Synergistic effect of cocoa and choline consumption on injured liver in experimental rats

Huda Ahmad AL-amer

Nutrition and Food Science Dep. Princess Nora Bint Abdul-Rahman University , Riyadh
huda.a.a@hotmail.com

Abstract: This study was conducted on seventy Sprague Dawley strain male rats, weighting 154 ±10 g. 10 rats served as control (-ve) group while 60 rats were subcutaneously administered a single dose of CCl4 in paraffin oil in dose 1ml/kg for two days from the start of the experimental period for inducing rat liver injury .The CCl4 rats were reclassified into control (+ve) and five treated rat groups which were cacao powder, cacao extract, choline, cacao powder with choline and cacao extract with choline. The study period was 8 weeks. In compared with control (-ve) group, the results revealed that the values of food efficiency ratio (FER), glycogen and superoxide dismutase (SOD) were significantly decreased but alkaline phosphatase (ALP) and bilirubin were significantly increased in all treated rat groups. Final body weight, body weight gain, protein efficiency ratio(PER), albumin ,total protein, albumin/ globulin ratio, liver triglyceride and glutathione peroxidase (GPX) were significantly decreased but the values of ALT,AST, creatinine ,low density lipoprotein cholesterol (LDLc) and liver total lipid were significantly increased in control (+ve) and rat groups which treated with cacao powder and cacao extract. On the other side , final body weight, body weight gain, (PER), albumin, GPX were significantly decreased but serum ALT, AST, creatinine, LDLc, liver malondialdehyde (MDA) levels were significantly increased in rat group which treated with choline. The values of hemoglobin (Hg) and packed cell volume (PCV) were significantly decreased in control (+ve) group. ALT and creatinine values were significantly increased in rat group which treated with cacao powder with choline while glutathione (GSH) was significantly decreased but serum total cholesterol, triglyceride, liver cholesterol were significantly increased in control (+ve) and rat group which treated with cacao powder. These results indicate that dietary intake of both cocoa and choline prevents CCl4 induced liver injury.

Huda Ahmad AL-amer. **Synergistic effect of cocoa and choline consumption on injured liver in experimental rats.** [Journal of American Science 2011;7(9):556-563]. (ISSN: 1545-1003). http://www.americanscience.org.

Key word: liver injury –CCl4-cacao –choline.

1 Introduction

The liver is the largest organ in the body and performs over 500 vital functions. Mainly , It processes all of the nutrients the body requires, including proteins, glucose, vitamins, and fats, manufactures bile that helps digest fats ,renders harmless potentially toxic substances, including alcohol, ammonia, nicotine, drugs, and harmful byproducts of digestion. In addition, removes old red blood cells from the blood and the iron contained in them is recycled to the bone marrow to make new red blood cells (Guyton and Hall 1996). Damage to the liver can impair these and many other processes. Hepatitis is a disorder in which viruses or other mechanisms produce inflammation in liver cells, resulting in their injury or destruction. Inflammation of the liver can also occur from medical problems, drugs, alcoholism, chemicals, and environmental toxins. Hepatitis varies in severity from a self limited condition with total recovery to a life threatening or life long disease (Jaeschke et al., 2002).

Choline is B complex vitamins, essential for the use of fats in the body. It is a precursor to acetylcholine, a nerve signal carrier in the brain. Choline also stops fats from being deposited in the liver and help move fats into the cells. Deficiency of choline can lead to cirrhosis with associated conditions such as bleeding; high blood pressure; cholesterolemia and atherosclerosis. Sources of dietary choline are liver, wheat germ, legumes, brewer's yeast, and egg yolk (Canty and Zeisel 1994).

Cocoa (Theobroma cacao) beans are rich source of polyphenols, contributing about 10% of the dry weight of the whole bean and its derivative chocolate, particularly dark chocolate, is considered one of the major contributors of antioxidants to the American diet after fruits and vegetables. Cocoa and cocoa products, namely, cocoa powder, cocoa liquor, and dark chocolate have protective effect on different cellular models of oxidative stress (Vinson et al., 1999). Numerous studies indicated that the health promoting properties of cocoa powder were attributed mainly to their polyphenolic compounds and methylxanthines. Numerous publications were reported on the health promoting properties of cocoa polyphenols, which were based on in vitro and in vivo studies. Extracts from cocoa seeds contain a good source of flavonoids, which contains epicatechin and its oligomers (Stephen et al., 2002 and Lecumberri et al., 2006).

In this respect, the objectives of the current study were to investigate the effects of cacao either

powder or extract with or without choline on carbon tetrachloride induced acute hepatitis in experimental rats.

