The Effect of aqueous extract of Cassia senna (Caesalpinaceae) on hyperlipidemic rats

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Abstract: Diet is the most important element in the treatment of hyperlipidemia. Hyperlipidemia is considered as a major risk factor for coronary heart disease (CHD). Wistar Albino Rats (N=27), were divided into three groups (n=9), as following: Group 1: fed on a normal pellet diet (NPD) for 7 months, Group 2: rats fed on high fat diet (HFD) for 7 months and denoted. Group 3: HFD- fed rats for 7 months, followed by aqueous C. Senna extract (1mL/Kg/day), for 2 months along with HFD. Triacylglyserols (TAGs), total cholesterol, LDL- and, HDL-cholesterols, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin, blood urea nitrogen (BUN), uric acid (UA) and creatinine were measured in the serum. Atherogenic index was calculated by LDL/HDL ratio. Livers and heart samples were collected for histopathological examination. This study revealed that aqueous C. Senna extract was effective in reducing body weight and serum lipid profiles as well as the increase in serum liver and kidney function biomarkers induced by HFD. The modulation in these biomarkers were coupled with improvement in histopathological pictures of liver and heart. aqueous C. Senna extract is potential hypolipidemic beside its therapeutic beneficial action against HFD induced liver and kidney dysfunction.

Key words: Antioxidants, cholesterol, kidney, lipids, liver, hyperlipidemia, Coronary heart diseases, flavonoids, high fat diet, casein.

1. Introduction

It has been agreed that hyperlipidemia is the main reason behind the incessant ingestion of high amounts of fat. As examples of the causative factors of the increasing ratio of coronary heart disease (CHD) and atherosclerosis are high levels of plasma total triacylglyserols (TAGs) and cholesterol (TC) (Chobanian et al., 1991, Farias et al., 1996, Jeong et al., 2005). Many researchers’ attempt to develop efficient and enhanced hypolipidemic drugs have resulted in the finding natural agents. A widely used alternative resolution to health problems is herbal medicine. Extracts from numerous plants have been shown to have strong hypocholesterolemic impact (Ghasi et al., 2000, Zhang et al., 2002, Vasu et al., 2005, Yousef et al., 2005, Bahramika et al., 2008).

Cassia senna (Caesalpinaceae), Known as Senna Mekki, cultivates in a variety of parts of Saudi Arabia (Adam et al., 2001). In addition, it was also grown in Egypt, Sinai desert, India and Sudan in similar environments (Hussain et al., 1979). Aerial parts of the plant are utilized in conventional medicine by local people within rural regions as antifungal and antibacterial as well as for treating constipation, fever, roundworm infection, edema, pleurisy, and pustular or eruptive skin conditions (Watt et al., 1962, Olver-Bever et al., 1986). C. senna contains anthrans, anthraquinone glycosides “Sennoside A, B, C and D”, rhein, proanthocyanidines, flavonoids (kaempferol), tannins, mucilages, sterols, as well as a variety of ions such as; Na⁺, K⁺, Ca²⁺ and Mg²⁺ (Patel et al., 1966, Acharya et al., 1972, Al-Masry et al., 1975, Hussain et al., 1979, Meelad et al., 1983, Al-Yahya et al., 1990, Agarwal et al., 2010).

The present study was designed to investigate the effect of the aqueous C. senna extract on serum lipid profiles, liver and Kidney functions in hyperlipidemic rats. Histomorphological pictures of rat livers and kidneys were also studied.

2. Material and Methods

Chemicals

All chemicals used were of high analytical grade, product of Sigma and Merck companies. Kits used for the quantitative determination of different parameters were purchased from Biogamma, Stanbio, West Germany.

Plants

Dried leaves of C. Senna were purchased from a local market.

