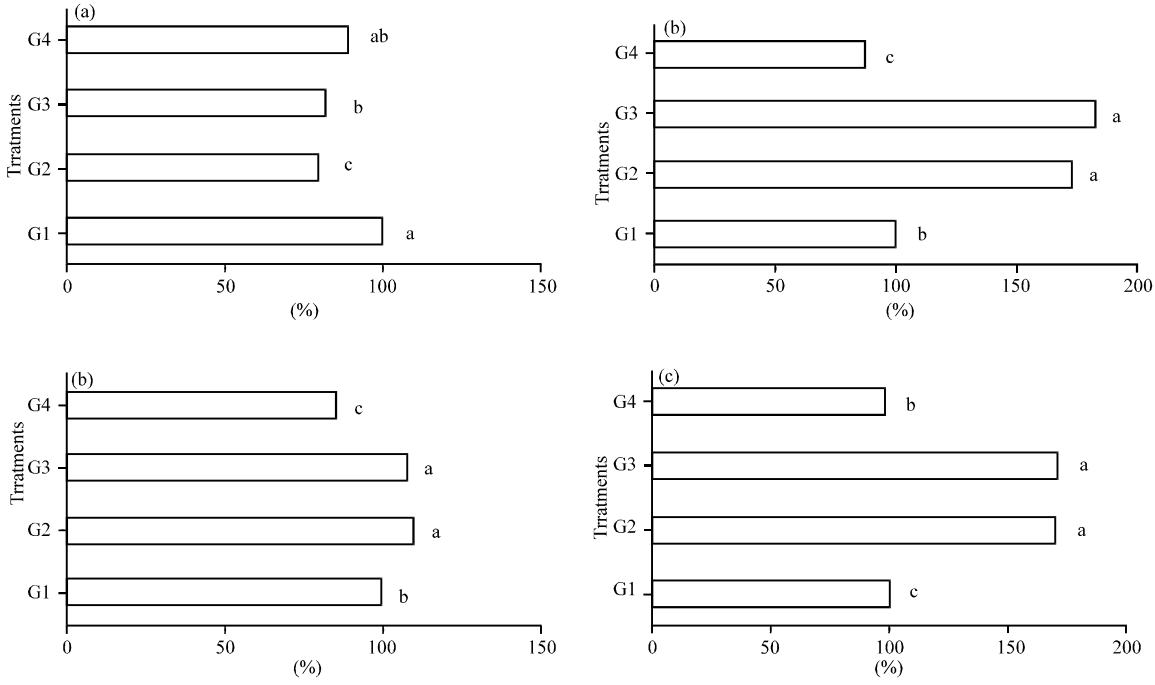

Fig. 3(a-d): Percentage changes of lipid profile (a) HDL, (b) LDL, (c) VLDL and (d) Atherogenic factor of rats fed frying oil with TBHQ or cinnamon volatile oil compared to the control, Letters displayed above the columns of figures are the significance variation among treatments at $p<0.05$

