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Phytochemical Constituents, Antioxidant Activity and Safety Evaluation of Kei Apple Fruit (*Dovyalis caffra*)

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Abstract

The present study was conducted to determine the phytochemical constituents of that fruit, to evaluate the antioxidant activity of *Dovyalis caffra* aqueous methanolic extracts and to examine the potential toxicity of aqueous methanolic extract after acute and sub chronic administration in rats. The results indicated that twenty-two phenolic compounds could be identified in dried *D. caffra* fruit and their whole fruit aqueous methanolic extract. Chlorogenic is the main identified phenolic compound in the dry fruit (2270 µg/g dry fruit), while pyrogallol was the most identified phenol in whole fruit aqueous methanolic extract. Malic acid was the most abundant organic acid in the fruit, its concentration reached (101.50 mg/g). In sub-chronic experiment, hemoglobin, red blood cells, total white blood cells and mean cell hemoglobin values did not significantly alter when male and female rats ingested at least 1000 mg/kg *D. caffra* whole fruit aqueous methanolic extract. Biochemical analysis showed some significant changes especially in female rats. When male rats treated with 1000 mg/g, 2000 mg/g or 4000 mg/g of *D. caffra* whole fruit aqueous methanolic extract, the values of sodium, potassium, total protein, albumin, glucose and alanine amino transferase (ALT) did not significantly differ when compared with control rats. No abnormal cases were recorded of internal organs between control and treated rats. The fatal dose of the extract is more than 12000 mg/kg.

***Keywords:** Dovyalis caffra; Phenolic profile; HPLC; Antioxidant; Acute and sub-chronic toxicity*

Introduction

D. caffrais a small tree with yellow edible fruits and commonly known as kei apple is [1]. It well known in African traditional medicine, that the roots and thorns of kei apple are used to treat chest pain and amenorrhea [2]. Different parts of Dovyalis species plants are stated to treat rheumatic fever pain [3]. The fruit which resemble apricot is more suitable for the purposes of food preservation because their high acidity content. Pharmacological studies demonstrated that fruit aqueous extract stimulates intestinal motility, inhibit uterine contractions and prolong the stimulation of ventricular contractions [4]. The same authors also mentioned that toxic dose of the fruit extract causes death through acute heart failure. The fruit juice of

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kei apple showed high polyphenolic content as well as high antioxidant potential [5]. The extracts of different parts of the plant have shown antibacterial activity [6-7].

Recently edible fruits such as pomegranate and mulberry and other fruits with unknown edible properties such as red dragon was evaluated for their potential toxicity in acute and sub-chronic studies on animal models [8-10]. In this respect, insufficient information on the potential toxicity of *D. caffra* lead to the present investigation which is aimed to determine the bioactive constituents in the dried whole fruit in addition to evaluate *in vitro* antioxidant potential of fruit aqueous extracts and safety assessment of whole fruit aqueous extract in rat model.

Materials and Methods

Fruit samples

Dovyalis caffra fruits were obtained from a local farm in Belbis, Sharkia governorate, Egypt. The fruits were carefully washed under running tap water, sliced into small pieces and dried at 50°C.

Chemical composition

Moisture, protein, fat, ash and fibers were determined in dried fruit samples according to the method described in AOAC [11]. Carbohydrates calculated by differences.

Chemical properties

Total acidity: Total acidity of *D. caffra* fruits was measured according to the method of AOAC [11]. Water extract of fruits was prepared in the ratio of 1:4 and titrated to pH 8.1 with 0.1N NaOH solution. Total acidity was expressed as percentage of malic acid.

Vitamin C determination: Vitamin C content of *D. caffra* fruits was estimated using colorimetric method described by [12].

Carotenoids extraction and total content: Total carotenoids were estimated according the method described in details by De Carvalho, et al. [13].

Phytochemical constituents of aqueous extracts

Extraction of the fruit: Both dried flesh part and the whole parts of the fruit included: seeds, skin and flesh parts were macerated with distilled water/methanol mixture (1000 g of fruit in 3 litre of distilled water/methanol mixture [4:1] for 4 h, four cycles). Thereafter, filtration was done and the resultant filtrates were concentrated under vacuum in a rotary evaporator to produce brown aqueous extracts of *D. caffra* whole fruit as well as the flesh part. The dried extracts were sealed in two bottles and stored in the deep freeze until further use. Sticky extracts were subsequently resolved in distilled water to required concentrations.

Preliminary phytochemical tests: Qualitative phytochemical tests were carried out on aqueous extract of whole parts of the *D. caffra* fruit as well as flesh aqueous extract to detect the presence of flavonoids, saponins, terpenes, alkaloids, phenols,

tannins and glycosides. All phytochemical screening tests of the mentioned components were carried out according to Harborne [14].

Quantitative determination of total flavonoids content: Total flavonoids content of *D. caffra* fruit aqueous extracts was quantitively determined according the method described by Lin and Tang [15]. Standard curve was plotted for yellow color absorbance values resulted from the reaction of quercetin in different concentrations [0.005 mg/ml to 0.1 mg/ml] with aluminum chloride. Total flavonoid content was expressed as milligram quercetin/g dry extract.