Preparation of plant aqueous extract

The aqueous C. senna extract was prepared by soaking 5 g of the powdered leaves of C. senna in 50 mL of distilled water (80°C) for 8 hours. The obtained extract was filtered and then lyophilized and administered to animals of third group (Akanmu et al., 2004).
Animals: In this study, twenty-seven Male Wister Albino Rats (100-150g) were obtained from animal house of King Fahd Medical Research Center, King Abdulaziz University, Jeddah. The animals were housed under standard environmental conditions of light, relative humidity and temperature and had free access to water and commercial pellet diet for 2 weeks (adaptation period). After wards, the rats were classified randomly to 3 groups, each group consists of 9 rats.

Group 1: Rats fed on a normal pellet diet (NPD) for 7 months.

Group 2: Rats fed on high fat diet (HFD) for 7 months, hyperlipidemic diet contains 25% palm oil (International Afia Company, KSA), 2% cholesterol (ACROS ORGANICS-New Jersey, USA), 0.08% bitartrate choline (250 mg, GNC-USA), 23% casein [BBA Lactalis Industrie-BOURGBARRÉ-France], yellow corn (34.22%), fibers (6%), sodium chloride (1.4%), vitamins A, D, E (0.1%, 0.02% and 0.18% respectively) and other salts and metals (8%) (Table 1).

The diet used in this study was modified from the diet described by Matos et al. (Matos et al., 2005). Group 3: HFD-fed rats for 7 months, then ingested orally aqueous C. Senna extract (1mL/Kg B.W./day), for 2 months along with HFD feeding.

After 9 months of total experimental period, the animals were fasted overnight (12-14 hours) (Wolford et al., 1986), the blood samples were collected from each animal in all groups into sterilized tubes for serum separation. Serum was separated by centrifugation at 3000 × g for 10 minutes and used for biochemical serum analysis. After blood collection, rats of each group were sacrificed under ether anesthesia and the livers and hearts samples were collected for histopathological examination.

Biochemical serum analysis

Different biochemical serum lipid profiles were measured such as triacylglycerols (TAGs) (Rautela et al., 1974), total cholesterol (TC) (Stadtman et al., 1957), low density lipoproteins (LDL) (Burtis et al., 1999), high density lipoproteins (HDL) [Burtis et al., 1999]. Liver function biomarkers for instance aspartate aminotransferase (AST) (Saris et al., 1978), alanine aminotransferase (ALT) (Bergmeyer et al., 1978), total bilirubin (Jendrassik et al., 1938) as well as kidney function markers including, blood urea nitrogen (BUN) (Talke et al., 1965), uric acid (UA) (Bulgar et al., 1941), and creatinine (Larsen et al., 1972), were measured by using Dimension® clinical chemistry system and its kits from Dade Behring company-New York, USA. Atherogenic index was calculated in term of the LDL/HDL ratio (Kaplan et al., 1983). In addition to, very low density lipoproteins (VLDL) was calculated from TAGs/5 Kaplan et al., 1983).

Histopathological examination

Livers and kidneys of different experimental groups were removed and kept in 10% formalin, embedded in paraffin wax, sectioned at 5 um and stained with hematoxylin and eosin (H&E) for histopathological examination (Bancroft et al., 1996).

Data analysis:

The results are presented as mean ± SD. The data were analyzed statistically using one way analysis of variance (ANOVA) followed by Student's t-test (SPSS program, version 10). A difference was considered statistically significant when the probability value (P-value) was < 0.05.

3. Results

The obtained results revealed that, feeding rats with high fat diet (HFD) for seven months markedly led to an increase in the body weight of HFD-fed rats compared with NPD-fed group. Oral ingestion of C. senna extract to HFD-fed rats successfully reduced their body weight compared to rats of HFD group (Table 2). Table 2 also showed significant increase in the levels of HDL, LDL, VLDL, TC and TAGs in HFD group compared with NPD group. While administration of C. senna extract to HFD fed rats daily for 2 months after HFD ingestion for 7 months showed significant reduction in the levels of these lipids in relation to HFD group except HDL which shows an increased level with respect to HFD group and NPD-fed group. Also, the results revealed an increase in the atherogenic index (Defined in term of LDL/HDL ratio) in HFD-fed rats and ingestion of C. senna extract beneficially decreased this index compared to rats of HFD group (Table 2).

Table 1. Composition of High Fat Diet [HFD].

