ROLE OF ALPHA LIPOIC ACID AND COCOA IN PREVENTION OF METABOLIC CHANGES INDUCED BY FRUCTOSE IN ADULT MALE ALBINO RATS

By

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ABSTRACT

Background: Obesity is one of the most critical threat to health and well-being. It contributes to elevated risk of cardiovascular diseases and diabetes mellitus.

Objective: Investigation of the possible role of some natural antioxidants alpha lipoic acid [α-LA] and cocoa in prevention of metabolic changes induced by fructose rich water in adult male albino rats.

Materials and methods: The current study has been carried on 50 adult male albino rats of average weights 130-160 g. They were divided into five equal groups: Group I (Control group), Group II (10 % fructose rich water “FRW”- fed group), Group III (FRW-fed group received 35 mg/kg α-LA three times / week, Group IV (FRW-fed group received 1 g/Kg cocoa 5 times / week), and Group V (FRW-fed group received both α-LA and cocoa). All rats were subjected to measurement of body weight (B.W) weekly, oral glucose tolerance test (OGTT), estimation of triglycerides “TAG”, total cholesterol “TC”, low density lipoprotein-cholesterol “LDL-c”, high density lipoprotein-cholesterol “HDL-c”, malondialdehyde (MDA), plasma level of catalase (CAT) activity and plasminogen activator inhibitor-1 (PAI-1). Abdominal adipose tissue (AAT) was dissected and weighed. Liver and abdominal adipose tissue were histopathologically examined.

Results: FRW-fed rats showed significant increase in BW, AAT weight, TC, LDL-c, TAG, MDA, OGTT at 0, 30, 60 and 120 min., PAI-1 and number of vacuolated hepatocytes, significant decrease in CAT activity and adipocytes number, and insignificant decrease in HDL-c.FRW fed rat receiving α-LA showed significant increase in BW, AAT weight and number of vacuolated hepatocytes, insignificant increase in lipid profile, CAT activity, MDA, PAI-1 and OGTT at 30 and 60 min., significant decrease in OGTT at 0 level and adipocytes number, and insignificant decrease in OGTT at 120 min.

Conclusion: Alpha lipoic acid and/or cocoa administration ameliorated the metabolic and oxidative stress changes. Co-administration of α-LA and cocoa produced non-significant effect over individual administration except in reducing serum LDL-c and increasing plasma CAT activity. Cocoa alone tended to be more effective in reducing body weight, AAT weight, adipocyte size, improving CAT and glucose tolerance than α-LA alone, while α-LA alone tended to reduce MDA to level insignificantly changed from control.

Key words: Fructose, metabolic changes, alpha lipoic acid, obesity, cocoa.

INTRODUCTION

Excessive fructose intake has been implicated as a driving force in metabolic syndrome and type 2 diabetes mellitus (Kho et al., 2014), inflammation and oxidative stress (Sivaraman et al., 2013), dyslipidemia (Van Buul et al., 2014),
hyperuricemia (Jalal et al., 2013), hypertension (Jalal et al., 2010), non-alcoholic fatty liver disease (Carrier et al., 2014), and obesity (Van Buul et al., 2014).

Alpha lipoic acid (α-LA) have antioxidant effect (Shay et al., 2008 and El-Nakib et al., 2013), metal chelating effect (Shay et al., 2009), anti-obesity effect (Carbonelli et al., 2010), improving effect on dyslipidemia and hyperglycemia in diabetes through its hypoglycemic, hypolipidemic and antioxidant properties (Kandeil et al., 2011), nephroprotective role (El-Nakib et al., 2013), cardioprotective effect (Patel et al., 2011 and Lee et al., 2012), and anti-inflammatory effect (Shinto et al., 2014).

Cocoa has beneficial health effects on oxidative stress (Nwichi et al., 2012), type 2 diabetes mellitus (Ali et al., 2014), obesity (Ali et al., 2014 and Watanabe et al., 2014), dyslipidemia (Jia et al., 2010). It has also immunomodulatory and anti-inflammatory effects (Pérez-Berezoet al., 2012).

Plasminogen activator inhibitor-1 (PAI-1) is the most important direct physiological inhibitor of t-PA and u-PA which increases PAI-1 activity and prevents tissue fibrinolysis (Yildiz et al., 2014).

The present work aimed to test the alteration in body weight gain, abdominal adipose tissue weight, lipid profile, oxidative stress markers, plasminogen activator inhibitor -1, and oral glucose tolerance test after α-LA and/or cocoa administration to fructose-rich diet (FRW) fed rats.

MATERIALS AND METHODS

This study was carried out at Physiology Department, Faculty of Medicine (Girls), Al-Azhar University, Cairo, Egypt.

Fructose was obtained from Unipharma Pharmaceuticals company; Cairo, Egypt. It was given as 10 % in drinking water. Water intake was measured daily (Farina et al., 2013).

Alpha lipoic acid was obtained from Sigma Pharmaceutical Company, Cairo, Egypt. It was given by oral route using a gastric gavage tube at a dose of 35 mg/kg dissolved in distilled water three times per week (Li et al., 2011).

Commercial cocoa powder was obtained from Royal Pack Company, Cairo, Egypt. It was given by oral route using a gastric gavage tube at a dose of 1 g/kg five times per week. Cocoa powder was dissolved in distilled water, boiled for 10 minutes in order to avoid lumps and cooled at room temperature (Noori et al., 2009a).

In the present study, fifty adult male albino rats of local strain weighing 130 to 160 grams were obtained from Nile Company for drugs, Cairo, Egypt. Rats were housed in cages (60x30x30 cm per 5 rats). Rats were kept for 2 weeks under prevailing atmospheric conditions to ensure laboratory acclimation. Also, they were fed on ordinary rat chow with free access to water. All the ethical protocols for animal treatment were applied. Rats were divided into 5 equal groups and subjected to the following regimens for 6 weeks:

**Group I (control group):** Rats fed on ordinary rat chow and left without any treatment.
Group II (fructose rich water "FRW" group): Rats fed on ordinary rat chow and fructose rich water in the form of 10% fructose in drinking water (Farina et al., 2013).

Group III (fructose rich water and α-lipoic acid group): Rats fed on ordinary rat chow and fructose rich water (10% fructose in drinking water) received α-lipoic acid at a dose of 35 mg/kg by gastric gavage three times per week (Li et al., 2011).

Group IV (fructose rich water and cocoa powder group): Rats fed on ordinary rat chow and fructose rich water (10% in drinking water) received natural cocoa powder at a dose of 1 g/Kg by gastric gavage five times per week (Noori et al., 2009a).

