Original research paper

Reduction of detrimental effect of soybean oil *in-vivo* using watermelon white rind extract

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Aim: To study the effect of white rind extract on decreasing soybean oil impact on calcium and phosphorous blood levels *in vivo.*

Method: Dried watermelon white rind was directed to mycotoxin and elemental determinations to assure its safe usage. Soybean oil was subjected to fatty acid and GC-MS analysis. Biological experiment was conducted using male albino rats fed diet prepared by soybean oil and supplied with aqueous watermelon white rind extract for two months' interval period. At the end of the experiment, the calcium and phosphorus in blood were determined.

Results: The rind was free from aflatoxin and ochratoxin. Watermelon white rind aqueous extract contained iron, copper, potassium, chromium and selenium at concentration ranges of 3.4, 0.53, 45.51, 0.0142 and 0.0985 ppm, respectively.

Soybean oil had free fatty acid, peroxide value, iodine number and anisidine value of 0.43%, 13.62 meq O_2/Kg, 132 and 0.7, respectively.

GC-MS analysis of soy oil ascertained the presence of twenty-four compounds: linoleic acid, methyl ester (25.27%), monensin (15.75%), elaidic acid (9.24%), nonadecanoic acid, methyl ester (7.04%), cis-13-eicosenoic acid (4.92%), cis-vaccenic acid (4.68%), linoleic acid (4.67%), palmitoleic acid (4.46%), 9-tetradecenal (4.42%) and cysteine (4.18%)were the most predominant.

Fatty acid profile of the oil showed that the ratio of saturated fatty acid to unsaturated fatty acids was 1:5.

Conclusion: Rats fed diet prepared by soybean oil had a decreased calcium level in comparison with negative control ($p<0.05$). Supplementation with watermelon white rind aqueous extract rendered calcium level to normal status as negative control. Phosphorus level wasn't affected by soya oil.

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KEYWORDS: watermelon white rind, fatty acid and GC-MS analysis, calcium and phosphorous blood levels.

INTRODUCTION

Watermelon (*Citrullus lanatus* var. *lanatus*, family Cucurbitaceae) is a flowering plant originally from southern Africa. Egypt is one of the top five watermelon producers with total amount of 1,874,710 tones yearly [1]. The white rind is thrown as unused-agro waste. Rind constitutes 30% of the weight of whole watermelon fruit.

Ola *et al.* [1] cited that ethanolic and aqueous extracts of watermelon white rind possessed antibacterial activity against *E. coli and Salmonella sp.* Gas Chromatography-Mass Spectrometry analysis revealed the existence of methionine, L-Aspartic acid, Glycyl-D-asparagine, 9-Cis-Retinoic acid, Stearic acid allyl ester and Ascorbic acid permethyl that contributed to its antibacterial activity.

The rind had total antioxidant activity of 297 mg AAE/100g, total phenols content of 139.6 mg GAE/100g and total flavonoids of 40.4 mg QE/100g. FRAP assay indicated the high reducing ability of the rind. Crude protein content amounted to 13.3%, crude fiber (14.7%) and fat (2.11%). The rind is a source of iron (30.4 mg/kg), potassium (6.95%), copper (9.4 mg/kg), chromium (85µg/100g) and selenium (542µg/100g). Unsaturated fatty acid amounted to 81.2%. Vitamins A and E valued 383.44 µg/100g and 43.72 mg/100g, respectively [2]. Wastes are source of sugars, minerals, organic acids, dietary fiber, and bioactive compounds [3] .

Fatty acids and their types play an important role in human’s health, high-fat diets, over long-term feeding regimes can affect the bone structure and bone health[9]. Soybean oil affected negatively bone structure as reported by Carlos [4]. A study investigated the adverse effect of soybean oil in rat found that oil induced significant fatty liver [5].

In the present work, biological experiment was designed to evaluate safety usage of watermelon white rind extract on decreasing soybean oil impact on calcium and phosphorous blood levels *In-Vivo.*

MATERIAL AND METHODS

Watermelon white rind was cut into small pieces, dried at 40˚C and pulverized into fine powder.

Preparation of white rind aqueous extract:

One gram of dried powder was mixed with one liter of hot water, stirred, filtered and used as the sole source of fluid.

Determination of Aflatoxin and Okratoxin

Total Aflatoxin and Ochratoxin were determined according to AOAC [6]. Total aflatoxins and ochratoxin A standards were purchased from Sigma (St. Louis, MO, USA). Stock solutions of each mycotoxin were prepared by dissolving solid commercial toxin. The presence of aflatoxins was detected by high performance liquid chromatography (HPLC, Agilent 1200) using C18 column of LiChrospher RP-18 (5µm × 25cm). The mobile phase consisted of water: methanol: acetonitrile (54:29:17, v/v/v) at flow rate of 1ml/min. The excitation and emission wavelengths for all aflatoxins were 362 and 460 nm.