<table>
<thead>
<tr>
<th>Content</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>2%</td>
</tr>
<tr>
<td>Casein</td>
<td>23%</td>
</tr>
<tr>
<td>Bitartrate choline</td>
<td>0.08%</td>
</tr>
<tr>
<td>Palm oil</td>
<td>25%</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>34.22%</td>
</tr>
<tr>
<td>Fibers</td>
<td>6%</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.4%</td>
</tr>
<tr>
<td>Other salts and metals</td>
<td>8%</td>
</tr>
<tr>
<td>Vitamin A, D, E</td>
<td>0.1%, 0.02%, 0.18% Respectively.</td>
</tr>
</tbody>
</table>

The levels of liver and kidney function biomarkers of the three experimental groups are shown in table 3. From the table, it can be seen that significant increase in serum liver function enzymes,
AST and ALT in HFD group compared with NPD group. However, non significant change in serum total Bilirubin was observed in HFD group versus NPD group. Oral administration of *C. senna* extract to HFD group markedly down-modulated the increase in these enzymes with respect to HFD group. Furthermore, significant decrease in serum total bilirubin level was noticed in comparison with HFD group (Table 3).

The levels of kidney functions biomarkers in different experimental groups are also illustrated in table 3. Significant increase in serum creatinine and UA levels in HFD group, while significant decrease in serum BUN was observed in relation to NPD group.

Ingestion of *C. senna* extract to HFD group effectively reduced the increase in creatinine and UA levels in relation to HFD untreated group.

The results of histopathological examination of liver section of hyperlipidemic rats showed fatty infiltration around the dilated and congested portal vessels. Some nuclei have signs of degeneration (Fig. 1b). Also, fat vacuoles appear in the wall of heart blood vessels (Fig. 2b), but treated group demonstrated that, *C. senna* extract lead to decrease in fatty infiltration in hepatocytes (Fig. 1c), and fat vacuoles in the wall of heart blood vessels (Fig. 2c).

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**Fig. 1a,b,c:**
- a: Section in the liver of normal male albino rats (NPD group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.
- b: Section in the liver of hyperlipidemic male albino rats (HFD group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.
- c: Section in the liver of male albino rats after treated with *C. senna* extract (HFD+Se group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.

**Fig. 2a,b,c:**
- a: Section in the heart and blood vessel wall of normal male albino rats (NPD group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.
- b: Section in the heart and blood vessel wall of hyperlipidemic male albino rats (HFD group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.
- c: Section in the heart and blood vessel wall of male albino rats after treated with *C. senna* extract (HFD+Se group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.
### Table 2. Changes in the body weight and serum lipid profile of rats which were fed normal pellet diet (NPD), high fat diet (HFD) and high fat diet + senna extract (HFD+Se).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NPD</th>
<th>HFD</th>
<th>HFD+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gm)</td>
<td>383.50±19.26</td>
<td>455.50±61.78*</td>
<td>329±32.44**</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>65.77±4.53</td>
<td>80.67±3.75**</td>
<td>67.17±9.07**</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>12.89±.22</td>
<td>36.34±2.05***</td>
<td>11.55±1.86***</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>18.17±.39</td>
<td>20.75±3.51</td>
<td>26.19±3.21*</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>0.71±.01</td>
<td>1.79±.32***</td>
<td>0.45±.03***</td>
</tr>
<tr>
<td>TAG (mg/dl)</td>
<td>66.67±1.02</td>
<td>101.77±12.17*</td>
<td>74.63±18.93*</td>
</tr>
</tbody>
</table>

Data are presented as mean ±S.D.; S.D. = Standard deviation; *Significant \( P < 0.05 \); ** Highly significant \( P < 0.01 \); *** Very highly significant \( P < 0.001 \)