I- Materials:

Carbon tetrachloride (CCl_4) was used to induce experimental acute hepatitis in rats. It was purchased from Somatco Co., in Riyadh in the form of 40% liquid dispensed in 1 L plastic bottles. Choline was obtained from Somatco Co., in Riyadh. The human recommended daily allowance was 600 mg. The rat dose was 50mg /kg body weight of rats according to previous study (Zeisel, 1999). Cacao powder was obtained from local market in Rayaid and administered to rats at dose 10% in diet. Seventy Sprague Dawley strain male rats were purchased from animal house in King Saud University in Riyadh. The average weight was 154 ±10 g. The basal diet was prepared according to Reeves et al., (1993) which consists of 14% casein (protein 80%), 4%,soybean oil 0.20% choline chloride,1.0% vitamin mixture, 3.5%mineral mixture, 5% fibers, 0.18%L-Cystine, 10%sucrose and the reminder was corn starch. The study was occurred in animal house of king Khaled University hospital.

II-Methods:

A - Preparation of methanol extracts:

100 gram of cacao powder was added to 1000 ml of 70% methanol (v/v) at room temperature for 20 hours with slowly rotated during this time. After filtration, ethanol was evaporated at low pressure at 30 centigrade degree (WHO 1983). The methanol extract was dissolved in normal saline and was immediately administered to rats at dose 500 mg/kg body weight by stomach tube.

B - Biological Investigation:

Experimental rats were fed on basal diet for one week for adaptation then divided into two main groups. The first main group (10 rats) fed on basal diet as a control (-ve) group. The second main group (60 rats) were subcutaneously administered a single dose of CCl4 in paraffin oil in dose 1ml/kg for two days from the start of the experimental period for inducing rats liver injuries according to the method described by Lee et al .,(2005). The CCl4 rats were reclassified into control (+ve) and five treated rat groups which were cacao powder, cacao extract, choline, cacao powder with choline and cacao extract with choline. During the study period (8 weeks), the daily food intake and weekly body weight gain were recorded. Rats were sacrificed to obtain blood and liver. First half of blood samples were heparenized for estimation of hemoglobin (Hg) and packed cell volume (PCV) according to Drabkin, 1949 and Mc Inory 1954. The others were centrifuged to obtain serum for estimation of some biochemical parameters. Serum aminotransferase (ALT, AST), alkaline phosphates (ALP) enzymes activity; total

2. Materials and Methods

bilirubin and creatinine were estimated according to Bergmeyer and Harder (1986), Kind and King (1954), Jendrassik (1938) and Bonsens and Taussky, (1984), respectively. Serum total protein and albumin were determined according to the method described by Weichselbaum (1946) and Bartholomev and Delany (1966), respectively. In addition, serum cholesterol, triglycerides (TG) and high density lipoprotein cholesterol (HDL-c) were determined by using enzymatic colorimetric methods according to Allain et al., 1974, Buccolo and David (1973) and Kostener, 1977, respectively. Livers of rats were perfused with 50 to 100 of ice cold 0.9%NaCL solution for estimation of liver cholesterol , total lipids, triglyceride and glycogen according to Abell et al., (1952)., Folch et al.,(1957), Young and Pestaner ,(1975) and Rerup and Lundquist, (1967),respectively. Liver glutathione (GSH), glutathione peroxidase (GPX), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were determined according to Reed (1999), Weiss et al. (1980) Beuchamp and Fridovich (1971) and Uchiyama and Mihara (1978), respectively.

C - Calculation of some parameters:

Food and protein efficiency ratios (FER&PER) were calculated according to (Chapman et al., 1950). Serum globulin (G) value was determined by subtracting the albumin from the total protein according to Coles (1974). A/G ratio was calculated using albumin and globulin values for each individual sample while low density lipoprotein cholesterol (LDL-c) was calculated as following [$LDL\text{-}_C$= Total cholesterol –$HDL\text{-}_C$ –$VLDL\text{-}_C$] according to Fruchart, (1982).

D -Statistical analysis:

Collected data were subjected to analysis according to SPSS program according to Steel and Torrie (1980).

3. Results

The nutritional results showed a significant decrease in final body weight, body weight gain, food intake, food efficiency ratio (FER)and protein efficiency ratio(PER) ($p< 0.01$&0.001) in control (+ve) group compared with control (-ve) group. The rat groups which treated with cacao powder, cacao extract and choline showed a significant decrease in final body weight, body weight gain, FER and PER at $p< 0.05$, 0.01&0.001 compared with control (-ve) group. The rat groups which treated with cacao powder with choline and cacao extract with choline showed a significant decrease in FER ($p< 0.05$) compared with control (-ve) group. All treated groups showed a significant increase in final body weight, body weight gain, food intake, food efficiency ratio

and protein efficiency ratio compared with control (+ve) rat group as shown in table (1).