Quantitative determination of total polyphenolic content: Total phenolics of *D. caffra* fruits aqueous extracts were determined according to Folin-Ciocalteu method as described by Singleton et al. [16]. After the incubation of reaction mixture for one hour at 25°C, the developed blue color was measured at 765 nm and total polyphenols content were expressed as mg gallic acid per gram dry extract.

HPLC analysis

HPLC analysis was conducted in The Laboratories of Food Technology Research Institute, Giza, Egypt.

Determination of organic acids: Organic acids in *D. caffra* fruit were determined by HPLC technique according to the method of Wodecki et al. [17].

Identification and quantification of flavonoids: Flavonoids were identified and quantified in dried fruits as well as in *D. caffra* whole fruit aqueous extract by An Agilent 1100 Series high-performance liquid chromatography equipped with diode array detector. The conditions of the analysis were described in detail by Mattila et al. [18].

Identification and quantification of polyphenols: Phenolic compounds were extracted from dried fruits as well as from aqueous extract of *D. caffra* and then injected into reversed phase HPLC/diode array detection (Hewlett Packard 1050) with a guard column Alltima C18, 5 mm. Identification and quantification of *D. caffra* polyphenols were done according to the method described in details by Goupy et al. [19].

Antioxidant activity

DPPH radical assay: Free radical scavenging activity of *D. caffra* fruit aqueous extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated according the method described in detail by Shirwakar et al. [20]. Absorbance of the resultant hydrazine was measured at 517 nm using a UV-VIS spectrophotometer. Percentage of free radical scavenging activity was calculated according to the following equation:

$$\% \text{ scavenging activity} = [(Ac - As)/Ac] \times 100$$

Where Ac=absorbance of control and As=absorbance of sample.

Nitric oxide scavenging ability: At physiological pH, nitrite ions could be formed as a result of interaction of oxygen with nitric oxide which was generated from sodium nitroprusside solution; and quantified according to Griess Illosvoy reaction

[21]. Various concentrations of *D. caffra* fruitaqueous extracts ranged between (25-200 µg/mL) were examined as nitric oxide scavengers. The generated pink chromophores were measured colorimetrically at 540 nm. Quercetin was used as a positive control. Nitric oxide scavenging ability as (%) was calculated by using above formula for DPPH assay.

Reducing power: Reducing power of *D. caffra* fruit aqueous extracts at various concentrations ranged between (31.25-1000 µg/mL) was determined according to the developed method of Kajaria et al., [22]. The reducing power of tested samples were standardized against gallic acid and expressed as difference in optical density. High absorbance values indicate the stronger reducing property.

Experimental animals

Male and female Sprague-Dawley (SD) rats were used for both types of toxicology studies. Rats were adapted to laboratory environment for 10 days prior to acute and sub chronic experiments. Animals were kept around 25 C with 75 ± 5% relative humidity in polyethylene cages (five animals/cage), with free access to normal diet and tap water ad labium. Percentages of chemical constituents of meal pellets for the experimental animals were 48.0, 21.0, 3.0, 9.0% and 12.0% for carbohydrate, crude protein, crude fat, crude ash, crude fiber and moisture, respectively.

Acute toxicity study in rats: Rats of both sexes, aged ten weeks old and weighing 120 g to 150 g were used. The aqueous extract of *D. caffra* whole fruit was dissolved in distilled water and administered orally in the concentrations of (3 g/kg, 6 g/kg and 12 g/kg) in single doses to both female and male rats (4 males and 4 females), whereas the control group received only distilled water. Body weight, toxicity signs such as mortality were recorded after single oral doses at the first, second, third and sixth hour and once daily for next 14 days, then all rats were kept fasted for 18 h and were killed by inhalation. Rats were dissected and different organs were observed. The relative organ weight (ROW) of each examined organ (i.e., liver, kidney and heart etc.) was then calculated as follows:

ROW=Weight of organ/Body weight of rat × 100%

Subchronic toxicity study in rats: Male and female rats of were randomly divided into four groups: a control group and three treated groups (n=10; 5 males and 5 females). The *D. caffra* whole fruit extract was resolved in distilled water and orally administered for 28 days at single doses of 1000 mg/kg, 2000 mg/kg and 4000 mg/kg (5 ml/kg), while control group received only distilled water. Rat's behavior was daily observed and they were weighed once a week. After 28 days of feeding period, all rats were anesthetized under inhalation and blood was collected *via* orbital plexus in the eyes into non-heparinized and EDTA-containing tubes for biochemical and hematological analyses, respectively. Thereafter, rats were killed; organs were removed then weighed and the relative organ weight was than calculated. Liver and kidneys were preserved for histopathological study. Hematological parameters were determined using an automated hematology analyzer. Serum biochemical examinations were performed using commercial kits. Serum total cholesterol (TC) [23], triglycerides (TG) [24], glucose [25], creatinine [26], uric acid [27,28] and GPT [29], sodium [30], potassium [31], total protein [32] were estimated using Spin react Company, Spain Kits. Albumin [33] was determined using Human company German Kit. Alkaline phosphatase estimated by the detection of liberated phosphate [34] using spectrum company kit.