Group V (fructose rich water, α-lipoic acid and cocoa powder group): Rats fed on ordinary rat chow and fructose rich water (10% in drinking water) received α-lipoic acid at a dose of 35 mg/kg three times per week in addition to natural cocoa powder at a dose of 1 g/Kg by gastric gavage five times per week.

Blood samples were obtained from the orbital sinus of an overnight fasted rats under light ether anesthesia using capillary tubes (Simmons and Brick, 1970). Blood was collected in two centrifuge tubes. The first blood sample was allowed to clot for an hour at room temperature, and then centrifuged at 3500 rpm for 15 minutes to separate serum for estimation of lipid profile and malondialdehyde. The second blood sample was collected in EDTA coated tube and rapidly centrifuged at 3500 rpm for 15 min. to separate plasma for estimation of catalase and plasminogen activator inhibitor-1. Samples were stored at -80°C up to the time of use.

At the end of experimental period, all groups were subjected to determination of body weight weekly, oral glucose tolerance test (Fouad et al., 2013), abdominal adipose tissue weight, total cholesterol (Schettler and Nussel, 1975), HDL-c (Trinder, 1969), LDL-c (Assmann et al., 1984) and triacylglycerol (Mc Gowan et al., 1983), antioxidant system (Catalase - Aebi, 1984), oxidative stress biomarker (malondialdehyde-Ohkawa et al., 1979) and plasminogen activator inhibitor-1 (Andreasen et al., 1986). Histopathological examination of liver and adipose tissue was done (Bancroft and Stevens, 1996).

Statistical analysis was done using statistic package for social science version 16 (SPSS, 16) for windows. Quantitative data were expressed as mean ± standard error (S.E.) of mean. Data were analyzed using one-way analysis of variance (ANOVA) followed by LSD as a post-hoc test. The level of significance between mean values was set at P value ≤ 0.05.

RESULTS

Administration of FRW to rats significantly increased body weight gain by 23.4%. Administration of α-LA to FRW fed rats significantly increased body weight gain by 22.6% when compared to control group, whereas insignificantly decreased by 0.7% when compared to FRW fed group. Administration of cocoa or both α-LA and cocoa to FRW fed rats insignificantly increased body weight gain by 12.1% and by 6% respectively when compared to control group. Significant decrease by 9.1% and by 14% respec-
respectively occurred when compared to FRW fed rats. Administration of both α-LA and cocoa to FRW fed rats significantly decreased body weight gain by 13.5% when compared to FRW fed rats received α-LA alone. However, insignificant decrease by 5.4% when compared to FRW fed rats received cocoa alone was reported (Table 1).

Feeding FRW to rats significantly increased AAT weight by 104% when compared to control rats. Administration of α-LA to FRW fed rats significantly increased AAT weight by 40% when compared to control group. However, a significant decrease by 31.3% was observed when compared to FRW fed rats. Administration of cocoa or both α-LA and cocoa to FRW fed rats insignificantly increased AAT weight by 16%, and by 12% respectively when compared to control group, whereas a significant decrease by 43% and by 45% respectively when compared to FRW fed rats. Administration of both α-LA and cocoa to FRW fed rats significantly decreased AAT weight 25% when compared to FRW fed rats receiving α-LA alone. However, insignificant decrease by 3.4% when compared to FRW fed rats received cocoa alone was noticed (Table 1).

Feeding FRW to rats significantly increased serum total cholesterol level by 107% when compared to control rats. Administration of α-LA or cocoa to FRW fed rats insignificantly increased serum total cholesterol level by 6.2%, and by 7.3% respectively when compared to control group. On the other hand, a significant decrease by 48.7% and by 48.2% respectively when compared to FRW fed group was reported. Administration of both α-LA and cocoa to FRW fed rats insignificantly decreased serum total cholesterol level by 9.3%, by 14.6%, and by 15.4% when compared to control group and FRW fed rats received α-LA alone or cocoa alone respectively. However, a significant decrease by 56.2% when compared to FRW fed rats was reported (Table 2).

Feeding FRW to rats significantly increased serum LDL-c level by 207% when compared to control group. Administration of α-LA or cocoa to FRW fed rats insignificantly increased serum
ROLE OF ALPHA LIPOIC ACID AND COCOA IN PREVENTION OF...

LDL-c level by 11.1% and by 22.2% respectively when compared to control group. Significant decrease by 63.7% and by 60.1% when compared to FRW fed group was noticed. Administration of both α-LA and cocoa to FRW fed rats insignificantly decreased serum LDL-c level by 16.7% when compared to control group. However, a significant decrease by 72.8%, by 25% and by 31.9% when compared to FRW fed group and FRW fed rats received α-LA alone or cocoa alone respectively (Table 2).

Feeding FRW to rats insignificantly decreased serum HDL-c level by 11.9% when compared to control group. Administration of α-LA or cocoa to FRW fed rats insignificantly increased serum HDL-c level by 11.1% and by 13.3% respectively when compared to control group. However, a significant increase by 26.2% and by 28.6% respectively when compared to FRW fed group were reported. Administration of both α-LA and cocoa to FRW fed rats insignificantly increased serum HDL-c level by 5.2% when compared to control group. However, a significant increase by 19.5% when compared to FRW fed rats was reported. On the other hand, insignificant decrease by 5.2% and by 7% when compared to FRW fed rats received α-LA alone or cocoa alone respectively (Table 2).

FRW fed rats showed a significant increase in serum TAG level by 101.9% when compared to control group. Administration of α-LA to FRW fed rats insignificantly increased serum TAG level by 7.3% when compared to control group. However, a significant decrease by 46.8% when compared to FRW fed rats was noticed. Administration of cocoa to FRW fed rats insignificantly decreased serum TAG level by 4.5% when compared to control group. However, a significant decrease by 52.7% when compared to FRW fed group was observed. Administration of both α-LA and cocoa to FRW fed rats insignificantly decreased serum TAG level by 8.8% and by 4.4% when compared to control group and FRW fed rats received cocoa alone respectively. However, a significant decrease by 54.8% when compared to FRW fed rats was observed. Also, a significant decrease by 15% when compared to FRW fed rats received α-LA alone was noticed (Table 2).