Elemental analysis of rind aqueous extract

Iron, copper, potassium, chromium and selenium were determined according to AOAC [7]. Minerals in the different samples were determined using atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc., Norwalk, CT, USA).

Chemical analysis of soybean oil

Quality of oil was assessed by determining anisidine value, iodine number, peroxide value and free fatty acid according to AOAC [7]. Fatty acid composition was determined according to AOAC [7]. The derivatization was conducted following the procedure described by AOAC Official Method 991.39 (AOAC, 2012), with modification in the amount of initial sample, the kind of extracting solvent and the temperature of heating. GC Analysis FAMEs from external standard or FAMEs resulted from sample derivatization were injected separately into Gas Chromatography instrument (GC). The GC analyses were performed on 7890A Gas Chromatography System (Agilent Technologies, California, US) equipped with flame ionization detector and splitless injector (1 µL). Injector and detector temprature were set at 270 °C and 280 °C, respectively. The utilized column was a DB-23 (60 m × 0.25 mm, with film thickness of 0.25 µm). This column was purchased from J and W Scientific (Folsom, CA). The GC oven program was as follows: 130 °C (hold 2 min), to 170 °C at 6.5 °C/min (hold 5 min), to 215 °C at 2.75 °C/min (hold 12 min), to 230 °C at 30 °C/min (hold 30 min). Helium and nitrogen of ultrahigh purity grade were used as carrier gases at flow rates of 11.07 and 31.24 mL/min.

The chemical constituents of the samples were identified using GC (Agilent Tecnologies 7890A) connected to a mass-selective detector (MSD, Agilent 7000). The flow of helium used as carrier gas was retained at 1 ml/min during the run. The components were confirmed by coordinating their mass spectra and retention time with the database of National Institute of Standard and Technology (NIST) library. The names, molecular weights and chemical structure of each of the components of the test materials were determined.

Total Aflatoxin and ochratoxin standards were purchased from Sigma (St. Louis, MO, USA). Stock solutions of each mycotoxins were prepared by dissolving toxin in the appropriate solvent at concentration of 1 mg/mL. AFs in toluene/acetonitrile 99:1 and OTA in toluene/acetic acid 99:1. Extraction and identification of aflatoxins from

Biological experiment

Eighteen rats were distributed into three groups:

Group (1) served as negative control and fed normal diet [8] and supplied with drinking water.

Group (2) served as positive control fed normal diet to which 150 ml soybean oil was added per kilo and supplied with drinking water.

Group (3) fed diet as group (2) supplied with aqueous watermelon white rind extract.

Diet and fluids were supplied *ad-libitum* for all groups.

At the end of the experiment, blood samples were collected centrifuged at 4000 rpm and serum was subjected to the analysis of calcium and phosphorus.

Statistical Analysis

Statistical analysis of biology experiment was done using SPSS and the means were compared by ANOVA at significance level ($p<0.05$).

RESULTS AND DISCUSSION

Elemental analysis of watermelon white rind aqueous extract (Table 1) ensured the presence of iron (3.4 ppm), cupper (0.53 ppm), potassium (45.5 ppm), chromium (0.014 ppm) and selenium (0.098 ppm). Aqueous rind extract was a source of mineral needed for health maintenance as clearly demonstrated.

Data in Table (2) revealed that soybean oil had anisidine value of 0.7, iodine number 131.2, free fatty acid 0.43% and peroxide value of 13.62 meq O_2/Kg. Results ensured low values of free fatty acid and anisidine and best soybean oil quality.

Twenty-four compounds were detected in the GC-MS chromatogram of soybean oil. Linoleic acid (25.27%) was the most predominant in the tested oil, followed by monensin (15.75%), elaidic acid (9.24%), nonadecanoic acid (7%), cis-vaccenic acid (4.63%), palmitoleic acid (4.46%), 9-tetradecenol (4.42%) and cysteine (4.18%) and accounted for 59.34% of oil constituent (Table 3).

Linoleic acid is a doubly unsaturated fatty acid, known as an omega-6 fatty acid, occurring widely in plant glycosides. Linoleic acid is an essential fatty acid in human nutrition because it cannot be synthesized by humans [9].

Elaidic acid is the major trans fatty acid in margarine and partially hydrogenated oils and also occurs in small amount in cow milk [10].

Nonadecanoic acid is a long chain saturated fatty acid derived from plant sourcesand can be found in fats and vegetable oils [11].

