Protective Effect of Baobab Fruit Pulp (*Adansonia digitata* L.) from Oxidative Stress Induced in Rats by High-Fat Diet

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Abstract: High dietary fat intake associates with abnormal lipid metabolism and oxidative stress. As traditional remedies for these conditions medicinal plants used. The aim of this study was to examine the effect of Baobab (*Adansonia digitata*) fruit pulp extract on oxidative stress and hyperlipidemia induced by high fat diet (HFD). Male adult Wistar rats fed on HFD, normal laboratory diet or HFD supplemented with Baobab (*Adansonia digitata*). Three groups of rats fed with HFD supplemented with extracts with concentrations (2.5, 5.0, 10.0%) in drinking water. Upon characterization of Baobab fruit pulp found rich in total phenols (48.10 mg /g), total flavonoids (42.7 mg/g), and vitamin C (67.3mg/100g). The scavenging activity of lipid peroxides was 96.36%. Feeding rats in HFD for nine weeks resulted in a significant (P <0.05) increase body weight (93.0%). Total cholesterol, triglycerides, LDL, MDA also increased (p<0.05) while, HDL level showed a significant (p<0.05) decrease. Glutathione (GSH) and total antioxidant capacity in serum also reduced. Liver homogenate analysis showed significant increase in triglycerides (p< 0.05). Alteration in activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) also found. Supplementation of extract resulted in variable restoration of the above-mentioned parameters to their normal values. The restoration was a dose-dependent effect. The best improvement in studied parameters achieved with the 10% baobab extract supplementation. In nutshell, the fruit pulp of Baobab is a rich source of phytochemicals (e.g. total phenols, total flavonoids, and Vitamin C). Those phytochemicals managed to overcome the deleterious effects of HFD.
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1. Introduction

Food consumption pattern and total daily energy expenditure in Saudi Arabia, as in most Arab countries, has changed dramatically as manifested by an increased consumption of high fat diet [1] and decreased total daily energy expenditure. Healthy foods with low calories are often replaced with fast foods, snack foods and processed foods [2,3]. A high-fat diet (HFD) has been reported to have adverse effects on human health [4-6]. Recently Gujjala [7] reported that HFD promotes the generation of free radicals, which have deleterious effect on the reproductive system in mammals. Hyperlipidemia is a condition in which the level blood lipids are higher than the normal ranges. HFD efficiently increases the lipid constituents of blood. Hence, that could contribute hyperlipidemia [8]. Hyperlipidemia is associated with the major risk factors such as high blood pressure, high blood cholesterol, hyperglycemia and leading ultimately to cardiovascular diseases, diabetes, stroke and certain types of cancers [9]. Some reports also claim that hyperlipidemia could by associated with graft rejection, atherosclerosis and renal injury in diabetic rat [10-12]. A major consequence of manifestation hyperlipidemia is oxidative stress due to formation of free radicals and other oxidants [13, 14]. In recent years, the strategy of supplementing diet with antioxidants for prevention of damages caused by free radicals, is gaining popularity. Most of the antioxidants derived from natural sources and used as functional food [14]. Several studies has been directed toward the evaluation of the nature and properties of antioxidants derived from plants and herbs [15].

Baobab (*Adansonia digitata* L.) is a deciduous tree belonging to the plant family *Bombacaceae*. It is found in the savannas of Africa and India and known to be rich in micronutrients and phytochemicals [16, 17].

Baobab (*Adansonia digitata)* fruit pulp has been used as food ingredient in many countries [16]. Various part of the tree such as bark, leaves fruit seeds etc. are used for treating diseases, such as malaria, diarrhea, fever and inflammation etc. [18, 19]. Due to their uses in foodstuff and various traditional medicine, Baobab is also named “The Small Pharmacy” or “Chemist Tree” [20, 21].

Afolabiand Popoola [22] and Chadare [23] studied the composition and the nutritional value of many parts of baobab tree and demonstrated that the pulp

was particularly rich in vitamin C. Reports suggest the content of vitamin C is as higher as 10 times of that is oranges [24, 25].

Results reported by Vertuani [26] clearly indicated the interesting antioxidant properties of the baobab fruit. Therefore, the aim of the present investigation was to study the protective effect of the Baobab (*Adansonia digitata L.*) fruit pulp administration at different concentrations in drinking water on blood and liver lipid parameters and oxidative stress markers in rats fed HFD.

2. Material and Methods

This study was carried out in rats in year 2016 at the department of food science and human nutrition, faculty of agriculture and veterinary medicine, Qassimuniversity, Saudi Arabia. Rats were fed HFD to induce hyperlipidemia and oxidative stress and then studied the ameliorative effect produced by baobab fruit pulp in drinking water of rats for nine weeks before slaughtering.