Table (2): Changes in serum total cholesterol (mg/dL), LDL-c (mg/dL), HDL-c (mg/dL), TAG (mg/dL) of control group, FRW fed rats and FRW fed rats received α-LA and/or cocoa.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group</th>
<th>FRW Group II</th>
<th>FRW+α-LA Group III</th>
<th>FRW+cocoa Group IV</th>
<th>FRW+α-LA+cocoa Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.E.M</td>
<td>Mean ± S.E.M</td>
<td>Compared to control</td>
<td>Compared to control</td>
<td>Compared to control</td>
<td>Compared to control</td>
</tr>
<tr>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
<tr>
<td>total cholesterol (mg/dL)</td>
<td>75.2 ±4.7</td>
<td>156 ±11.7</td>
<td>+107%</td>
<td>0 ±8</td>
<td>+6.2%</td>
</tr>
<tr>
<td>LDL-c (mg/dL)</td>
<td>30.5 ±2.7</td>
<td>93.7 ±4.9</td>
<td>+207%</td>
<td>33.9 ±2.2</td>
<td>+11.1%</td>
</tr>
<tr>
<td>HDL-c (mg/dL)</td>
<td>51.1 ±3.1</td>
<td>45 ±2.9</td>
<td>-11.9%</td>
<td>56.8 ±2.5</td>
<td>+11.1%</td>
</tr>
<tr>
<td>TAG (mg/dL)</td>
<td>187.7 ±7.9</td>
<td>379 ±13.6</td>
<td>+101.9%</td>
<td>201.3 ±10.4</td>
<td>+7.3%</td>
</tr>
</tbody>
</table>

(a) Significant values versus control (group I).
(b) Significant values versus FRW (group II).
(c) Significant values versus FRW+α-LA (group III).
(d) Significant values versus FRW+cocoa (group IV).
Feeding FRW to rats significantly decreased plasma CAT activity by 38.6% when compared to control rats. Administration of α-LA to FRW fed rats insignificantly increased plasma CAT enzyme activity by 3.8% when compared to control group. However, a significant increase by 69.3% when compared to FRW fed rats was observed. Administration of cocoa to FRW fed rats significantly increased plasma CAT enzyme activity by 38.6% and by 126.1%, when compared to control and FRW fed groups respectively. Administration of both α-LA and cocoa to FRW fed rats significantly increased plasma CAT enzyme activity by 52.2%, 148.3%, 46.6%, and 9.7% when compared to control, FRW fed rats and FRW fed rats received α-LA alone or cocoa alone respectively (Table 3).

Feeding FRW to rats significantly increased serum MDA by 278.8 % when compared to control rats. Administration of α-LA to FRW fed rats insignificantly increased serum MDA by 13.4% when compared to control group. However, a significant decrease by 70% when compared to FRW fed rats was noticed. Administration of cocoa or both α-LA and cocoa to FRW fed rats significantly increased serum MDA level by 43.2% and by 34.6% when compared to control group. However, a significant decrease by 14.4% when compared to FRW fed rats was reported (Table 3).

Table 3: Changes in plasma catalase (CAT) activity (µ/L), malondialdehyde (MDA) (nmol/mL), plasminogen activator inhibitor -1 (PAI-1) (ng/L) of control, FRW fed rats and FRW fed rats received α-LA and/or cocoa.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Group I</th>
<th>FRW Group II</th>
<th>FRW+α-LA Group III</th>
<th>FRW+cocoa Group IV</th>
<th>FRW+α-LA+cocoa Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT (µL)</td>
<td>Mean ± S.E.M.</td>
<td>Mean ± S.E.M.</td>
<td>% change</td>
<td>Mean ± S.E.M.</td>
<td>% change</td>
</tr>
<tr>
<td></td>
<td>a 213.9 ± 3.9</td>
<td>b 219.9 ± 3.1</td>
<td>-38.6%</td>
<td>ab 488.3 ± 15</td>
<td>+30.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/</td>
<td>Mean ± S.E.M.</td>
<td>Mean ± S.E.M.</td>
<td>% change</td>
<td>Mean ± S.E.M.</td>
<td>% change</td>
</tr>
<tr>
<td>mL)</td>
<td>a 20.1 ± 0.7</td>
<td>b 20.3 ± 0.2</td>
<td>+15.4%</td>
<td>a 14.9 ± 1.5</td>
<td>-13%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PAI-1 (ng/</td>
<td>Mean ± S.E.M.</td>
<td>Mean ± S.E.M.</td>
<td>% change</td>
<td>Mean ± S.E.M.</td>
<td>% change</td>
</tr>
<tr>
<td>mL)</td>
<td>a 25.2 ± 0.3</td>
<td>b 25.3 ± 0.2</td>
<td>+3.1%</td>
<td>a 25.9 ± 0.3</td>
<td>-1.2%</td>
</tr>
</tbody>
</table>

(a) Significant values versus control (group I).
(b) Significant values versus FRW (group II).
(c) Significant values versus FRW+α-LA (group III).
(d) Significant values versus FRW+cocoa (group IV).
ROLE OF ALPHA LIPOIC ACID AND COCOA IN PREVENTION OF...

Administration of FRW to rats significantly increased blood glucose levels at 0, 30, 60 and 120 minutes of OGTT when compared to control rats. Administration of α-LA to FRW fed rats significantly decreased blood glucose level at 0, and 60 minutes, and insignificantly decreased at 120 minutes of OGTT when compared to control rats. Cocoa administration to FRW fed rats and FRW fed rats received both α-LA and cocoa showed a significant decrease in blood glucose level at 0 and 120 minutes, and insignificant increase at 30 and 60 minutes of OGTT when compared to control rats. Administration of α-LA and/or cocoa to FRW fed rats induced insignificant decrease in blood glucose level at 0, 60 and 120 minutes, and significant decrease at 30 minute of OGTT when compared to FRW fed rats. However, it produced insignificant increase in blood glucose level at 0, 30, and 120 minutes, and insignificant decrease at 60 minute of OGTT when compared to FRW received cocoa alone (Table 4).

Liver changes:

In fructose rich water fed group, histological examination showed that the general liver architecture was similar to the control, but some of the hepatocytes appeared vacuolated. The vacuolated cells tended to be more at the periphery of the lobule. The vacuolation was most abundant in this group of animal (Fig. 1). Fructose rich water fed group received α-lipoic acid showed a general architecture of liver similar to control. The vacuolated hepatocytes were less prominent than the FRW group, and were situated at the periphery of the lobule (Fig. 2). Fructose rich water fed group received cocoa showed that vacuolated hepatocytes were rarely seen (Fig. 3). Fructose rich water received both α-lipoic acid and cocoa showed that the vacuolated hepatocytes were less prominent than the FRW group (Fig. 4).