Results and Discussion

Chemical analysis of kie apple fruit

Moisture content of dried kie apple whole fruit was 15.50%. Chemical analysis showed that dried kie apple fruit contained 4.0, 7.45, 16.3, 3.0 and 54.05 of ash, protein, fiber, crude ether extract and carbohydrates, respectively. The presence of seeds in the fruit increased the content of crude fiber**.** There were no previously researches dealt with the chemical composition of dried kie apple fruits.

Some chemical properties of kie apple fruits were estimated such as titratable acidity, ascorbic acid (mg/100 g) and carotenoids content. Percentage of titrable acidity of kie apple fruit as shown in TABLE 1 was 3.49%. So, fruit is however considered too acidic to beat directly fresh. There were no previously reports about titrated acidity of kie apple fruit. Colorimetric method for the determination of vitamin C content demonstrated that *D. caffra* fruits contained 162.50 (mg/100 g). The obtained value for ascorbic acid was higher than that recorded by Morton [35]. Total carotenoids content in pet ether layer calorimetrically estimated and calculated by a known equation. It could be seen from TABLE 1 that dried kie apple fruit contained 59.89 µg carotenoids per gram.

TABLE 1. **Chemical properties of kie apple fruit.**

Chemical properties	Value
Titratable acidity % (as maleic acid)	3.49%
Ascorbic acid (mg/100 g)	162.50
Carotenoids content µg/g	59.89

Phytochemical constituents of *D. caffra* fruit aqueous extracts

Phytochemical screening: The phytochemical screening of *D. caffra* WFAME and FAME which were extracted with mild hot maceration revealed the presence of glycosides, phenolics, tannins and flavonoids. Alkaloids, terpenes and saponins were not detected.

Lavonoids and polyphenols: TABLE 2 showed the quantitative analysis of total polyphenols (as mg of gallic acid equivalent/gram extract) and total flavonoids (as mg of quercetin equivalent/gram extract) of *D. caffra* WFAME and FAME. Whole fruit extract showed higher polyphenols (29.01) and flavonoids (13.71) than those obtained by flesh aqueous extract. This might be due to the presence of the skin and the seeds of fruits which have a considerable amount of phenolics. In this respect, Beer [36] showed that high amount of phenolic compound in dry weight kie apple are found in the seeds as 1990 mgGAE/100 g followed by the peels (as 1126 mgGAE/100 g) followed by the flesh of the fruit (as 521 mgGAE/100 g).

TABLE 2. **Total polyphenols, total flavonoids of aqueous extracts of *D. caffra* fruit.**

	Total polyphenols	Total flavonoids
Flesh aqueous extract	18.50	11.44
Fruit aqueous extract	29.01	13.71

Analysis

Identification and quantification of organic acids in kie apple fruit: The concentrations of the detected organic acids in *D. caffra* whole dried fruit are shown in TABLE 3. Malic acid was the most abundant organic acid, its concentration reached

(101.50 mg/g). Low concentrations of propionic, citric acid, oxalic acid, succinic and lactic acids were detected. No previous papers identified organic acids of dried *D. caffra* fruit by HPLC technique.

TABLE 3. **Organic acids in dried kie apple fruit.**

Organic acid	Succinic acid	Malic acid	Citric acid	Oxalic acid	Lactic acid	Propionic acid
(mg/g)	3.57	101.50	5.74	4.21	3.42	6.4

Identification and quantification of phenolics in kie apple whole fruit: Twenty-two phenolic compounds of *D. caffra* whole fruits and its aqueous extract could be identified and quantified by HPLC technique as shown in TABLE 4. The phenolic profile contained eleven phenolic acid derivatives, three bound flavonoids and three free flavonoids with variable concentrations. Chlorogenic acid is the predominant phenolic acid in *D. caffra* dry fruit (2270 µg/g dry fruit). While, the results for aqueous extract revealed that pyrogallol is the predominant phenolic acid (5070 µg/g dry fruit). This could be interpreted by the higher solubility of pyrogallol in water (40 g/100 ml) than chlorogenic acid (4 g/100 ml).

TABLE 4. **Identification and quantification of flavonoids and other phenolics of aqueous extract of *D. caffra* fruit by HPLC.**

	Aqueous extract µg/g	Dry fruit µg/g
Hesperidin	0077	79.00
Rutin	0077	26.00
Rosmarinic	0077	6.00
Quercetrin	0077	4.00
Luteolin	0077	22.00
Catechin	292	196.00
Gallic acid	8077	28.00
Pyrogallol	**5070.00**	**1140.00**
Protocatchuic	292	210.00
Coumarin	0077	2.80
Catechol	0077	275.00
Chlorogenic	**2730**	**2270**
Caffeic	00077	32.00
vanillic	247.00	39.00
Ferulic	6.00	61.00
Iso-ferulic	41.00	30.00
Reversetrol	6.00	6.00
Ellagic	24.00	41.00
3, 4, 5 methoxy cinnamic	3.00	6.00
p-coumaric	2.00	72.00
Cinnamic	2.00	1.00
Epicatechin	230.00	101.00
p-OH benzoic	9.00	61.00

Chlorogenic acid, the most abundant phenolic compound in *D. caffra* dry fruit, exerts many biological activities, including antibacterial, antioxidant, anticarcinogenic and regulating glucose and lipids metabolism activities [37-40]. Other notable phenolics in freeze dried aqueous extract were chlorogenic, catechin, protocatchuic, vanillic, epicatechin, in the ratio of 2730, 292, 292, 247 and 230 (µg/g dry aqueous extract), respectively. Different results obtained by, Loots et al., [5] whom found that Kei-apple juice contained high concentration of caffeic acid. Other remarkable polyphenols such as p-coumaric acid, protocatechuic acid and p-OH phenyl acetic acid were identified in moderated concentrations.