Vaccenic acid, an isomer of oleic acid, is the principal ruminant *trans* fatty acid. It is produced through the biohydrogenation of linoleic acid and α-linolenic acid by microorganisms in the rumen and is found naturally in foods such as dairy and ruminant meat products.

As in Table (4), fatty acid profile of soybean oil showed the existence of linoleic acid (54.28%), oleic acid (22.85%), linoleic acid (6.2%) and gadolic acid (0.21%) as unsaturated fatty acids accounting for 83.54% of total oil content. Saturated fatty acids comprised palmitic acid (10.99%), stearic acid (4.82%), arachidic acid (0.36%) and behenic acid (0.29%) representing 16.46% of soybean oil content. These results are in accordance with Friedman and Brandon [12] who stated that soybean had low level of saturated fat and high content of linoleic acid [13].

As shown in Table (5), a significant difference ($p<0.05$) existed between negative control (G1) and rats group fed diet with soybean oil (G2). A decrease in calcium level was observed indicating that soybean oil affected calcium blood level.

Soybean had high phytate level [14]. Phytates can block the uptake of essential minerals as calcium, copper, iron, zinc and magnesium in intestinal tract that may contribute to mineral deficiencies [15].

There was non-significant difference between negative control (G1) and Group 3 fed soybean oil and drunk rind extract, nor between G2 and G3.

Data revealed that phosphorus blood level was not affected by any treatment and non-significant differences existed between G1and both groups G2 and G3.

The extraction of plant material and isolation of biologically active compounds are essential to understand their role in disease prevention and treatment.

CONCLUSION

Watermelon white rind aqueous extract is a source of iron, copper, potassium, chromium and selenium. Soybean oil decreased blood calcium level, while phosphorus was stable in all treated groups. Supplementation with watermelon white rind aqueous extract rendered calcium level to normal status as negative control.

Table (1): Elemental analysis of watermelon rind aqueous extract

Element	Result
Copper (ppm)	0.5
Iron (ppm)	3.4
Potassium (ppm)	45.5
Chromium (ppm)	0.014
Selenium (ppm)	0.098

Table (2): Chemical evaluation of soy oil

Tested parameters	Result
Free fatty acid (%)	0.43
Peroxide number (meq O_2/Kg)	13.62
Iodine number	131.8
Anisidine value	0.7

Table (3): GC-MS analysis of soy oil

RT	Compound name	Area sum (%)
3.88	Chicoric acid	0.29
5.7	Phytanic acid	0.59
6.187	3,2′,4′,5′-Tetramethoxyflavone	0.27
8.04	Gardenin	0.49
8.96	Isovitexin	0.59
11.7	Lutein	1.33
12.03	Stevioside	0.57
13.23	Hexadecanoic acid, methyl ester	2.63
13.43	Pentadecanoic acid	0.73
13.5	Monensin	15.75
13.9	Zearalenone	1.59
14.17	Oleic acid	2.83
14.35	Cis-vaccenic acid	4.68
14.52	Linoleic acid, methyl ester	25.27
14.59	Elaidic acid	9.24
14.66	Cis-13-eicosenoic acid	4.92
14.75	Nonadecanoic acid, methyl ester	7.0
14.93	Linoleic acid	4.67
15.14	Quinine	0.5
15.33	3-(3,4-dimethoxyphenyl)-4,6-dimethylcoumarin	0.98
15.9	Di-γ-linolenin	1.97
16.009	Palmitoleic acid	4.46
16.04	Cystine	4.18
16.79	9-tetradecenal, (Z)-	4.42

Table (4): Fatty acid analysis of soybean oil

Fatty acid	Classification	Relative distribution
Palmitic acid C16:0	Saturated fatty acid	10.99%
Stearic acid C18:0	Saturated fatty acid	4.82%
Arachidic acid C20:0	Saturated fatty acid	0.36%
Behenic acid C22:0	Saturated fatty acid	0.29%
Oleic acid C18:1n9	Unsaturated fatty acid	22.85%
Linoleic acid C18:2n6	Unsaturated fatty acid	54.28%
Linolenic acid C18:3n3	Unsaturated fatty acid	6.2%
Gadolic acid C20:1ω9	Unsaturated fatty acid	0.21%

Table (5): Serum calcium and phosphorus levels in treated rat groups

Groups / Parameters	Group 1 (n=6)	Group 2 (n=6)	Group 3 (n=6)
Calcium (mg/dl)	13.2±0.64	11.3±0.48 *	12.8±0.62
Phosphorus (mg/dl)	10.5±0.66	10.38±0.76	11.96±0.44

*Significant difference ($p<0.05$) in comparison with negative control

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