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). Wister 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.

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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 anthrons, anthraquinone glycosides "Sennoside A, B, C and D", rhein, proanthocyanides, flavonoids (kaempferol), tannins, mucilages, sterols, as well as a variety of ions such as; Na^+, K^+, Ca^{2+} and Mg^{2+} (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-BOURGBARRE-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%) (Table1). 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 triacylglyserols (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 exmamination

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].

Content	%
Cholesterol	2%
Casein	23%
Bitartrate choline	0.08%
Palm oil	25%
Yallow corn	34.22%
Fibers	6%
Sodium chloride	1.4%
Other salts and metals	8%
Vitamin A, D, E	0.1%, 0.02%, 0.18% Respectively.

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).

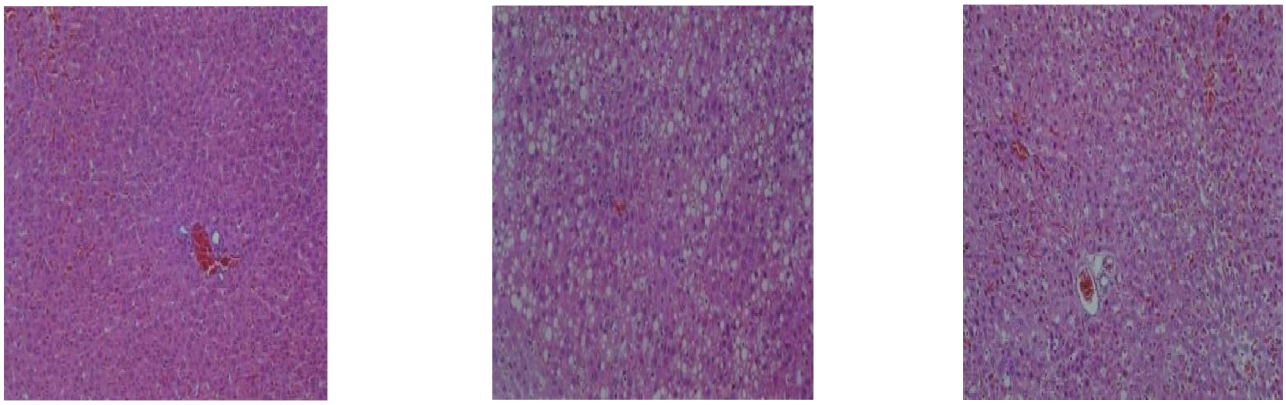

Fig. 1a,b,c: a: Section in the liver of normal male albino rats (NPD group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.
b: Section in the liver of hyperlipidemic male albino rats (HFD group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.
c: Section in the 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.

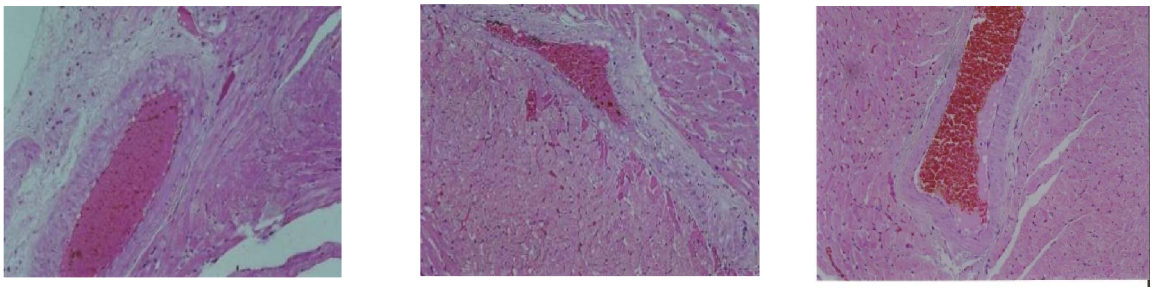

Fig. 2a,b,c: a: Section in the heart and blood vessel wall of normal male albino rats (NPD group) using Hematoxylin & Eosin (H& E). The magnification power was X 200.
b: Section in the heart and blood vessel wall of hyperlipidemic male albino rats (HFD group) using Hematoxylin & Eosin (H& E). The magnification 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).