### Table 3. Changes of liver and kidney functions in rats fed with: normal pellet diet (NPD), high fat diet (HFD) and high fat diet + senna extract (HFD+Se)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NPD</th>
<th>HFD</th>
<th>HFD+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST activity (U/L)</td>
<td>116.8±14.45</td>
<td>153.33±17.39**</td>
<td>134.67±6.11</td>
</tr>
<tr>
<td>ALT activity (U/L)</td>
<td>79.6±6.23</td>
<td>152±5.29***</td>
<td>92.67±9.81***</td>
</tr>
<tr>
<td>Total Bilirubin(mg/dl)</td>
<td>0.13±0.03</td>
<td>0.12±0.00</td>
<td>0.06±0.00**</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>19.79±1.06</td>
<td>14.62±0.78***</td>
<td>12.42±0.43**</td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td>0.9±0.03</td>
<td>1.12±0.37</td>
<td>1.27±0.20</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.44±0.03</td>
<td>0.6±0.06**</td>
<td>0.47±0.08**</td>
</tr>
</tbody>
</table>

Data are presented as mean ±S.D.; S.D. = Standard deviation; *Significant \( P < 0.05 \); ** Highly significant \( P < 0.01 \); *** Very highly significant \( P < 0.001 \)

### 4. Discussion

Obesity is regarded as a disorder of energy balance, occurring when energy expenditure is no longer equal to daily energy ingestion, thus to ensure body weight homeostasis (Van Herpen et al., 2008). Although the etiology of obesity is complex, a risk factor for its growth is dietary factors, specifically the consumption of HFD (Kim et al., 2000).

The current results showed that body weight increased significantly in the HFD group compared with the normal group (Table 2) which may be related to the increased food ingestion. This was confirmed by the current experiments, science it was observed that rats which fed on HFD consumed more diets than the control rats, indicating that the excess energy resulted in the buildup of adipose tissue which may lead to the increase in body weight. This result is in accordance with Xu et al. (Xu et al., 2008). Previous investigation stated that ingestion of the HFD led to obesity because it facilitates the development of a positive energy which coupled with an increase in visceral fat deposition; this led to fat deposition particularly in abdominal region. Moreover, Schrauwen-Hinderling et al. (Schrauwen-Hinderling et al., 2005) found that HFD feeding is accompanied by molecular adaptations that prefer fat storage in muscle rather than oxidation.

Administration of *C. senna* extract to HFD group caused a significant reduction in body weight versus NPD group which is in agreement with the results of Amin and Nagy (Amin et al., 2009). This impact may be referred to hydroxyl-anthracene glucosides, particularly sennosides A and B in *C. senna* which enhance gastrointestinal motility and affect colonic motility thereby reducing fluid absorption and eases weight loss.

The increased levels of serum lipid profiles, TAGs, TC and LDL beside lowered HDL level is a common metabolic disorder, that is considered a risk factor for many diseases, especially atherosclerosis (Carpentier et al., 1997).

The current study revealed an increase in serum lipid profiles, TAGs, TC, LDL and HDL level as well as LDL/HDL in HFD-group compared with NPD-group. Abnormal increased of lipid profiles induced by HFD is considered serious risk factors and important early events in the pathogenesis of atherosclerosis in both peripheral and coronary circulation (Maxfield et al., 2005, Mallika et al., 2007, Paez et al., 2009).

A reasonable therapeutic approach to deal with atherosclerosis and decrease the occurrence of CHD is diet/or lipid-lowering drugs which may be considered as a target for treating the hyperlipidemia. The present study indicated that ingestion of *C. senna* extract to the HFD group led to a significant reduction in body weight versus NPD group which is in agreement with the results of Amin and Nagy (Amin et al., 2009). This impact may be referred to hydroxyl-anthracene glucosides, particularly sennosides A and B in *C. senna* which enhance gastrointestinal motility and affect colonic motility thereby reducing fluid absorption and eases weight loss.
extract significantly reduce the levels of the above serum lipid profiles in HFD group except HDL which shows an increased level with respect to HFD group and NPD-fed group, indicating its hypolipidemic effect. In the cells, the reduction of the concentration of TGs and the differentiation of the adipocytes occurs in the presence of sennoside B and rhein (Choi et al., 2006) In humans, the concentrations of lower TC and LDL are related to the dietary products that are rich in flavonoids, polyphenols (Tokunaga et al., 2002, Abe et al., 2000, Arai et al., 2000, Koo et al., 2007). Recent research suggests that the hypolipidemic effect of C. senna extract due to the presence of polyphenols, flavonoids, sennosides and rhein which were confirmed by chromatographic studies (Patel et al., 1966, Acharya et al., 1972, Al-Masry et al., 1975, Hussain et al., 1979, Meelad et al., 1983, Al-Yahya et al., 1990, Agarwal et al., 2010).