### Abdominal adipose tissue (AAT)

In FRW fed group, the fat cells markedly enlarged (Fig. 5). In fructose rich water fed group receiving α-lipoic acid, the fat cells were smaller than FRW group but still larger than the control group (Fig. 6). In fructose rich water fed group receiving cocoa, fat cells showed broad similarity to the control group (Fig. 7). In fructose rich water receiving both α-lipoic acid and cocoa (FRW+ α-LA+cocoa), fat cells were more or less similar to the control group (Fig 8).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control Group I</th>
<th>FRW Group II</th>
<th>FRW+α-LA Group III</th>
<th>FRW+cocoa Group IV</th>
<th>FRW+α-LA+cocoa Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGTT (min.)</td>
<td>Mean ± S.E.M</td>
<td>% change</td>
<td>Mean ± S.E.M</td>
<td>% change</td>
<td>Mean ± S.E.M</td>
</tr>
<tr>
<td>0</td>
<td>109.6 ± 3.3</td>
<td>95 ± 1.4</td>
<td>125.7 ± 2.6</td>
<td>134.1 ± 3.9</td>
<td>109.3 ± 2.1</td>
</tr>
<tr>
<td>60</td>
<td>144.6 ± 2.8</td>
<td>151.3 ± 3.8</td>
<td>156.7 ± 3.8</td>
<td>156.7 ± 3.8</td>
<td>151.3 ± 3.8</td>
</tr>
</tbody>
</table>

(a) Significant values versus control (group I).
(b) Significant values versus FRW (group II).
Compared to the control group, vacuolated hepatocytes count were the highest and fat cells size were the largest in FRW group, while the number of vacuolated hepatocytes and the size of adipocytes were closely approximated to the control group in FRW receiving both α-LA and cocoa group. Feeding FRW to rats significantly decreased adipocytes number (cell/HPF) by 102.6% and significantly
increased number of vacuolated hepatocytes (cell/HPF) when compared to control group. Administration of \(\alpha\)-LA to FRW fed rats significantly decreased adipocytes number (cell/HPF) by 22.4% when compared to control group, while a significant increase by 57.1% when compared to FRW fed rats was observed. Moreover, it significantly increased number of vacuolated hepatocytes (cell/HPF) by 73.3% when compared to control group, while a significant decrease occurred by 90.2% when compared to FRW fed rats was reported.

Administration of cocoa to FRW fed rats insignificantly increased adipocytes number (cell/HPF) by 4.8% when compared to control group, while significant increase by 112.5% when compared to FRW fed rats was noticed. Moreover, it significantly increased number of vacuolated hepatocytes (cell/HPF) by 700% when compared to control group, while a significant decrease by 90.6% when compared to FRW fed rats was observed.

Administration of both \(\alpha\)-LA and cocoa to FRW fed rats produced insignificant increase in adipocytes number (cell/HPF) by 5.7% when compared to control group, while a significant increase by 114.2% when compared to FRW fed rats was reported. Moreover, a significant increase by 36.3% when compared to FRW fed rats received \(\alpha\)-LA alone and insignificant increase by 0.8% when compared to FRW fed rats received cocoa alone were observed.

Furthermore, administration of both \(\alpha\)-LA and cocoa to FRW fed rats insignificantly increased vacuolated hepatocytes number (cell/HPF) by 333.3% when compared to control group, while a significant decrease by 94.9% when compared to FRW fed rats was noticed. Moreover, insignificant decrease by 48% and by 45.8% when compared to FRW fed rats received \(\alpha\)-LA alone or cocoa alone respectively were reported (Table 5).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control Group I</th>
<th>FRW Group II</th>
<th>FRW+(\alpha)-LA Group III</th>
<th>FRW + cocoa Group IV</th>
<th>FRW+(\alpha)-LA + cocoa Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Adipocytes (cell/HPF)</td>
<td>Mean ± S.E.M.</td>
<td>n</td>
<td>ab</td>
<td>b</td>
<td>bc</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Compared to control</td>
<td>17.6 ± 9.0</td>
<td>-22.4%</td>
<td>+57.1%</td>
<td>+4.8%</td>
<td>+112.5%</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>-22.4%</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>23.8 ± 8.8</td>
<td></td>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Compared to FRW</td>
<td></td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
<tr>
<td></td>
<td>Mean ± S.E.M.</td>
<td>23.8 ± 8.8</td>
<td></td>
<td>b</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Compared to FRW</td>
<td></td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
<tr>
<td></td>
<td>Compared to control</td>
<td>24 ± 1</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
<tr>
<td></td>
<td>Mean ± S.E.M.</td>
<td>24 ± 1</td>
<td></td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
<tr>
<td>No. of Vacuolated Hepatocytes (cell/HPF)</td>
<td>Mean ± S.E.M.</td>
<td>0.3 ± 0.1</td>
<td>25 ± 7.5</td>
<td>+8666.6%</td>
<td>+733.3%</td>
<td>+90.2%</td>
</tr>
<tr>
<td></td>
<td>Compared to control</td>
<td>25 ± 7.5</td>
<td>+8666.6%</td>
<td>+733.3%</td>
<td>+90.2%</td>
<td>+700%</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>25 ± 7.5</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
<tr>
<td></td>
<td>Group III</td>
<td>25 ± 7.5</td>
<td>+8666.6%</td>
<td>+733.3%</td>
<td>+90.2%</td>
<td>+700%</td>
</tr>
<tr>
<td></td>
<td>Compared to FRW</td>
<td>32 ± 8.8</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
<tr>
<td></td>
<td>Mean ± S.E.M.</td>
<td>32 ± 8.8</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
<td>% change</td>
</tr>
</tbody>
</table>

(a) Significant values versus control (group I).
(b) Significant values versus FRW (group II).
(c) Significant values versus FRW+\(\alpha\)-LA (group III).
DISCUSSION

Fructose rich diet has been associated with visceral adiposity, elevated blood pressure, hypertriglyceridemia, insulin resistance and excess weight gain that are typical of metabolic syndrome and may predispose to T2DM. These changes were observed in FRW fed rodents that made this animal model an important tool for studying metabolic disorders. Obesity results from the deposition of fat in subcutaneous and visceral adipose tissue. It occurs usually over several months to years as a result of an imbalance between energy intake and energy expenditure (Tappy et al., 2013).

Alpha lipoic acid is one of the natural molecules known to prevent or retard oxidative stress. It is considered as a universal antioxidant that acts in both lipid and aqueous phase and regenerates other cellular antioxidants (Sena et al., 2008).

Cocoa-derived products have been identified to be rich in flavonoids which may act as potent antioxidants. Researches have indicated that the flavanols found in cocoa are associated with short and long-term health benefits (Vertuani et al., 2014).

The present work was an attempt to study the possible preventive or protective effect of alpha lipoic acid and cocoa powder either individually or combined together on metabolic changes induced by FRW in rats.

The results of the present study showed a significant increase in body weight after intake of 10 % FRW in drinking water for 6 weeks in comparison to control rats. This result was in agreement with Senthilkumar et al. (2013).