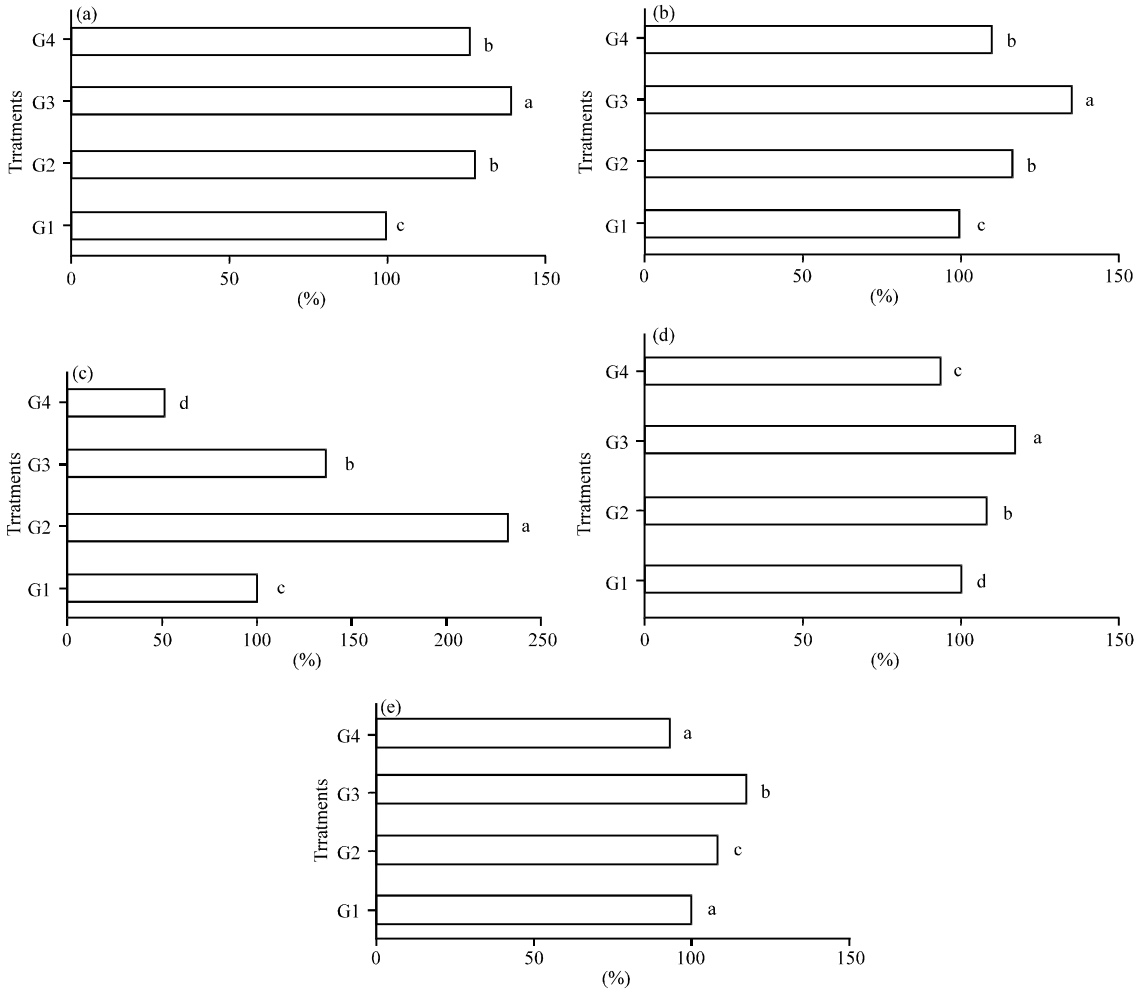

Fig. 4(a-e): Percentage changes of serum estimations parameters (a) Uric acid, (b) Creatinine, (c) Total bilirubin, (d) Serum glucose and (e) Total protein) of rats fed fried oil with TBHQ or cinnamon volatile oil compared to the control, Letters displayed above the columns of figures are the significance variation among treatments at $p<0.05$

-3.44 % over G1, in contrast there was significant increase (38.92, 36.99 mg dL^{-1}) in both G3 TBHQ and G2 fried oil which represented 82.38 and 73.33%, respectively in comparison to G1 (Fig. 3).

As shown in Fig. 3 G4 showed the lowest VLDL 25.36 mg dL^{-1} representing significantly decrease percentages of -13.85% compared to G1 control group (29.44 mg dL^{-1}), furthermore VLDL 32.4 and 31.86 were found in G2 fried oil and G3 fried oil with TBHQ, respectively representing significantly increase percentages of 10.05 and 8.22 % compared over G1 control group (29.44 mg dL^{-1}).

Atherogenic factor (AF) is a good indicator for atherosclerosis and heart disease risk. The results in Fig. 3 indicated that G2 and G3 had the highest atherogenic factor which were 3.36 and 3.34, respectively, representing significantly increased percentages of 70.55 and 69.54% compared to G1 control group (1.97), moreover, FA in G4 cinnamon oil were the lowest (1.97) comprising -2.53% of G1.