Antioxidant activity of *D. caffra* fruit aqueous extracts

The potential antioxidant activity of aqueous extracts of *D. caffra* was evaluated by nitric oxide assay, DPPH radical assay and reducing power activity methods (TABLE 5).

TABLE 5. **DPPH and NO radical assays for *D. caffra* fruit aqueous extracts.**

		25	50	100	200	IC_{50}
NO radical	Flesh aqueous extract	0.72	13.21	24.71	48.01	208.33
	Fruit aqueous extract	3.05	22.43	40.82	83.55	120.80
	Quercetin	0.56	20.45	53.83	95.54	104.05
DPPH assay	Flesh aqueous extract	1.51	15.17	27.11	50.62	187.12
	Fruit aqueous extract	8.07	29.76	52.11	87.00	95.09
	BHT	37.66	78.20	99.2	100	32.25

DPPH radical scavenging assay: Discoloration of DPPH radical; as a result of their scavenging by different antioxidant in the examined samples; to stable yellow color hydrazine indicates the radical-scavenging potential [41]. In this experimental test, *D. caffra* whole fruit aqueous extract exhibited high antioxidant activity (TABLE 5 and FIG. 1). The antioxidant potentials were highest for BHT (IC50: 32.25 µg/ml), followed by the whole fruit aqueous extract (IC50: 95.09 µg/mL) and lastly flesh aqueous extract (IC50: 187.12 µg/mL).

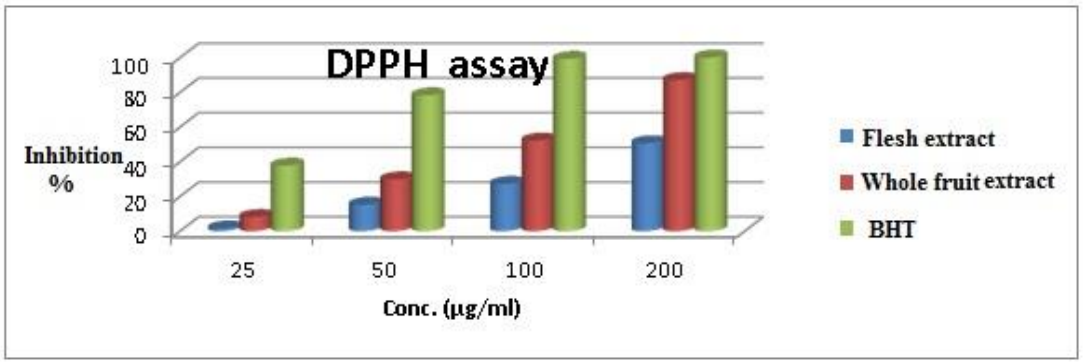

FIG. 1. **DPPH radical assay for *D. caffra* fruit aqueous extracts and synthetic antioxidant.**

NO radical scavenging assay: Nitric oxide (NO) is an effective pleiotropic mediator of smooth muscle relaxation and inhibition of platelet aggregation and other physiological manners [42]. Fruit aqueous extracts inhibit nitric oxide in a dose dependent manner (FIG. 2). Quercetin had the best IC50 value (as 104.05 µg/ml) followed by whole fruit aqueous extract (as 120.80 µg/ml), while *D. caffra* flesh aqueous extract showed a relatively high IC50 value (208.33 µg/ml).

Reducing power potential: Bioactive antioxidant phyto molecules have an ability to donate electrons which reflecting their reducing power. Antioxidants are reductant which in activate oxidants as described as redox reactions [43]. Different phenolic compounds reductant in the samples reduce Fe^{3+} ferricyanide complex to Fe^{++} form. It could be noticed from TABLE 6 and FIG. 3 that reducing power of *D. caffra* aqueous extracts relatively increased with graded concentrations, which suggests that the electron donating capacity of the aqueous extracts is concentration dependent. Higher absorbance values of whole *D. caffra* fruit aqueous extract than gallic acid at low concentrations suggests that whole fruit aqueous extract, especially at such concentrations, has high redox activities and can acts as reducing agent, hydrogen donating particles and quencher for high energy form singlet oxygen. Although the same classes of phytochemicals were noticed in both aqueous extracts of *D. caffra*, higher antioxidant activity of whole fruit aqueous extract could be interpreted by their high phenolic content. In this respect, Taher et al., [44] reported that different polyphenolic contents of *T. stans* fractions which ranged from 102.49 to 279.41 gallic acid/g dry weight significantly effect on the levels of non-enzymatic antioxidant parameters in liver homogenates. The reducing power of reducing power test followed the order: gallic acid>*D. caffra* whole fruit aqueous extract>flesh aqueous extract of the same fruit.