Administration of α-LA at a dose of 35 mg/kg 3 times per week to rats fed on FRW in the present work induced insignificant reduction of body weight gain when compared to FRW fed group, whereas a significant elevation in comparison to control was observed. These findings were in agreement with Cummings et al. (2010) and Castro et al. (2013).

This significant increment in body weight gain of FRW fed group received α-LA compared to control rats is due to ineffective role of the used dose of α-LA in competing the effect of FRW.

The results of the present study revealed that administration of cocoa to rats fed on FRW significantly decreased body weight gain in comparison to FRW fed group, while insignificant increase in comparison to control group was observed which was in agreement with Noori et al. (2009b). This decrease may be attributed to its polyphenols content which enhances lipolysis in the skeletal muscle through induction of medium-chain acyl-CoA dehydrogenase and carnitine palmitoyl transferase-2 (Watanabe et al., 2014). This reduction may be explained by stimulation of cellular energy, inhibition of lipid and carbohydrate absorption by inhibiting the activity of pancreatic lipase and secreted phospholipase A2 (Gu et al., 2014a and Gutiérrez-Salme?n et al., 2014a). Administration of both α-LA and cocoa to FRW fed rats significantly decreased body weight gain in comparison to FRW fed rats and FRW fed rats received α-LA, while insignificant changes in comparison to control group and FRW fed rats received cocoa. These results suggested that the decrement in the
ROLE OF ALPHA LIPOIC ACID AND COCOA IN PREVENTION OF...

709

body weight gain noticed in FRW received both α-LA and cocoa was due to cocoa effect only.

Regarding to relative AAT weight in FRW fed rats compared to control rats, significant increase was observed. This may be attributed to FRW-induced increase in TAG and LDL-c concentration which furtherly deposited in adipose tissue leading to increase AAT weight and cell size as evidenced by histological results of the present study. Pollock et al. (2012) and Kho et al. (2014) reported that increased exposure to TAG and remnant lipoproteins resulted from fructose metabolism led to greater visceral fat accumulation.

On the other hand, administration of α-LA to rats fed on FRW significantly decreased in relative AAT weight compared to FRW fed group, and a significant increase in comparison to control group. These results were in line with Halici et al. (2012), Soe et al. (2012) and El-Senousey et al. (2013). They demonstrated that α-LA supplementation lowered body fat deposition in a dose-dependent manner due to increased fatty acid β-oxidation and elevated hormone-sensitive lipase activity which furtherly decreased circulating lipids and consequently their deposition in AAT (Fernandez-Galilea et al., 2012).

This study revealed that administration of cocoa to rats fed on FRW significantly decreased relative AAT weight compared to FRW fed group and insignificant increase in comparison to control group was noticed. The reduction in AAT in FRW fed rats received cocoa was supported by Gu et al. (2014 a). These results may be explained by reduction in arachidonic acid, pro-inflammatory cytokines (TNF-α and IL-6) and macrophage surface marker (which are inflammatory lipid mediators positively correlated with mass and fat depot content of AAT) by cocoa supplementation (Gu et al., 2014 b). The present results indicated that cocoa administration alone has a significant effect on reducing AAT weight than α-LA alone. So, the significant reduction in AAT weight obtained by co-administration of α-LA and cocoa was mainly due to the effect of cocoa.

Regarding to the lipid profile, present work showed a significant increase in total cholesterol, LDL-c and TAG, and insignificant decrease in HDL-c after intake of 10 % of FRW in drinking water for 6 weeks when compared to control rats. Özdoğan et al. (2012), Schultz et al. (2012), Senthilkumar et al. (2013) and Schultz et al. (2013) explained these findings by FRW-induced insulin deficiency or insulin resistance.

The increment in LDL-c in the present results may be explained by the reduction in the rate of LDL-c clearance from circulation due to defective LDL-c receptors which was associated with increased plasma total cholesterol concentration and the significant rise in serum TAG level to decreased TAG clearance from plasma secondary to reduction of lipase activity which involved in TAG lipolysis into glycerol and fatty acids (Schaefer et al., 2009).

Administration of α-LA or cocoa to rats fed on FRW significantly decreased total cholesterol, LDL-c and TAG and significantly increased HDL-c compared to FRW fed rats, while insignificant changes in total cholesterol, LDL-c,
HDL-c and TAG in comparison to control were observed. These results were in agreement with Park et al. (2012) and Carrier et al. (2014) who explained lipid lowering effect of α-LA by reduction in cholesterol synthesis and enhanced cholesterol clearance through increasing lipoprotein lipase, lecithin cholesterol acyl transferase and reduction in the mRNA expression and activity of HMG-CoA reductase. The improvement of lipid profile by α-LA may be also ascribed to activation of AMPK and PGC1-α. They activate the respiratory chain and fatty acid oxidation genes thus improving dyslipidemia (Park et al., 2008). In addition, Castero et al. (2013) explained the hypolipidemic effect of α-LA by its ability to decrease the activation of PPARγ which promotes TAG synthesis and metabolism within the adipocytes. The effect of cocoa in the present work was in agreement with Osakabe & Yamagishi (2009) and Gutiérrez-Salmeñ et al. (2014b) who explained this effect of coccacatechinsto inhibition of the intestinal absorption of dietary cholesterol and TAG.

Administration of both α-LA and cocoa to rats fed on FRW significantly decreased total cholesterol, LDL-c and TAG, and significantly increased HDL-c in comparison to FRW fed rats, while insignificant changes in total cholesterol, LDL-c, HDL-c and TAG in comparison to control were observed. Also, there were insignificant changes in total cholesterol and HDL-c and a significant decrease in LDL-c, and TAG when compared to FRW fed rats received α-LA alone. Moreover, insignificant changes in total cholesterol, HDL-c and TAG and a significant decrease in LDL-c in comparison to FRW fed rats received cocoa alone were reported. These results demonstrated that there were a synergistic effect of co-administration of α-LA and cocoa in lowering LDL-c level. This may be due to reducing plasma malondialdehyde-modified LDL-c (MDA-LDL-c), a chemical modification thought to reflect naturally occurring oxidation of LDL-c level, through synergistic antioxidant effect (Ishimitsu et al., 2009). In addition, cocoa inhibits intestinal cholesterol absorption thus prevents synthesis of LDL-c (Gu et al., 2014 a).