Serum estimation: Serum analyses demonstrated the results (Fig. 4) concerning the serum uric acid in rats fed on fried oils with adding synthetic antioxidants and cinnamon volatile oil. The data showed that the serum uric acid level was significantly increased from 5.09 mg dL^{-1} for the control animals to 7.07 and 6.53, mg dL^{-1} in G3 TBHQ and G2 fried oil, respectively. In G4 (cinnamon oil) there was least increase in serum uric acid 6.42 mg dL^{-1} which represented 26.13% over G1.

The results showed that serum creatinine was significantly increased in groups G3 TBHQ (0.42) and G2 fried oil (0.36 mg dL^{-1}) representing 35.48 and 35.48% in comparison to the G1 control group, But there was no significant differences between the G1 and G4 cinnamon oil 0.34 mg dL^{-1} in percentage 9.68% compared with G1 control group (Fig. 4).

Compared with the normal diet, adding cinnamon oil G4 produced a significant reduction in total bilirubin levels (0.13 mg dL^{-1}) by -48.0% of G1, Moreover there was significant increase in total bilirubin levels for both G2 fried oil (0.58 mg dL^{-1}) and G3 TBHQ (0.34 mg dL^{-1}) representing 132 and 36% respectively over G1 control group (Fig. 4).

There was significant reduction in the serum glucose level (155.19 mg dL^{-1}) by (-6.52%) for G4 cinnamon oil when compared with G1 control group, while other two groups (G2 and G3) have shown a significantly increase from 166.02 mg dL^{-1} for the control group to 195.35 and 179.60 mg dL^{-1} for G3 TBHQ and G2 fried oil groups, respectively which represent 17.67 and 8.20 % when compared to the G1 control group (Fig. 4).

G3 and G2 produced a significant elevation in the serum total protein by 1.27 and 4.36%, respectively compared to G1, however there was no difference between G1 and G4 as shown in Fig. 4.

DISCUSSION

Historically it was mentioned that cinnamon has been used in food applications by ancient Egyptians and Ancient Chinese and also in medical applications. In this study we examined the effects of using cinnamon volatile oil and TBHQ synthetic antioxidant with fried oil-on the lipid profiles and haematological parameters in rats to get the possibility for using cinnamon oil as natural antioxidants with the fried oils. The recent studies reported that Cinnamon has potential lipid lowering properties in animal and human studies (Kannappan *et al*., 2006).

El-Baroty *et al*. (2010) reported that Cinnamon oil was characterized with high amounts of oxygen-containing monoterpenes (69.65% of the total oil). Of which, cinnamyl aldehyde (45.13%), this data agreed with our study. And also Mallavarapu *et al*. (1995) mentioned that a commercial sample of essential oils from Cinnamon contained approximately 63% cinnamaldehyde, 8% limonene, 7% eugenol. Similar results were obtained by Ooi *et al*. (2006), who reported that the analysis of hydro-distilled Chinese cinnamon oil by GC-MS in comparison with pure cinnamaldehyde, cinnamaldehyde was found in a percentage of 85%.

When studying the previous results it can be noted that feeding the rats with fried oil without any additions led in most cases to negative impacts related to lipid profile. These harmful results are due to the effect of heating the oil. The feeding on fried oil resulted to increasing in total lipids which may lead to increase the risk factor of atherosclerosis (Abd El-Wahed, 2002) reported that rats fed on smoked cottonseed oil, total lipids were higher than in rats fed on fresh cottonseed oil. Adding TBHQ and the essential oils opposed the harmful effect of heating the oil during frying by scavenging of formed free radicals or protected the unsaturated fatty acids from oxidations and decomposition as well as lowering the formation of free fatty acids, trans fatty acids and oxidation products which have adverse effect on the health and nutritional status of living beings. On the other hand it is important to compare between TBHQ and essential oils. Looking at the effects of cinnamon oil in different assays one would conclude that cinnamon essential oils contains relatively high amounts of phenolic compounds (18.2%, of the oil), this phenolic group plays an important role in antioxidant activity which act as hydrogen donor. There are many reports emphasize that the positive correlation between volatile phenolic compounds in the essential and its antioxidant activity (Farag *et al*., 1989).