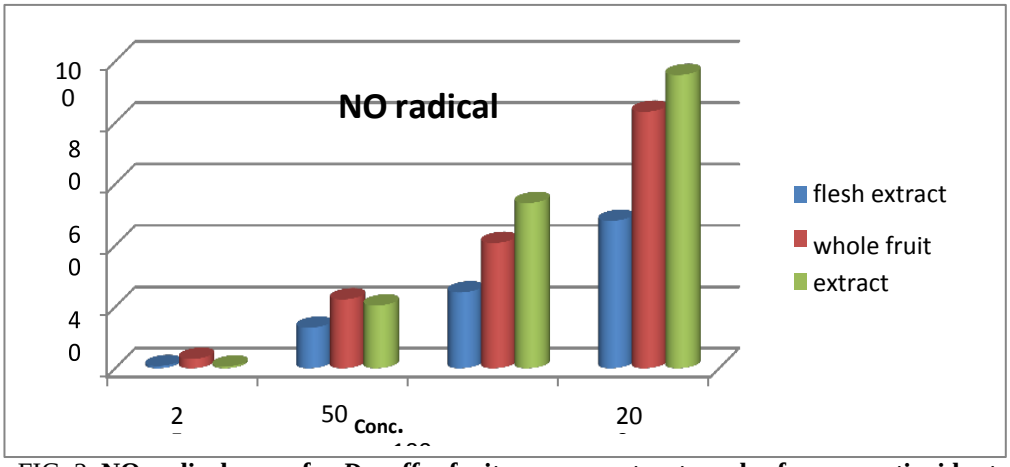

FIG. 2. **NO radical assay for *D. caffra* fruit aqueous extracts and reference antioxidant.**

TABLE 6. **Reducing power of *D. caffra* fruit aqueous extracts.**

	31.25	62.5	125	250	500	1000
Fruit aqueous	0.419	0.641	0.701	1.211	1.751	2.111
Flesh aqueous	0.293	0.341	0.511	0.677	0.981	1.526
Gallic acid	0.392	0.598	0.721	1.622	2.421	3.132

Generally, it is known that total polyphenols content, are highly correlated with the antioxidant activity [45,46]. Furthermore, chlorogenic acid (CGA), the most abundant polyphenol compound in *D. caffra* dry fruit, exerts antioxidant effect in

hyperlipidemic mice where, malondialdehyde (MDA) content was decreased while; the activities of antioxidant enzymes in serum and liver were increased [47]. Our data demonstrated an inverse correlation between the number of polyphenols and the values of IC50 for scavenging radicals. So it could be suggested that, high polyphenols content in particularly chlorogenic acid in whole *D. caffra* dry fruit aqueous extract is responsible for their strong antioxidant activity.

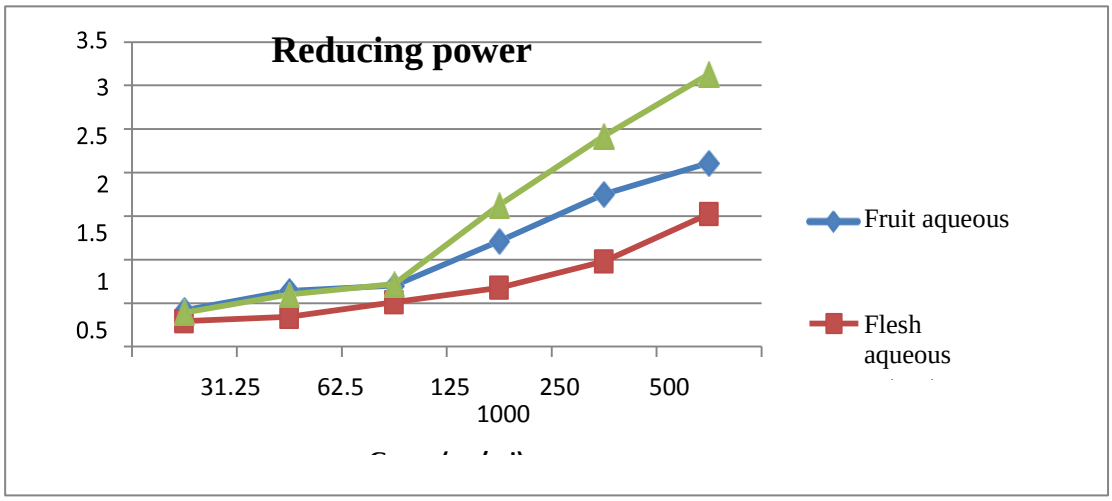

FIG. 3. **Reducing power assay for *D. caffra* fruit aqueous extracts and gallic acid as reference antioxidant.**

Safety assessment

Acute toxicity study: The acute effect of the single oral dose of *D. caffra* whole fruit aqueous extract at 3000 mg/kg, 6000 mg/kg and 12000 mg/kg did not create any death case nor change the behavior of examined rats during the monitoring period. Body weight increased gradually throughout the period of acute study in male and female of all groups. Rats showed no notable changes in organ weight and relative organ weights between the control and the single oral dose treated rats. The observations of acute study elucidate that *D. caffra* fruit aqueous extract is far save. Therefore, the LD50 value for oral administration of *D. caffra* whole fruit aqueous extract in male and female rats is more than 1.2 g/kg body weight. Our results conflict with those obtained by Gomaa et al., [4]. They reported that in acute toxicity experiment, LD50 of *D. caffra* fruit alcoholic extract was 175 mg/Kg body weight and the cause of death was due to heart failure (TABLE 7).