Chemicals and kits

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) Chemical Co. Commercial kits were purchased from (bio-Merieux Laboratory Reagents and Products, France).

Rats housing

Fifty male adult Wistar rats, seven weeks old, weighing between 150-200 g were used in the present study. Rats were purchased from the Animal house, College of pharmacy, King Saud University, Saudi Arabia. The animals were housed in clean poly acrylate plastic cages and allowed to acclimatize to the laboratory environment for one week under the same laboratory conditions of photoperiod (12-h light:12-h dark cycle), a minimum relative humidity of 40-45 % and room temperature 23 ± 2 ◦C.

Rats were provided *adlibitum* with tap water and fed with standard commercial rat chow (Number 648-General Organization for Grain Silos and Flour Mills – Riyadh).

Rats diet

Two types of diet were prepared. The composition of the standard and HFD is presented in Table 1. High fat diet was prepared (42% of energy from fat, fat source: animal fat.)

Chemicals and kits

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) Chemical Co. Commercial kits were purchased from (bio-Merieux Laboratory Reagents and Products, France).

Sample preparation

Edible portion of Baobab (pulp) was collected, hand pounded to pass through sieve 40 mesh size, and kept for analysis and preparation of syrup.

The powdered pulp sample, 100 g was weighed, dispersed in 500 mL of deionized hot water. The mixture was then centrifuged and the supernatant filtered through rapid fluted filter paper. From the Baobab stock 2.5, 5.0, 10.0 % (V/V) solutions were prepared in tap water and stored in refrigerator -20^{0}C till used.

Table 1. Composition of experimental diets

Diet composition	Control	HF
Nutrient % (wt/wt)		
Protein	20	20
Carbohydrate	62.7	46.3
Fat	5	21.4
Kcal from fat (%)	11.97	42.07
Ingredient (g/1000 diet)		
Casein	200	200
L-cystine	3	3
Corn starch	527	363
Sucrose	100	100
Cellulose	50	50
Animal fat	0	164
Soybean oil	50	50
Mineral Mixture (AIN-76)	35	35
Vitamin Mixture (AIN-76)	10	10
Choline bitartrate	25	25
Total	1000	1000

Experimental design

After a period of adaption (one week), rats were randomly classified into five groups. The experimental groups were subjected to the following treatments:

Group (1): Negative control; rats were fed standard diet and *ad libitum* tap water.

Group (2): Positive control; rats were fed on HFD and *ad libitum* tap water.

Group (3): Rats fed on HFD + 2.5% baobab syrup.

Group (4): Rats fed on HFD + 5.0% baobab syrup.

Group (5): Rats fed on HFD + 10.0% baobab syrup.

The experiment continued for 9 weeks, starting from feeding on the high fat diet. During the experimental period, animal weights were recorded every week per each group. At the end of the experimental period (9 weeks), rats were fasted overnight, anesthetized by diethyl ether, bled and sacrificed. Blood samples were collected in plane tubes and centrifuged at 3000 rpm for 10 min. The obtained clear serum was collected and stored at -20 ºC pending for analysis.

After decapitation, livers were rapidly dissected out, washed immediately with ice saline, samples of liver tissues were taken from all groups and quickly labeled and stored at -20 ºC.

Determination of total phenolic content:

The total phenolic content of the extract was determined by colorimetry, using the method of **Singleton** [27].

Determination of total Flavonoids content:

The content of flavonoids of water extract of Baobab fruit powder was measured using method described by Jia *et al.* [28].

Determination of ascorbic acid (Vitamin C) content:

Vitamin C (ascorbic acid) content was determined by the standard titrimetric method by titration of extract against 2, 6-dichlorophenol indophenol [29].

Measurement of antioxidant activity (DPPH free radical scavenge)

The ability of the extracts to scavenge DPPH free radicals was determined by the method described by **Blois** [30]**.**

Serobiochemical analysis:

Total Cholesterol [31], triglycerides [32]**,** HDL [33] were measured in serum. The concentration of LDL was calculated according to **Friedwald.** [34]

Malondialdehyde (MDA) was determined according to the method described by Namıduru [35]. Total antioxidant capacity was determined in serum according to Koracevic [36]**.**

Evaluation of hepatic antioxidant enzyme activity:

Lipid extraction from liver tissues was performed according to the method of Folch [37]. Triglyceride (TG) were determined according to Stein and Myers [32] using standard diagnostic kits (Reckon Diagnostic Ltd., India).