Whatever the results that are produced by the TBHQ, it must not be forgotten that the systemic products have always side effects. So it is advisable to counterpart the effects of heating the oil during frying by adding the essential oil as natural sources. Of course the choice of these medicinal plants is according to the preference of the consumer in relation to colour, taste and aroma as well as expects. It was reported that tert-butylhydroquinone (TBHQ) is highly effective cancer chemopreventive agent. These agents have been shown to act against tumour formation by a variety of carcinogens in animal models of human cancers. (King and McCay, 1983; Lam *et al*., 1980).

The major finding reported in this study is that cinnamon volatile oil has positive effects on blood lipid profile and these good effects may due to the major constituent of the cinnamon essential oil. It was reported that cinnamon exerts clinically significant lipid-lowering effects; it has reduced mean total cholesterol by 12 to 26%, triglycerides by 23 to 30% and LDL-cholesterol by 7 to 27%. (Khan *et al*., 2003). These data refer to that adding essential oils have antioxidant activity which led to reduce heart diseases risk.

Moreover, the increasing of serum uric acid due to the effects of fried oil (Abd El-Wahed, 2002), found that rats fed on hypercholesterolemic factors increased serum uric acid largely 183% than the control animals.

Bilirubin results from hemoglobin decomposition in spleen, liver and bones, in small amount. It is the base of bile composition; and is a good indication of the liver performance. Bilirubin percentage increases by the diets containing hypercholesterolemic factors (Abd El-Wahed, 2002). The results show that using cinnamon essential oils has a reduction effect.

From the previous results, it appears that fried oil caused an increase in glucose level. These results match other studies which reported that feeding on hypercholesterolemic diets increased serum glucose level (Abd El-Wahed, 2002).

Based on the results of this study and the published researches, we propose that cinnamon essential oil in the insulin transduction pathway leading to the beneficial effects in people with type 2 diabetes. Cinnamon polyphenols affect multiple steps of the pathway, first, cinnamon polyphenols activate insulin receptors by increasing their tyrosine phosphorylation activity and by decreasing phosphatase activity that inactivates the receptor (Imparl-Radosevich *et al*., 1998); the second cinnamon polyphenols increase the amount of insulin receptor protein; third cinnamon polyphenols increase the amount of GLUT4 protein glucose transporter the increases of GLUT4 protein by this polyphenols compounds may therefore suggest a positive effect of these compounds on the long-term regulation of glucose transport.; fourth cinnamon polyphenols increase glycogen synthase activity and glycogen accumulation (Cao *et al*., 2007). Cinnamon exerts clinically significant glucose lowering effects, it has reduced mean fasting serum glucose by 18-29% (Khan *et al*., 2003).

Since cinnamon essential oils help protect the polyunsaturated fatty acids from being destroyed so it advisable to consider including these aromatic oils, the diets especially with patients with cardiovascular diseases or among diabetics.

In summary, this study reports novel findings that cinnamon volatile oil polyphenols exhibit the potential to increase of lipid profiles total cholesterol, Total lipids, triglyceride and Atherogenic factor which consider heart disease risk factors which caused by contentious consuming for fried foods, More over Cinnamon volatile oil improve some serum estimations which has been associated with fried oil feeding and using synthetic antioxidants (TBHQ).

CONCLUSION

From these results it may be concluded that addition of cinnamon volatile oils to fried oil has improved the lipid profiles and also haematological parameters than using TBHQ synthetic antioxidants and these effects may due to the main component of the cinnamon oil. Therefore, more research on this point should be carried out aiming to replace the synthetic chemical antioxidants with natural one.

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