Subchronic toxicity study: There was no mortality in all rat experimental groups during 28 days of treatment. Oral administration of *D. caffra* whole fruit aqueous extract did not produce any noticeable effect of on the body weight or common behavior of the treated rats after 28 days of treatment. There were no significant differences in organ weight and relative organ weights between the control and all treated rats (TABLE 8). Moreover, control ABLE and *D. caffra* extract oral administrated rats appeared homogeneously healthy throughout the 28 days treatment period.

TABLE 7. **Organ weight and relative organ weights of rats treated with single dose of aqueous extract of *D. caffra* fruit.**

	Control		FEX 3000		FEX 6000		FEX 12000	
	O. W	**R.O. W**	**O.W**	**R.O.W**	**O.W**	**R.O.W**	**O.W**	**R.O.W**
Male								
Heart	1.03	0.30	1.05	0.29	1.04	0.30	1.05	0.29
Liver	7.60	2.23	7.59	2.16	7.77	2.26	7.68	2.41
Spleen	0.77	0.22	0.78	0.22	0.75	0.21	0.75	0.20
Kidney (right)	0.76	0.22	0.78	0.22	0.76	0.22	0.76	0.22
Kidney (left)	2.48	0.72	2.56	0.73	2.61	0.70	2.42	0.67
Testis (right)	2.50	0.72	2.57	0.73	2.64	0.77	2.42	0.67
Testis (left)	2.51	0.72	2.57	0.73	2.64	0.77	2.41	0.67
Female								
Heart	0.86	0.29	0.84	0.28	0.86	0.28	0.88	0.29
Liver	6.41	2.22	6.50	2.22	6.83	2.29	6.27	2.12
Kidney (right)	0.63	0.21	0.58	0.19	0.57	0.19	0.56	0.18
Kidney (left)	0.62	0.21	0.59	0.20	0.57	0.19	0.58	0.19
Ovaries	0.73	0.52	0.70	0.23	0.75	0.25	0.69	0.23

TABLE 8. **Organ weight and relative organ weights of rats treated with repeated dose of *D. caffra* whole fruit aqueous extract for 28 days.**

	Control		1000		2000		400	
	O.W	**R.O.W**	**O.W**	**R.O.W**	**O.W**	**R.O.W**	**O.W**	**R.O.W**
Male								
Heart	0.93	0.33	0.91	0.33	1.04	0.30	1.06	0.29
Liver	7.65	2.23	7.59	2.16	7.77	2.26	8.68	2.41
Kidney (right)	0.77	0.22	0.78	0.22	0.75	0.21	0.75	0.20
Kidney (left)	0.76	0.22	0.78	0.22	0.76	0.22	0.74	0.20
Testis (right)	2.48	0.72	2.56	0.73	2.61	0.70	2.42	0.67
Testis (left)	2.50	0.72	2.57	0.73	2.64	0.77	2.42	0.67
Female								
Heart	0.86	0.29	0.84	0.28	0.86	0.28	0.88	0.29
Liver	6.41	2.22	6.50	2.22	6.83	2.29	6.27	2.12
Kidney (right)	0.63	0.21	0.58	0.19	0.57	0.19	0.56	0.18
Kidney (left)	0.62	0.21	0.59	0.20	0.57	0.19	0.58	0.19
Ovaries	0.73	0.52	0.70	0.23	0.75	0.25	0.69	0.23

Histopathological study: No abnormal macroscopic observations were found related to *D. caffra* aqueous extract treatments. Treatments did not form any gross defects. No histological changes were recorded for liver and kidney analyzed tissues (data not shown).

Hematological parameters: It's well known that alternations in hematological parameters have higher predictive importance for human toxicity when the data are transformed from animal studies [48]. The results of hematological parameters as showed in TABLE 9 indicated that hemoglobin, red blood cells, total white blood cells and mean cell hemoglobin values did not significantly alter when male and female rats ingested at least 1000 mg/kg body weight *D. caffra* whole fruit aqueous extract. Platelet count of female rats treated with *D. caffra* whole fruit aqueous extract (1000 mg/kg, 2000 mg/kg and 3000 mg/kg) did not differ significantly from those of control female rats. The obtained data for hematological parameters in sub-chronic experiment indicate that aqueous extract is so far save especially at 1000 mg/kg where, the fruit extract does not interfere in the formation of red blood cells and white blood cells nor does it cause microcytic anemia or enlarge erythrocytes than normal in rats. There were no previously reports examined the hematological parameters of *D. caffra* whole fruit aqueous extract.