The activity of SOD was measured in liver homogenate according to the method of Minami and Yoshikawa [38]. The activities of GSHPx and CAT were determined by the methods described by Cohen [39] and Paglia and Valentine [40] respectively. Commercial reagents purchased from Nanjing Jiancheng Bioengineering Company, Nanjing, Chinawere used.

Statistical analysis:

Results are presented as mean±SD. Two-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons using a computer-based fitting program (Prism, GraphPad). Differences of means considered significant only with $p<0.05$.

3. Results

Antioxidant contents and activity of Baobab fruit pulp extract:

Five samples of baobab powder were analyzed for their antioxidant contents. As shown in Table 2, baobab powder contains high levels of total phenols and total flavonoids and very high level of vitamin C. Scavenging activity (%) was found to be very high.

Body weight (BW) changes of rats fed HFD:

Body weights changes are presented in Table 3After nine weeks of feeding, positive control group that received HFD only was heavier than all other groups. However, administration of baobab at three levels to the rats resulted in a decrease in body weights compared to the positive group of rats (Figure1).

Table 2. Antioxidant contents and activity of Baobab fruit pulp extract

Compound	**Concentration**
Total phenols (mg/g)	48.10±1.08
Total flavonoids (mg/g)	42.7±0.43
Vitamin C (mg/100g)	67.3±0.11
Scavenging activity%	96.36±0.53

Data are means ± SD, n=5

Table 3. Bodyweight changes (g) of different treatment groups

Groups	Initial body weight (g)	Final body weight (g)	gain in body weight (g)	Body weight gain%
-ve control	151.4	241.7	90.3	6.14
+ve control	154.5	298.2	143.7	93.00
2.5% Baobab	155.6	234.3	78.7	50.57
5% Baobab	152.8	231.2	78.4	51.30
10% Baobab	156.4	226.4	70.0	44.75

-ve control: normal diet,+ve control: fed on HFD only

As shown in Table 3the percentage body weight gain decreased in groups that received baobab syrup and the lowest gain was observed in the group that received 10.0 % syrup of baobab.

Effect of baobab on Serum levels of cholesterol, triglycerides, HDL and LDL in rats in different treatment groups.

The present observations provide further evidence showing that consumption of HFD can also induce abnormal changes in serum cholesterol, triglycerides, HDL, LDL in rats. Table 4 shows levels of serum TC, TG, LDL, HDL of rats in different experimental groups. Rats in the positive control group displayed a significant (p<0.05) increase in the levels of the serum TC, TG, LDL and a significant decrease (p<0.05) in the level of HDL when compared with negative control group.

Baobab administration resulted in a significant decrease in the levels of serum TC, TG, LDL and a significant increase in the level of HDL in a dose-dependent manner of baobab after 63 days treatment when compared with the positive control group (II) (p<0.01).

Table 4. Serum Lipid profile of rats following treatment

Treatments	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
-ve Control	74.24±2.6[a]	46.48±3.76[a]	42.82±1.8[a]	23.42±1.8[a]
+ve Control	130.62± 6.4[b]	118.26±6.4[b]	25.66±2.4[b]	82.14±3.6[b]
2.5% Boabab	104.28± 4.8[c]	74.64± 4.8[c]	30.8±2.2[c]	59.42±2.2[c]
5% Boabab	82.92±5.4[a]	52.8±6.8[a]	38.4± 3.4[a]	34.88±1.64[d]
10% Boabab	78.46±8.2[a]	48.63±2.6[a]	40.8±1.8[a]	25.36±0.92[a]

-ve control: normal diet, +ve control: fed on HFD only.
Means on the same column with different letters are significantly different at p<0.05.

Effect of experimental treatments on serum antioxidants and oxidative stress parameters in rats.

The effect of treatment with baobab in serum lipid peroxidation indicators e.g. malondialdehyde (MDA), reduced glutathione (GSH) and total antioxidant capacity (TAC) are presented in Table 5.

There was a significant (p<0.05) increase in the level of MDA in the positive control group. The level of MDA in the control group was 1.56±1.18 µmol/L. This level increased significantly (p<0.05) to 6.94±0.87 µmol/L in the positive control group that received HFD only. Administration of baobab at different concentrations resulted in a significant (p<0.05) decrease in the levels of MDA and the reduction was dose- dependent.

In the present investigation, the total antioxidant capacity was determined in serum of the experimental rats –as presented in Table 5. It was found that the TAC of the negative control group was 0.45±0.02 mM/L and this level decreased significantly (p<0.05) to 0.13±0.04 mM/L in the positive control group indicating lipid peroxidation.