The reduction of serum cholesterol by tannins, flavonoids may be related to the changes in some parameters of cholesterol metabolism including fecal excretion of steroids (Grundy et al., 1975), 3-hydroxy-3-methylglutaryl coenzyme A reductase (Grundy et al., 1970) and lipoprotein structure and metabolism (Cooper et al., 1982).

HDL levels are anti-atherogenic, whereas its decreased levels are related with raised risk for CHD (Zilva et al., 1991). Moreover, HDL controls the lipid metabolism and able to remove free cholesterol from the peripheral cells, esterify and transport it in the neutral lipid, also, inhibit LDL-oxidation to protect the endothelial cells from the cytotoxic effects of oxidized LDL (Assman et al., 2003, Harrison et al., 2003). Furthermore, HDL raise triglyceride catabolism rate (Tiez et al., 1986). Our results obviously showed that C. senna extract is able to increase the HDL concentration in the treated rats relative to HFD group. The hypolipidemic effect of this extract may be related to its phytochemicals with antioxidant impacts; flavonoids and tannins (Larkins et al., 2004).

Numerous clinical trials have reported that beneficial modifications of the LDL/HDL ratio after consume flavonoid-containing food products. Weggemans and Trautwein (Weggemans et al., 2003) have reported that feeding on flavonoids decreased LDL and increased HDL in hypercholesterolemic humans. Taking these facts into account, it may be possible that these active components are responsible for the reduction of TG, TC, LDL and increasing HDL in HFD+Se group. The hypolipidemic effect of the used extract may reflect on histomorphological picture of heart which showed a decrease in fat vacuoles in the wall of heart blood vessels induced in rats by HFD.

The current study also demonstrated that HFD induce liver tissue injury indicated by obvious increase in serum transaminases (AST and ALT) which are considered as sensitive indicator of hepatic injury (Molander et al., 1955). Injury to the hepatocytes changes their transport functions and membrane permeability, resulting in the leakage of enzymes from their cells (Krishna Mohan et al., 2007). This leakage leads an increase in activities of serum ALT and AST (Zimmerman et al., 1970). Treatment with C. senna extract appears to down-modulate the liver function enzymes to near normal levels. The therapeutic beneficial effect of the used extract may be related to presence of some tannins, flavonoids and anthocyanins in C. senna extract which have antioxidants properties and scavenging free radicals that induce liver tissue injury (Larson et al., 1988, Wolniak et al., 2002). This curative potential action of the current extract was accompanied with improvement in the histopathological picture of livers of HFD rats which showed normal architecture after C. senna extract administration.

The increase of creatinine level in serum is one of the most sensitive and remarkable signs of kidney injury. As shown from the results, HFD administered rats showed highly significant increase in creatinine level when compared with NPD group, also the obtained results showed that, administration of the high caloric diet might have weakened the absorption of protein and other nutrients indicated by lowering level of serum BUN in HFD group relative to NPD group. It is founded that a lower intake of protein leads to a lower urea production since amino acids used for protein synthesis and not degraded (Woo et al., 1996). Nakagawa et al. (Nakagawa et al., 2005) pointed out that proanthocyanidin capable to improve the renal dysfunction. In this context, the observed decrease in the levels of serum BUN and creatinine in treated rats shows that C. senna extract enhanced the renal function from HFD induced damage.

Conclusion

The dose of C. senna extract, used in present study is preferable in lowering lipid profile and improving liver and kidney functions as well as the histopathological pictures of both liver and heart. Further scientific efforts are certainly required to establish the exact mechanism of action using the purified active components of the C. senna.

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