TABLE 9. **Blood hematology of rats treated with *D. caffra* whole fruit aqueous extract for four weeks.**

	Control	**FEX1000**	**FEX2000**	**FEX3000**
Male				
Hemoglobin g/l	$13.62^{(ab)}$	$14.16^{(a)}$	$13.86^{(a)}$	$13.55^{(ab)}$
Total red blood cell 10^{12}/l	$8.27^{(ab)}$	$8.39^{(a)}$	$8.15^{(bc)}$	$7.8^{(c)}$
Packed cell volume l/l	$44.46^{(a)}$	$44.06^{(a)}$	$42.69^{(b)}$	$42.83^{(b)}$
mean cell volume	$53.8^{(a)}$	$52.65^{(b)}$	$51.56^{(c)}$	$51.37^{(c)}$
Mean cell hemoglobin pg	$16.68^{(ab)}$	$16.89^{(a)}$	$16.90^{(a)}$	$17.05^{(a)}$
Mean cell hemoglobin concentration g/dl	$30.09^{(c)}$	$31.22^{(b)}$	$31.68^{(ab)}$	$32.13^{(a)}$
red cell distribution width RDW-CV%	$17.34^{(a)}$	$16.62^{(b)}$	$16.82^{(b)}$	$17.53^{(a)}$
Total white blood cell 109/l	$12.00^{(a)}$	$11.43^{(ab)}$	$17.58^{(b)}$	$17.65^{(b)}$
Granulocyte %	$16.2^{(b)}$	$15.58^{(c)}$	$16.5^{(b)}$	$16.96^{(a)}$
Lymphocytes%	$71.3^{(b)}$	$71.5^{(b)}$	$72.3^{(a)}$	$70.5^{(c)}$
Mid cells %	$12.5^{(a)}$	$12.92^{(b)}$	$11.2^{(a)}$	$12.54^{(a)}$
Platelet count 109/l	$680^{(a)}$	$623^{(b)}$	$574^{(c)}$	$599.4^{(bc)}$
Female				
Hemoglobin g/l	13.92	13.61	13.56	13.46
Total red blood cell 10^{12}/l	$8.04^{(a)}$	$7.76^{(ab)}$	$7.58^{(b)}$	$7.54^{(b)}$
Packed cell volume l/l	$43.22^{(a)}$	$41.81^{(b)}$	$40.72^{(c)}$	$41.40^{(b)}$
mean cell volume	$55.00^{(a)}$	$53.90^{(b)}$	$52.94^{(c)}$	$53.68^{(b)}$
Mean cell hemoglobin pg	$17.28^{(ab)}$	$17.58^{(a)}$	$17.00^{(b)}$	$17.70^{(a)}$
Mean cell hemoglobin concentration g/dl	$31.36^{(c)}$	$31.74^{(bc)}$	$32.72^{(a)}$	$32.06^{(b)}$

red cell distribution width RDW-CV	17.76$^{(a)}$	17.84$^{(a)}$	17.46$^{(ab)}$	17.36$^{(b)}$
Total white blood cell 109/l	8.22	8.48	8.80	8.42
Granulocyte %	16.6$^{(a)}$	00.02$^{(b)}$	16.70$^{(a)}$	16.80$^{(a)}$
Lymphocytes%	72.3$^{(a)}$	71.78$^{(b)}$	72.60$^{(a)}$	70.94$^{(c)}$
Mid cells %	11.10$^{(a)}$	12.20$^{(b)}$	10.70$^{(a)}$	12.06$^{(b)}$
Platelet count 109/l	685.5	679.2	677.00	673.60

Biochemical parameters: The clinical biochemistry parameters for glucose, lipid pattern, liver and kidney function tests are presented in TABLE 10. When male rats treated with 1000, 2000 or 3000 mg/g of *D. caffra* whole fruit aqueous extract, the values of sodium, potassium, total protein, albumin, glucose and alanine amino transferase (ALT) did not significantly differ when compared with control rats. On contrast, a significant decrease in total cholesterol and uric acid were found when experimental male rats treated with *D. caffra* whole fruit aqueous extract at a dose of 2000 and 3000 mg/kg. However, the levels of blood total protein and ALT in female rats did not significantly changed even rats ingested the high dose of *D. caffra* whole fruit aqueous extract. Other biochemical analysis in female rats experiment showed more distinct significantly changes, including uric acid, alkaline phosphatase, sodium, potassium, total cholesterol and triglycerides levels.