However, administration of baobab syrup resulted in significant (p<0.05) increases in TAC. The increase was again dose-dependent. In rats that received 10.0% baobab, the level of TAC was similar to the level in the negative control group.

Table 5: Effect of treatments on serum antioxidant and oxidative stress parameters in rats.

Treatments	MDA (µmol/L)	T. Antioxidant Capacity (mM/L)	Reduced glutathione (GSH) µmole/L)
-iveControl	1.56±1.18[a]	0.45±0.02[a]	4.36± 0.46[a]
+iveControl	6.94±0.87[b]	0.13±0.04[b]	1.82± 0.66[b]
2.5% Boabab	4.18±1.33[c]	0.24±0.02[c]	2.42± 0.48[c]
5% Boabab	3.00±1.63[a]	0.35±0.02[a]	3.64± 0.26[a]
10% Boabab	2.61±0.33[a]	0.45±0.01[a]	4.46± 0.88[a]

-ve control: normal diet, +ve control: fed on HFD only.
Means on the same column with different letters are significantly different at p<0.05.

Reduced glutathione levels in serum of the experimental rats are presented in Table 5. The level of GSH in the negative control rats was 4.36±0.46 µmole/L. This level decreased significantly (p<0.05) in the positive control group to 1.82± 0.66 µmole/L. Administration of baobab at three levels recorded resulted in increases in the level of reduced glutathione to a varying degrees. The best improvement was achieved with 10% administration of baobab (Table 5).

Effect of Baobab on the activities of antioxidant enzymes and triglycerides in liver homogenate of control and experimental rats fed high fat diet are presented in Table 6.

There was a significant decrease (p<0.05) in the activities of SOD, CAT, GSHPx and a significant increase (p<0.05) in the level of triglycerides in the liver homogenate in the rats fed HFD only when compared with the negative control. Administration of baobab showed a significant (p<0.05) increase in the activities of these enzymes and a significant (p<0.05) decrease in the level of triglycerides.

4. Discussions

In the present work, *in vivo* antioxidant activity of Baobab (*Adansonia digitata* L.) was evaluated in designed HFD rat's model. Studies had shown that phyto-polyphenolic compounds are effective nutrients and could be used for prevention of diseases contributed by oxidative stress [41]. Phenolic compounds are potent antioxidants and this activity is due to their redox properties that enable them to act as reducing agents, hydrogen donors, metal chelators [42]. The phenolic acids and flavonoids present in the plants are natural antioxidants [43]. They proved to have, cardio protective properties, anti-inflammatory and antimicrobial activity [44]. We found that Baobab fruit pulp is very rich in antioxidants. It contains high amounts of total phenols, total flavonoids and very high levels of vitamin C (Table 2).

Table 6: Effect of Baobab on liver homogenate antioxidant enzyme activities and triglycerides of rats fed high fat diet.

Treatments	SOD (U/g)	CAT (µmol/g)	GSHPx-(µmol/g)	Triglycerides (mg/g)
-ive Control	12.42±0.24^{a}	82.64±3.22^{a}	11.72±0.62^{a}	47.6±3.7^{a}
+iveControl	6.26±0.62^{b}	46.48±4.68^{b}	6.25±0.82^{b}	86.5± 2.4^{b}
2.5% Boabab	8.82±0.72^{c}	58.64±2.88^{c}	8.64±0.44^{c}	82.6± 2.6^{c}
5% Boabab	10.34±0.82^{c}	68.94±1.92^{d}	10.28±1.84^{a}	60.8± 6.5^{d}
10% Boabab	12.68±16^{a}	80.98± 4.56^{a}	10.88±1.64^{a}	52.7±4.4^{d}

-ve control: normal diet, +ve control: fed on HFD only.
Means on the same column with different letters are significantly different at p<0.05.

Dietary fat intake was shown to be the primary factor in the development of human obesity [45]. Many studies have shown that high fat diet is associated with increased oxidative stress in mammals [46]. Several reports have pointed out to the fact that high fat diet may increase the incidence of diabetes, hypertension and other degenerative diseases [47,48]. Patients with cardiovascular and cerebrovascular diseases have been reported to consume diets rich in fat than the general population [49]. Bowen and Borthakur [50] suggested that high fat diet may be responsible for the development of these diseases.

In past few decades, adverse effects of synthetic hypolipidaemic drugs have been reported by Schwandt [51]. Due to that, interests of scientists shifted towards alternative therapeutic approach. Herbal drugs are extensively used nowadays in the treatment of many diseases and its complications due to their efficacy, low incidence of side effects and low cost.