The current study showed a significant increase in serum MDA level and a significant decrease in plasma CAT activity after administration of FRW in comparison to control rats. These results were in agreement with those of Castero et al. (2013) and Sivaraman et al. (2013). These effects may be ascribed to excessive generation of ROS by FRW fed rats. These ROS not only inactivates protein as CAT enzyme but also attacks the fatty acid component of cell membrane leading to pro-oxidative changes. These pro-oxidative changes lead to enhanced lipid peroxidation through oxidative conversion of polyunsaturated fatty acids to lipid peroxidesas MDA (Rahal et al., 2014). In addition, this peroxidation of lipids, protein and DNA increased release of free radicals causing rupture of the lysosomal membranes, release of lysosomal enzymes, necrosis of the cell, and destruction of parenchymal tissue. All these processes culminate in an increase in serum MDA levels (Brieger et al., 2012). MDA is not only highly toxic to cell but also has an inhibitory action on protective enzymes as CAT (Sharma et al., 2012).
The increased MDA level and decreased CAT activity can be attributed to FRW-induced hyperglycemia which furtherly leads to compensatory hyperinsulinemia. Patel et al. (2011) and Senthilkumar et al. (2013) reported that better antioxidant status in diabetic rats accompanied improved glycemic control. Also, lowering glycemia might improve the ability of the rats to produce more antioxidants that removed excess free radicals. Also, the elevated MDA recorded with FRW may be attributed to increased cholesterol level by FRW as evidenced by biochemical analysis of the current study and previously proved by Choi et al. (2010).

The current study showed that administration of α-LA to rats fed on FRW significantly decreased serum MDA level and significantly increased plasma CAT activity in comparison to FRW fed rats. On the other hand, insignificant changes in serum MDA level and plasma CAT activity in comparison to control group were noticed. Hussein et al. (2012), Mignini et al. (2013) and Pradhan et al. (2013) showed that administration of α-LA significantly decreased MDA level and increased CAT activity. They attributed this to high reactivity of α-LA towards ROS and its capability to increase levels and bioavailability of anti-oxidant enzymes. α-LA also scavenges ROS and lipid peroxides effectively at their mitochondrial source and in cell membranes since it is both lipid and water soluble (Castero et al., 2013). Also, α-LA, through its reduced form of DHLA can regenerate vitamin E which acts as a powerful antioxidant that can neutralize ROS at the cellular membrane (Patel et al., 2011).

The increase in glucose tolerance and subsequently insulin resistance produced by α-LA administration to FRW fed rats in the current work may be the cause of increased CAT activity and diminished MDA level. This was previously supported by the study of Deiuliis et al. (2011), Li et al. (2013) and Moreno and Hong (2013) who reported that oxidative stress, lipid peroxidation and insulin resistance illustrated a vicious circle that was broken by α-LA administration.

Administration of cocoa to rats fed on FRW significantly decreased serum MDA level and increased plasma CAT activity in comparison to FRW fed rats. Whereas, a significant increase in both serum MDA level and plasma CAT activity in comparison to control group were observed. In agreement with these result, Schinella et al. (2010), Martorell et al. (2011) and Desideri et al. (2012) attributed this to increasing resistance to hydrogen peroxide (H₂O₂) free radical which was the substrate of CAT enzyme thus preserved CAT activity.

The increased CAT activity by cocoa administration may be ascribed to modulating the action of oxidant-responsive transcription factor NF-kB and reducing DNA oxidative damage (Jenny et al. 2009). Also, reduction in the inflammatory marker (hs-CRP, IL-6 and TNF-α) may be another cause of decreased MDA level by cocoa supplementation, since there was a positive correlation between MDA and inflammatory markers (Parsaeyan et al., 2014). Co-administration of α-LA and cocoa did not reduce MDA level compared to individual administration.
This might be explained by production of MDA by many sources including arachidonic acid metabolism for the synthesis of prosta-glandins and polyunsaturated fatty acid peroxidation through ROS generation and free radical scavenging enzymes reduction. Also, MDA could combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA (Jayasekharan et al., 2014). Thus, the synergistic effect of α-LA and cocoa in reducing MDA level might require higher doses or longer duration of both. The results also revealed that co-administration of α-LA and cocoa significantly increased CAT activity than individually treated groups or control group. This indicated that both α-LA and cocoa produced synergistic effect in neutralization of CAT enzyme substrate H₂O₂ leading to increased CAT activity.

The present findings showed that FRW significantly increased plasma PAI-1 level when compared to control group. Administration of α-LA or cocoa to FRW fed rats significantly decreased plasma PAI-1 level when compared to FRW fed rats, while insignificant increase in comparison to control group was observed. Administration of both α-LA and cocoa to FRW fed rats significantly decreased plasma PAI-1 level in comparison to FRW fed rats. Insignificant changes in comparison to control group, FRW fed rats received α-LA alone or cocoa alone were observed. The obtained results coincided with Alzamendi et al. (2012) who found that circulating level of PAI-1 and its mRNA expression in adipose tissue were significantly higher in FRW fed rats compared to control group. They attributed this to adipose tissue dysfunction secondary to increased offer of a metabolic substrate (fructose).

OGTT of the present work showed significant increase in blood glucose level in rats fed on FRW at 0, 30, 60 and 120 minutes when compared to control rats. Administration of α-LA to FRW fed rats significantly decreased blood glucose level at 0, 30, 60 and 120 minutes when compared to FRW fed rats. A significant decrease at fasting (0) and insignificant changes at 30, 60 and 120 minutes in comparison to control group were noticed. Administration of cocoa to rats fed on FRW significantly decreased blood glucose level at 0, 30, 60 and 120 minutes in comparison to FRW fed rats, while a significant decreases at 0 and 120 minutes and insignificant increases at 30 and 60 minutes when compared to control group were noticed. Similar results were obtained following administration of both α-LA and cocoa. In addition, it induced insignificant changes at 0, 30, 60, and 120 minutes in comparison to FRW fed rats received α-LA alone or cocoa alone. Hsieh et al. (2013) and Senthilkumar et al. (2013) explained FRW-induced impairment in glucose tolerance by elevation in body weight, liver weight and adipose tissue fat deposition which seemed to be responsible for insulin resistance. Therefore, the improvement in glucose tolerance by α-LA and/or cocoa can be attributed to their ability to reduce body weight gain and adipose tissue mass with subsequent reduction in insulin resistance (Scarpulla et al., 2012). The impaired glucose tolerance by FRW can be explained by increased TAG production as evidenced by the biochemical results of this study and supported by Kho et al. (2014). They
carried out OGTT to check insulin resistance in FRW fed rats after 8 weeks of experimental period. They attributed this impairment in OGTT to decreased activation of AMPK that increased TAG production. The increased TAG production led to impaired glucose tolerance by multiple mechanisms including decreased glycogen synthesis, increased glycogenolysis and glucogenesis and rise in intestinal glucose production. So, the improvement in glucose tolerance by α-LA and/or cocoa can be explained by their ability to decrease TAG level, as evidenced by the biochemical results of this study, and increase AMPK expression in the plasma membrane of skeletal muscle and brown adipose tissues (Yamashita et al., 2012a).