Parameters	Groups		
	NPD	HFD	HFD+Se
Body weight (gm)	383.50±19.26	455.50±61.78*	329±32.44**
TC (mg/dl)	65.77±4.53	80.67±3.75**	67.17±9.07**
LDL (mg/dl)	12.89±.22	36.34±2.05***	11.55±1.86***
HDL (mg/dl)	18.17±.39	20.75±3.51	26.19±3.21*
LDL/HDL	0.71±.01	1.79±.32***	0.45±.03***
TAG (mg/dl)	66.67±1.02	101.77±12.17*	74.63±18.93*
	13.33±0.20	20.35±2.43*	

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

Tabe 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)

Parameters	Groups		
	NPD	HFD	HFD+Se
AST activity (U/L)	116.8±14.45	153.33±17.39**	134.67±6.11
ALT activity (U/L)	79.6±6.23	152±5.29***	92.67±9.81***
Total Bilirubin(mg/dl)	0.13±0.03	0.12±0.00	0.06±0.00**
BUN (mg/dl)	19.79±1.06	14.62±0.78***	12.42±0.43**
UA (mg/dl)	0.91±0.03	1.12±0.37	1.27±0.20
Creatinine (mg/dl)	0.44±0.03	0.6±0.06**	0.47±0.08**

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 as 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 inhance 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, VLDL 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 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 existence 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-hydroxyl-3-methylglutaryl coenzyme A reductase (Grundy *et al.,* 1970) and lipoprotein structure and metabolism (Cooper *et al.,* 1982).

HDL levels are anti-atherogenic, wherease 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). Numerouse 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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References

1. Abe I, Seki T, Umehara K, Miyase T, Noguchi H, Sakakibara J, Ono T (2000). Green tea polyphenols: novel and potent inhibitors of squalene epoxidase. Biochemical and Biophysical Research Communications 268: 767–771.
2. Acharya TK, Chatterjee IB (1972). Isolation of chrysophanic acid-anthrone, the major antifungal principle of Cassia tora. Lloydia 38: 218-220.
3. Adam SE, Al Yahya MA, Al Farhan AH (2001) Combined toxicity of *Cassia senna* and *Citrullus colocynthis* in rats. Vet-Hum-Toxicol 43: 70 – 72.
4. Agarwal V, Bajpai M (2010) Pharmacognostical and biological studies on senna and its products: An overview. International Journal of Pharma and Bio Sciences 1: 1-10.
5. Akanmu MA, Iwalewa EO, Elujoba AA, Adelusola KA (2004). Toxicity potentials of *Cassia Fistua* fruits as laxative with reference to Senna. African Journal of Biomedical Research 7: 23–26.
6. Al-Masry H: Al-Aqrabazin wa almostahdarat alsaydalaniah [Pharmacology and phormaceutic preparations]. First Ed. *Dar Al-Qualm,Al-Kuwait* 1975.
7. Al-Yahya MA, Al-Meshal IA, Mossa JS, Al-Badr AA, Tarig M: Saudi Plants (1990): A phytochemical and Biological Approach, King Abdulaziz City for Science and Technology, King Saud University Press, Riyadh.
8. Amin KA, Nagy MA (2009). Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. Diabetology & Metabolic Syndrome 1:17.
9. Arai Y, Watanabe S, Kimira M, Shimoi K, Mochizuki R, Kinae N (2000). Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. Journal of Nutrition 130: 2243–2250.
10. Assmann G, Nofer J (2003). Atheroprotective effects of high-density lipoproteins. Annu Rev Med 54: 321–341.
11. Bahramikia S, Yazdanparast R (2008). Effect of hydroalcoholic extracts of Nasturtium officinale leaves on lipid profile in high-fat diet rats. J Ethnopharmacol 115: 116–1217.
12. Bancroft JD, Stevens A (1996). Theory and practice of histological technique. 4th Ed. Churchill, Livingston Edinburgh, London, Melbourne and New York 50-56.