Kadowaki [45] have reported that the human obesity is largely contributed by high dietary fat beyond the genetic factors. An increase in body weight and shift of lipid profile from normal to higher Cholesterol, LDL, TG levels and a decrease in HDL level was observed [52, 53]. Contrarily, due to the weight loss in human a decrease in Cholesterol, LDL, TG levels have been reported [54]. In the present study, we showed that feeding HFD resulted in increase in body weight of rats and the gain in body weight was 93% in the group that did not received baobab. There was a decrease in body weight gain in all rats that received Baobab fruit pulp extract. Also, we found an increase in circulating cholesterol, LDL, TG levels and body weight, and a decrease in HDL level of animals fed high-fat diet like those reported previously. This finding suggests that HFD accelerated oxidative stress in present study. Ohkawa [55] had reported that, diet supplementation with natural antioxidants is linked with a reduction in the

level of oxidized lipoproteins. Similarly, supplementation of diet with Baobab fruit extract in present study showed a significant reduction in oxidative stress. Hence, from the safety view point, phytotherapeutic approach could be the most attractive for the development of a natural agent to reduce overweight or obesity associated disorders. Also, Baobab fruit pulp could be a promising functional food against obesity and high fat consuming population.

Results of the present investigation pointed out to the fact that lipid peroxidation is the main cause of the ill effects accompanying feeding high fat diet. There was a significant increase in the levels of peroxidation indicators. exemplified by a significant increase in the concentration of MDA and a significant reduction in reduced glutathione and serum total antioxidant capacity, The reduction in the level of GSH indicate high consumption of GSH in neutralization of free radicals generated following consumption of high fat diet. These changes were accompanied by changes in the, activities of the antioxidant enzymes (SOD, CAT and GSHPx) that act as free radical scavenging system. These enzymes prevent cells damage by free radicals and provide a repair mechanism for oxidized membrane components.

The inhibition of SOD, CAT and GSHPx activities are found to be involved in many degenerative diseases [56]. Likewise, in the present study, high fat diet caused a marked decrease in SOD, GSHPx, CAT activities and non-enzymatic antioxidant (GSH) levels in rats. In addition, asignificant increase in MDA level in serum was observed in rats fed HFD. In addition, increased peroxidation in rats fed HFD could be due to elevation of blood glucose. A relationship between glucose concentration and oxidative stress has been shown in red blood cells [57] and cultured bovine endothelial cells [58]. This suggests that a high consumption of dietary fat may be detrimental to the intrinsic antioxidant defense system in rats.

The present results showed that baobab administration at different concentrations could significantly improve the activity of antioxidant enzymes (SOD, CAT and GSHPx) and the levels of non-enzymatic antioxidants in rats fed HFD.

This study showed that increasing the concentration of baobab would increase the antioxidative activity. Baobab is rich in natural antioxidant e.g. total phenols, total flavonoids and in particular Vitamin C (Table 3). The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components [59]. Polyphenols are potent inhibitors of LDL oxidation and this type of oxidation is considered to be a key mechanism in development of atherosclerosis. Polyphenols may also exert antithrombotic effects by means of inhibiting platelet aggregation [60].

The antioxidant activity of vitamin C develops in two ways: (a) directly, by scavenging oxygen free-radicals, more generally known as reactive oxygen species (ROS) and (b) indirectly, by regenerating other antioxidant systems [61].

Intake of ascorbic acid and other antioxidant micronutrients have been linked to good health. This is because of their capability of trapping ROS which is the cause of a broad spectrum damages to biological systems Chadare. [23]

This study pointed to the beneficial effects of baobab fruit pulp in ameliorating hyperlipidemia induced by HFD. This finding can be useful and baobab can be applied as a substitute for imported western drugs specially in Africa for treatment of diseases such as cardiovascular diseases. In the present study three levels of baobab fruit pulp were triedhowever, the exact appropriate level was not determined. The toxic level need to be determined. Baobab fruit pulp was approved in 2009 by the Food and Drug Administration as a food ingredient in the United States of America.

In conclusion Baobab (*Adansonia Digitata* L.) fruit pulp extract, induced a noticeable improvement in lipid profile concentration and lipid peroxidation induced by HFD intake (groups III, IV, V). The extract was able to boost activity of antioxidant enzymes (SOD, CAT and GSHPx) which was declined due to HFD. In addition, that increases the levels of non-enzymatic antioxidants along with lowering LDL cholesterol levels. This was found to be due to its high content of total phenols, total flavonoids and vitamin C. Hence, Baobab fruit pulp could be used as functional food for natural treatment and prevention hyperlipidemia associated health abnormalities.

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