The present glucose intolerance can be attributed to FRW-induced oxidative stress, dysfunction and damage of the islet β-cell (Cummings et al., 2010 and Maiztegui et al., 2011) secondary to AAT dysfunction (Alzamendi et al., 2009). So, the improving effect of α-LA and/or cocoa on OGTT can be explained by their powerful antioxidant effect as proved by reduction in MDA level and elevation in CAT activity reported in the present study. This was previously proved by Tian et al. (2013). Impaired glucose tolerance observed in FRW fed rats can be also explained by high leptin production and gene expression secondary to increased adipocyte cell size. Leptin signif\(_{\text{icantly affects insulin binding to its receptor, expression of IRS-1/IRS-2 downstream of the insulin receptor and reduced intracellular mediators IRS-1/2 (Farina et al., 2013 and Ma et al., 2013).}}\)

The effect of α-LA and/or cocoa administration in glucose tolerance can be explained by decreased adipocyte mass and cell size as evidenced by histopathological examination of the present study. This was in line with the previous study of Jung et al. (2012).

The results of this study demonstrated that rats fed on FRW exhibited a significant increase in the number of vacuolated hepatocytes. This vacuolation is most probably due to excess lipid droplets accumulated in hepatocytes that dissolved during preparation with no signs of inflammation or fibrosis. On the other hand, a significant decrease in adipocytes number with increased cell size when compared to control group were reported. The changes induced by FRW in this work can be considered as NAFL.

The increased fatty infiltration in the liver can be attributed to increased circulating level of PAI-1 in FRW fed rats. This elevated PAI-1 caused NAFLD through inhibition of u-PA that prevented the maturation of pro-hepatocyte growth factor to hepatocyte growth factor. Decreased hepatocyte growth factor signaling led to a decrease in the expression of apolipoprotein B and microsomal TAG transfer protein. This resulted in decreased secretion of lipoproteins and intrahepatocellular TAG accumulation that are no longer shuttled out of the cells via ApoB/VLDL (Arteel, 2008).

FRW induced dyslipidemia especially elevated TAG level may give another explanation of these results as excessive plasma TAG will prefer its deposition in many tissues including adipose tissue (leading to adipocyte hypertrophy), liver
SAMIHA D. BADR et al.

(leading to NAFLD) and muscle (Saponaro et al., 2015).

In addition, NAFLD noticed in FRW fed rats may be attributed to insulin resistance as proved by impaired glucose tolerance in the present study which is used as indicator for insulin resistance. Insulin resistance leads to resistance to the antilipolytic effect of insulin in adipose tissue which furtherly increased circulating free fatty acids. These free fatty acids induce mitochondrial dysfunction and hepatic TAG synthesis and deposition (Gaggini et al., 2013). The increased lipid peroxidation (MDA) and decreased antioxidant enzymes (CAT) by FRW in the present study may be a possible explanation of this hepatic fatty infiltration.

The increased fatty liver infiltration by FRW in the present study can be explained by decreased expression of adiponectin in adipose tissue as well as its receptor 1 in the liver. Adiponectin stimulates fatty acid oxidation in liver and improves hepatic microcirculation and oxygen availability (Kondo et al., 2010).

Administration of α-LA to rats fed on FRW significantly increased adipocytes number and significantly decreased vacuolated hepatocytes number in comparison to FRW fed rats. However, a significant decrease in adipocytes number and a significant increase in vacuolated hepatocytes number when compared to control group were observed. These histological findings were supported by the biochemical results of the present work. Decreased liver fatty infiltration by α-LA can be ascribed to the antioxidant effects of α-LA as evidenced by decreased MDA level and increased CAT activity. These antioxidant effects furtherly lead to reduction of pro-inflammation cytokines by radical scavenging, metals chelating and restoration of intracellular GSH which improved not only fatty infiltration, but also prevent further progression to inflammation and fibrosis (Min et al., 2013 and Tian et al., 2013).

The reduction in TAG level by α-LA administration to FRW may be a possible explanation of these results. These reduction in circulating free fatty acids and TAG reduced lipid accumulation in non-adipose (liver) as well as in adipose tissue (Schultz et al., 2012). Also, it was noticed that α-LA significantly decreased plasma PAI-1 level thus reversed FRW-induced fatty liver (Ritze et al., 2013). Furthermore, the improved glucose tolerance and consequently insulin resistance by α-LA in the present study might explain the reduction of fatty liver infiltration (Gaggini et al., 2013).

Administration of cocoa to rats fed on FRW significantly increased adipocytes number and significantly decreased vacuolated hepatocytes number when compared to FRW fed rats. Insignificant increase occurred in adipocytes number and a significant increase in vacuolated hepatocytes number when compared to control group. These histological findings were supported by the biochemical results of the present work. Decreasing PAI-1 level and reducing mRNA expression of pro-inflammatory genes as NF-KB, TNF-α and interleukins by cocoa administration may explain decreased fatty liver infiltration in the present study (Gu et al., 2014b). In addition, decreased TAG level, improving glucose tolerance and insulin resistance may give another explanation
of this improvement in hepatic lipid accumulation. In addition, cocoa supplementation increased mRNA expression of liver fatty acid binding protein (LFAB) which played an important role in suppressing hepatic lipid accumulation and it decreased activation of hepatic stellate cells which was responsible for secreting scar tissue collagen that furtherly led to fibrosis (Janevski et al., 2011). The improving effect of cocoa on FRW-induced fatty liver may be explained by modification of lipid digestion through increasing fecal lipid content (Gu et al., 2014a) and its increasing effect of systemic adiponectin level which antagonizes excess lipid storage in the liver (Buechler et al., 2011).

As regards the effect of cocoa on FRW induced adipocyte hypertrophy, this may be attributed to increased AMPK activation in fat cells, diminished adipocyte PPAR-γ which was implicated in decreased adipogenesis and adipocyte cell size (Yamashita et al., 2012b). Administration of both α-LA and cocoa to rats fed on FRW significantly increased adipocytes number and significantly decreased vacuolated hepatocytes number in comparison to FRW fed rats, whereas insignificant increase in adipocytes and vacuolated hepatocytes number in comparison to control group were observed. Moreover, a significant increase in adipocytes number and insignificant decrease in vacuolated hepatocytes number in comparison to FRW rats received α-LA alone were reported. Insignificant changes in adipocytes and vacuolated hepatocytes number in comparison to FRW fed rats received cocoa alone were noticed. Co-

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**REFERENCES**


ROLE OF ALPHA LIPOIC ACID AND COCOA IN PREVENTION OF...