13. Bergmeyer HU, Scheibe P, Wahlefeld AW (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clin Chem 24: 58-73.
14. Bulgar HA, Johns HE (1941). The determination of plasma uric acid. J Biol Chem 140: 427-440.
15. Burtis CA, Asshwood ER: Tietz Textbook of Clinical Chemistry. 3rd ed., WB Saunders Co., Philadelphia 1999, PA, 42-72 [specimen collection and processing], 843-845 [Friedewald Equation], 39-40 [biological hazards], and p. 849 [specimen storage].
16. Carpentier YA (1997). Dietary intake and hyperlipoproteinemia. Rev Med Brux 18: 32.
17. Chobanian A, Single V (1991). Risk factor intervention may be inadequate to inhibit atherosclerosis progression when hypertension and hypercholesterolemia coexist. Hypertension 18: 130–131.
18. Choi SB, Ko BS, Park SK, Jang JS, Park S (2006) Insulin sensitizing and α-glucoamylase inhibitory action of sennosides, rheins and rhaponticin in Rhei Rhizoma. Life Sciences 78: 934–994.
19. Cooper AD, Shrewsbury MD, Erickson SK (1982). Comparison of binding and removal of triglyceride rich lipoproteins of intestinal and hepatic origin by rat liver *in vitro*. Am J Physiol 243: 389.
20. Farias RAF, Neto MFO, Viana, GSB, Rao VSNI (1996). Effects of Croton cajucara extract on serum lipids of rats fed a high fat diet. Phytother Res 10: 697–699.
21. Ghasi S, Nwobodo E, File JO (2000). Hypocholesterolemic effects of crude extract of leaf of Moringa oleifera Lam in high-fat died fed Wistar rats. J Ethnopharmacol 69: 21–25.
22. Grundy SM (1975). Effects of polyunsaturated fats on lipid metabolism in patients with hypertriglyceridemia. J Clin Invedst 5: 269.
23. Grundy SM, Ahrens JR (1970). The effects of unsaturated dietary fats on absorpation,excretion, synthesis and distribution of cholesterol in man. J Clin Invest 49: 1135.
24. Harrison D, Kathy KG, Hornig B, Drexler H (2003). Role of oxidative stress in atherosclerosis. Am J Neuroradiol 91: 7–11.
25. Hussain F: Al-Nabatat Al-tipeia wa ziraatiha wa Mokawinatih [Medicinal plants, their cultivation and constituents], [Arabic]. Al-Doar Al- Arabia for books, Tunisia 1979.
26. Jendrassik L, Grof P (1938). Vereinfachte photometrische methoden zur bestimmung des blutbilirubin. Biochem 297: 281
27. Jeong YJ, Choi YJ, Kwon HM, Kang S, Park HS, Lee M, Kang YH (2005). Differential inhibition of oxidized LDL induced apoptosis in human endothelial cells heated with different flavonoids. Br J Nutr 2005, 93:581–591
28. Kaplan A, Szabo LL. Clinical Chemistry: Interpretation and techniques. 2nd ed. USA. 1983, 11: 323-344.
29. Kim JY, Nolte LA, Hansen PA, Han DH, Ferguson K, Thompson PA, Holloszy JO (2000). High-fat diet-induced muscle insulin resistance: relationship to visceral fat mass. Am J Physiol Regul Integr Comp Physiol 279: 2057-2065.
30. Koo SI, Noh SK (2007). Green tea as inhibitor of the intestinal absorption of lipids: potential mechanism for its lipid-lowering effect, Journal of Nutritional Biochemistry 18: 179–183.
31. Krishna Mohan G, Pallavi E, Ravi Kumar B, Ramesh M, Venkatesh S (2007). Hepatoprotective activity of *Ficus carica* Linn. leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. DARU 15: 162-166.
32. Larson RA (1988). The antioxidants of higher plants. Phytochemistry 27: 969-978.
33. Larsen K (1972). Creatinine assay by a reaction-kinetic principle. Clin Chim Acta 41: 209-217.