TABLE 10. **Biochemical parameters of rats treated with aqueous extract of *D. caffra* fruit for 28 days.**

	Control	**1000**	**2000**	**3000**
Male				
Sodium mmol/L	138.04$^{(a)}$	136.96$^{(a)}$	139.54$^{(a)}$	130.87$^{(a)}$
Potassium mmol/L	6.27$^{(a)}$	5.87$^{(a)}$	5.94$^{(a)}$	6.14$^{(a)}$
Creatininelmol/L	2.84$^{(a)}$	3.00$^{(a)}$	2.82$^{(a)}$	2.72$^{(b)}$
Uric acid mg/dL	2.14$^{(a)}$	1.77$^{(b)}$	1.72$^{(b)}$	1.53$^{(b)}$
Total protein g/dL	6.74$^{(a)}$	6.88$^{(a)}$	6.98$^{(a)}$	6.78$^{(a)}$
Albumin g/L	3.82$^{(a)}$	3.90$^{(a)}$	3.98$^{(a)}$	4.07$^{(a)}$
Alkaline phosphatase IU/L	298.1$^{(a)}$	276.36$^{(b)}$	284.22$^{(b)}$	273.66$^{(b)}$
ALT IU/L	35.58$^{(a)}$	38.46$^{(a)}$	38.98$^{(a)}$	37.00$^{(a)}$
Glucose	79.90$^{(a)}$	80.40$^{(a)}$	77.22$^{(a)}$	79.82$^{(a)}$
Cholesterol	77.50$^{(a)}$	75.06$^{(ab)}$	72.22$^{(b)}$	64.46$^{(c)}$
Triglycerides	100.5$^{(b)}$	95.86$^{(c)}$	103.74$^{(ab)}$	107.28$^{(a)}$
Female				
Sodium mmol/L	123.20$^{(b)}$	130.40$^{(ab)}$	130.96$^{(a)}$	135.42$^{(a)}$
Potassium mmol/L	5.08$^{(b)}$	5.20$^{(b)}$	5.64$^{(a)}$	5.84$^{(a)}$

Creatininelmol/L	$2.53^{(a)}$	$2.23^{(b)}$	$2.37^{(ab)}$	$2.33^{(ab)}$
Uric acid mg/dL	$2.12^{(b)}$	$2.54^{(a)}$	$2.03^{(b)}$	$2.40^{(a)}$
Total protein g/L	$6.92^{(a)}$	$6.80^{(a)}$	$6.94^{(a)}$	$6.94^{(a)}$
Albumin g/L	$4.34^{(a)}$	$3.90^{(b)}$	$4.14^{(ab)}$	$3.88^{(b)}$
Alkaline Phosphatase IU/L	$222.7^{(a)}$	$199.9^{(b)}$	$218.30^{(a)}$	$194.84^{(b)}$
ALT IU/L	$34.52^{(a)}$	$35.12^{(a)}$	$34.36^{(a)}$	$35.76^{(a)}$
Glucose	$81.94^{(a)}$	$83.96^{(a)}$	$78.66^{(ab)}$	$82.42^{(a)}$
Cholesterol	$84.78^{(ab)}$	$86.83^{(a)}$	$81.38^{(bc)}$	$77.10^{(c)}$
Triglycerides	$133.54^{(a)}$	$127.54^{(a)}$	$113.64^{(b)}$	$105.68^{(b)}$

ALT is an important serum enzyme in the liver and estimating their activity helps to notice any human chronic liver diseases [49]. The increase in the level of ALT is an important indicator of hepatocellular damage. In the present study, there were no significant differences in ALT level between control and *D. caffra* treated male and female rats at any dose. So, it could be suggested that *D. caffra* aqueous extract does not cause any remarkable liver injury. This positive effect was confirmed by histopathological studies.

It has been noted that any raise in serum proteins is an indicator of liver damage [50]. The principle for evaluating the synthetic capacity of the liver depend on the monitoring the levels of serum proteins especially albumin. Therefore, any reduction in serum proteins reflects chronic damage [51]. In the present data, the positive non-significant values for serum total protein in both sex's rats and albumin in male rats indicated that *D. caffra* whole fruit aqueous extract did not have any adverse effects on liver function.

Serum creatinine a metabolite resulting from the breakdown of creatine phosphate in skeletal muscle; increases when renal dysfunction occurred [52]. So, the high level of blood creatinine is a dependable indicator of impaired glomerular filtration and so a deteriorate in kidney function was expected. In the present data, non-significant decreases in serum creatinine values in male and female rats as shown in TABLE 10 demonstrated that *D. caffra* whole fruit aqueous extract had no negative impact on kidney. Similarly, other parameters like potassium and sodium did not differ between the male control rats and treated groups. The significant increased values of sodium, potassium and uric acid in female rats were toxically unrelated because these contrary results did not appear in both sixes and were not dose dependent. Moreover, the positive effect on kidney was confirmed by histopathological examination which showed normal architecture in male and female rats.

Blood lipid profile parameters such as total cholesterol and triglycerides with elevated concentrations are general indicators for monitoring human diseases i.e., type 2 diabetes mellitus, hyperlipidemia and hypercholesterolemia [44]. In the present study, a dose dependent significant decrease in serum cholesterol values in male and female rats as shown in TABLE 10 revealed that *D. caffra* whole fruit aqueous extract had positive effects against cardiovascular diseases. Abdel-Fattah et al [53] stated that *D. caffra* fruits contained considerable percent of pectin substances, essentially as galacturonic acid. So, it

could be suggested that the hypolipidemic effect of *D. caffra* whole fruit aqueous extract; in the present study; is mainly due to considerable amount of pectic substances. It has been reported that pectin reduces blood cholesterol in a wide variety of volunteers and experimental animals [54]. Serum cholesterol reduced with a percent of 13% after 14 days of treatment in noemolipidemic and hyperlipidemic individuals [55].

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