78. Shay, K. P.; Moreau, R. F.; Smith, E. J.; Smith, A. R. and Hagen, T. M. (2009): Alpha-lipoic acid as a dietary supplement:


دور حامض الألفا ليبووك والكاكاو في منع التغيرات الأيضية التي يتسبب فيها الفركتوز في ذكور الجرذان البيضاء البالغة

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خلفية البحث: تعد البذلية "السمنة" واحدة من أخطر التهديدات على الصحة والرفاه. وتساهم في ارتفاع خطر الإصابة بأمراض القلب والأوعية الدموية والسكري.

الهدف من البحث: معرفة مدى التأثير الوقائي لتناول حامض الألفا ليبووك ومسحوق بودرة الكاكاو أو كلهما معا على الإجهاد التأكسدي والتغيرات الأيضية التي يحدثها الفركتوز على ذكور الفئران البالغة.

مواد وطرق البحث: أجريت هذا البحث على خمس مجموعات متساوية من ذكور الجرذان البالغة البيضاء يتراوح وزنها بين 130-160 جم، والتي تم تغذيتها بغذاء الجرذان العادي. واستمرت التجربة لمدة 6 أسابيع، وكانت المجموعات مقسمة كالآتي: المجموعة الأولى: لم تخضع لأي معالجات واعتبرت هي المجموعة الضابطة، والمجموعة الثانية: تغذت على ماء غني بالفركتوز (10% في مياه الشرب)، والمجموعة الثالثة: تغذت على ماء غني بالفركتوز بالإضافة إلى حامض الألفا ليبووك (25 مجم/كمجم) ثلاثية مرات أسبوعياً والتي تم إعطاؤها عن طريق أنبوب تصل من الفم إلى المعدة، والمجموعة الرابعة: تغذت على ماء غني بالفركتوز بالإضافة إلى مسحوق بودرة الكاكاو (1 جم/كمجم) خمس مرات أسبوعياً والذي تم إعطاؤه عن طريق أنبوب تصل من الفم إلى المعدة، والمجموعة الخامسة: تغذت على ماء غني بالفركتوز بالإضافة إلى حامض الألفا ليبووك ومسحوق بودرة الكاكاو.

وقد تم تقييم وزن الجسم أسبوعياً إبتداء من نهاية الأسبوع الأول حتى نهاية التجربة. وقبل إتمام التحلي بالفركتوز عن طريق الفم، وقف إنهاء التجربة بثلاثة أيام ثم عمل اختبار تحمل الجلوكوز عن طريق الفم.

وفي نهاية التجربة تم تخدير الجرذان كلياً، وتم تجميع عينات الدم من الوريد الحبيبي العيني، وقيمت كل عينة إلى جزأين: مصل الدم لقياس نشاط إنزيم CAT، وأيضا لقياس مستويات أدينوزين-1، ومصل لقياس مستوى دهنيات الدم (نسبة الكوليسترول الكلي والكوليسترول في البلازما).
البروتين الدهني منخفض الكثافة والكوليسترول في البروتين الدهني علاى الكثافة والدهون الثلاثية والمالونالدهيد. كما تم تحديد وزن النسيج الدهني البطني، و دعمت النتائج بالفحص التشخيصي لكل من الكبد والنسيج الدهني البطني.

النتائج: أحدث تعاطي الجرذان للماء الغني بالفركتوز زيادة ذات دلالة إحصائية في وزن الجسم ووزن النسيج الدهني البطني، وعدد خلايا الكبد الدهنية، كذلك حدث نقص للجولوكوز في الدم عند جميع النقاط الزمنية وPAI-I وعدد خلايا الكبد الدهنية. بالإضافة إلى ذلك، حدث نقص في وزن النسيج الدهني البطني وعدد خلايا الكبد الدهنية، بالإضافة إلى نقص في محتوى الجولوكوز عن طريق الفم. 

وقد أحدث إعطاء حامض اللايفا ليوبيك للجرذان التي تتغذى على الماء الغني بالفركتوز زيادة لها دلالة إحصائية في وزن الجسم ووزن النسيج الدهني وعدد خلايا الكبد الدهنية، كما حدث نقص للجولوكوز في الدم عند جميع النقاط الزمنية وPAI-I وعدد خلايا الكبد الدهنية. بالإضافة إلى ذلك، حدث نقص في وزن النسيج الدهني البطني وعدد خلايا الكبد الدهنية، بالإضافة إلى نقص في محتوى الجولوكوز عن طريق الفم.

وقد أحدث إعطاء مسحوق بودرة الكاكاو إلى الجرذان التي تتغذى بالماء الغني بالفركتوز زيادة لها دلالة إحصائية في كمية المياه المتناولة ونشاط إنزيم MDA، وعدد خلايا الكبد الدهنية، بالإضافة إلى نقص للجولوكوز في الدم عند جميع النقاط الزمنية وPAI-I وعدد خلايا الكبد الدهنية. بالإضافة إلى ذلك، حدث نقص في وزن النسيج الدهني البطني وعدد خلايا الكبد الدهنية، بالإضافة إلى نقص في محتوى الجولوكوز عن طريق الفم.

وقد أحدث إعطاء حامض اللايفا ليوبيك ومسحوق بودرة الكاكاو إلى الجرذان التي تتغذى بالماء الغني بالفركتوز زيادة في كمية المياه المتناولة يوميًا ونشاط إنزيم MDA، وعدد خلايا الكبد الدهنية، بالإضافة إلى نقص للجولوكوز في الدم عند جميع النقاط الزمنية وPAI-I وعدد خلايا الكبد الدهنية. بالإضافة إلى ذلك، حدث نقص في وزن النسيج الدهني البطني وعدد خلايا الكبد الدهنية، بالإضافة إلى نقص في محتوى الجولوكوز عن طريق الفم.

الاستنتاج: للعلاج الوقائي بحامض اللايفا ليوبيك ومسحوق بودرة الكاكاو أو كليهما معا دور هام في الإقلال من الإجهاد التأكسدي والشوارد الحرة والسمة وارتفاع نسب الدهون الضارة وزيادة المقاومة لهورمون الإنسولين وزيادة مستوى مثبطات منشطات الالافيرنسين-1 في الحالات التي تتناول الماء الغني بالفركتوز مما يقلل من ظهور الدهاء السكري، النوع الثاني، وكثير من أمراض القلب والشرايين والجلطات.