34. Larkins N, Wynn S (2004). Pharmacognosy: phytomedicines and their mechanisms. Vet Clin North Am Small Anim Pract 34: 291–327.
35. Mallika V, Goswami B, Rajappa M (2007). Atherosclerosis pathophysiology and the role of novel risk factors: a clinicobiochemical perspective. Angiology 58: 513–522.
36. Matos SL, de Paula H, Pedrosa ML, dos Santos RC, de Oliveira EL, Júnior DAC, Silva ME (2005). Dietary models for inducing hypercholesterolemia in rats. Brazilian Archives of Biology and Technology 48: 203-209.
37. Maxfield FR, Tabas I (2005). Role of cholesterol and lipid organization in disease. Nature 438: 612–621.
38. Meelad MH (1983). Some biological effects of gamma irradiation on Cassia senna. Ms Thesis. Department of Biological Sciences. Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia. 1983
39. Molander DW, Wroblewski F, La Due JS (1955). Transaminase compared with cholinesterase and alkaline phosphatase an index of hepatocellular integrity. Clin Res Proc 3: 20–24.
40. Nakagawa T, Yokozawa T, Satoh A, Kim HY (2005). Attenuation of renal ischemia-reperfusion injury by proanthocyanidin-rich extract from grape seeds. J Nutr Sci Vitaminol (Tokyo) 51: 283-286
41. Olver-Bever BEP (1986). Medicinal Plants in Tropical West Africa. Cambridge University Press, Cambridge 1986.
42. Paez FG, Gomez ABZ (2009). Endothelial dysfunction and cardiovascular risk factors. Diabetes Res Clin Practice 84: 1–10.
43. Patel MR, Agrawal YK, Raj KPS: **Varying content of metals in four leafy drugs.** *Chem Abst.* 1980, **94**: 109179g. Richter G: Side effect of senna preparation. *Chem Abst* 1966 67: 25370s
44. Rautela GS, Hall RG, Bekiesz CL, Wermus GR (1974). A kinetic method for the rapid and automatic measurement of triglycerides in biological fluids. Clin Chem 1974, 20:857.
45. Richter G (1966). Side effect of senna preparation. Chem Abst 7: 25370s.
46. Saris N (1978). "Revised IFCC method for aspartate aminotransferase". Adv Chin Chem 24: 720-721.
47. Schrauwen-Hinderling VB, Kooi ME, Hesselink MK, Moonen-Kornips E, Schaart G, Mustard KJ, Hardie DG, Saris WH, Nicolay K (2005). Intramyocellular lipid content molecular adaptations in response to a 1 week high-fat diet. Obes Res 13: 2088-2094.
48. Stadtman TC: Methods in Enzymology. Vol III, Colowick SP, and Caplan NO, [Eds], Academy Press, New York, NY 1957, PP 392-394,678-681.
49. Talke H, Schubert GE (1965)."Enzymatische harnstoffbestimmung in blut and serum in optischen test nach warburg". Klin Wschr 41: 174.
50. Tietz NM (1986). Textbook of clinical chemistry. 3rd ed., W.B. Saunders Company, Philadelphia, London and Toronto 1986, p. 510.
51. Tokunaga S, White IR, Frost C, Tanaka K, Kono S, Tokudome S, Akamatsu T, Moriyama T, Zakouji H (2002). Green tea consumption and serum lipids and lipoproteins in a population of healthy workers in Japan. Annals of Epidemiology 12: 157–165.
52. Van Herpen NA, Schrauwen-Hinderling VB (2008). Lipid accumulation in non-adipose tissue lipotoxicity. Physiol Behav 23: 231-241.
53. Vasu VT, Modi H, Thaikoottathil JV, Gupta S (2005). Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume aqueous extract in cholesterol fed rats. J Ethnopharmacol 101: 277–282.
54. Watt JM, Breyer-Brandwijk NG (1962). Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingstone, Edinburgh.
55. Weggemans RM, Trautwein EA (2002). Relation between soy-associated isoflavones and LDL and HDL cholesterol concentrations in humans: a Meta-analysis. Eur J Clin Nutr 57: 940–946.
56. Wolford ST, Schroer RA, Gohs PP, Gallobrodek M, Fall HB, Kuhrne R (1986). Reference range data base for serum chemistry and haematology values in laboratory. J Toxicol and Environmental Healthy 1986, 18: 161.
57. Wolniak M (2002). Mechanisms of antioxidative action of anthocyanins. Farm Pol 20: 931–934.
58. Woo J, Henry JB (1996). Metabolic Intermediates and Inorganic Ions in Clinical Diagnosis and Management by Laboratory Animals. Philadelphia: W. B. Saunders Company. pp. 162-193.
59. Xu RY, Wan YP, Tang QY, Wu J, Cai W (2008). The effects of high fat on central appetite genes in Wistar rats: A microarray analysis. Clin Chim Acta 397: 96-100.
60. Yousef MI (2005). Reproductive performance, blood testosterone, lipid peroxidation and seminal plasma biochemistry of rabbits as affected by feeding *Acacia saligna* under subtropical conditions. Food Chem Toxicol 43: 333–339.
61. Zhang Z, Ho WK, Huang Y, James AE, Lam LW, Chen ZY (2002). Hawthorn fruit is hypolipidaemic in rabbits fed a high cholesterol diet. J Nutr 132: 5–10.
62. Zilva JF, Pannoil PR, Mayne PD (1991). Clinical chemistry in diagnosis and treatment. 5th ed. ELBS with Edward Arnold, British Government Chapter 11, p. 233.
63. Zimmerman HJ, Seeff LB (1970). Enzymes in hepatic disease. In: Goodly E.L, ed. Diagnostic Enzymology. Philadelphia: Lea and Febige.

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