Table (1): Mean values ± SD of body weight gain, food intake and food efficiency ratio (FER) of the experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Cacao powder	Cacao extract	Choline	Cacao powder+ choline	Cacao extract + choline
Initial. weight(g)	155.41± 3.67[a]	145.91± 4.21[a]	155.51± 4.55[a]	173.14± 5.19[a]	157.16± 5.12[a]	158.31± 5.16[a]	159.11± 4.36[a]
Final weight(g)	255.02± 13.67[a]	188.52± 15.41[c**]	225.12± 14.41[b*]	232.41± 16.21[b*]	222.27± 15.21[b*]	240.48± 17.21[a b]	244.84± 16.41[a b]
Weight gain (g)	99.61± 5.87[a]	33.61± 3.65[e***]	69.61± 4.80[d**]	75.92± 5.22[c*]	65.11± 4.71[d**]	82.17± 5.27[a b]	85.71± 5.33[a b]
Food intake(g/w)	18.21 ± 2.14[a]	14.45± 1.16[b**]	17.81± 1.87[a]	17.31± 1.67[a]	18.11± 1.21[a]	18.31± 2.11[a]	18.87± 1.89[a]
FER	0.091± 0.003[a]	0.038± 0.002[d*]	0.065± 0.001[c**]	0.073± 0.002[b*]	0.059± 0.001[c**]	0.074± 0.003[b*]	0.075± 0.004[b*]
PER	27.36± 3.49[a]	11.62± 1.71[d***]	19.55± 2.16[b*]	21.94± 2.66[b*]	17.98± 2.11[c**]	22.45± 4.25[ab]	22.73± 3.41[a]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

The values of serum ALT, AST, ALP, bilirubin and creatinine were significantly increased in control (+ve) at p< 0.001, cacao powder, cacao extract and choline groups (p< 0.05&0.01) compared with control (-ve) group. The rat group which treated with cacao powder with choline showed significant increased in ALT, ALP, bilirubin and creatinine (p< 0.05) while rat group which treated with cacao extract with choline showed significant increased in ALP and bilirubin (p< 0.05) compared with control (-ve) group. All treated groups showed a significant decrease in serum ALT, AST, ALP, bilirubin and creatinine compared with control (+ve) rat group as shown in table (2).

Table (2): The Mean values ± SD of serum ALT, AST, ALP, bilirubin and creatinine of the experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Cacao powder	Cacao extract	Choline	Cacao powder+ choline	Cacao extract+ choline
ALT (µ /ml)	21.15± 3.21[d]	66.71± 7.14[a***]	41.16± 5.55[b**]	35.81± 3.24[bc*]	34.43± 4.21[bc*]	29.88± 2.27[c*]	25.31± 3.26[cd]
AST (µ /ml)	51.11± 6.21[c]	148.71± 13.24[a***]	87.76± 8.21[b*]	82.17± 7.99[b*]	83.71± 7.67[b*]	60.14± 6.51[c]	61.14± 7.01[c]
ALP (µ /ml)	39.33± 3.27[d]	117.75± 13.21[a***]	91.14± 10.17[b**]	92.36± 9.69[b**]	90.51± 10.21[b**]	75.16± 8.27[c*]	64.36± 6.21[c*]
Bilirubin (mg/dl)	0.52± 0.01[e]	2.67± 0.33[a***]	1.09± 0.22[b**]	0.98± 0.11[b**]	1.11± 0.12[b**]	0.77± 0.03[c*]	0.66± 0.02[d*]
Creatinine (mg/dl)	o.81± 0.07[c]	2.11± 0.43[a***]	1.12± 0.27[b*]	0.99± 0.03[b*]	1.13± 0.23[b*]	1.21± 0.22[b*]	o.85± 0.10[c]

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

The control (+ve) rat group showed a significant decrease in the values of serum total protein, albumin (p< 0.05) and globulin (p< 0.01) and a significant increase in albumin/ globulin ratio (p< 0.05) compared with control (-ve) group. The rat groups which treated with cacao powder and cacao extract showed a significant decrease in serum total protein, albumin and albumin/ globulin ratio (p< 0.05) compared with control (-ve) group. The rat group which treated with choline showed a significant decrease in serum albumin (p< 0.05) compared with control (-ve) group.

The rat groups which treated with choline ,cacao powder with choline and cacao extract with choline

showed a significant increase in serum total protein, while the rat groups which treated with cacao powder with choline and cacao extract with choline showed a significant increase in serum albumin compared with control (+ve) group. All treated groups showed a significant increase in serum globulin and a significant decrease in albumin/ globulin ratio compared with control (+ve) rat group as represented in table (3).

Table (3): The Mean values ± SD of serum total protein, albumin, globulin and albumin/globulin (A/G) ratio of the experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Cacao powder	Cacao extract	Choline	Cacao powder+ choline	Cacao extract+ choline
T. protein (g/dl)	7.21± 1.21^{a}	5.34± 0.96^{c*}	5.91± 0.34^{bc*}	5.81± o.44^{bc*}	6.01± 0.57^{ab}	6.21± 0.88^{ab}	7.11± 1.23^{ab}
Albumin (g/dl)	3.49± 0.42^{a}	2.96± 0.22^{b*}	2.69± 0.33^{b*}	2.60± 0.34^{b*}	2.89± 0.32^{b*}	3.01± 0.42^{a}	3.37± 0.37^{a}
Globulin (g/dl)	3.72± 0.16^{a}	2.38± 0.11^{b*}	3.22± 0.15^{a}	3.21± 0.42^{a}	3.12± 0.32^{a}	3.20± 0.17^{a}	3.74± 0.29^{a}
Albumin / Globulin	0.93± 0.03^{b}	1.24± 0.22^{a**}	0.83± 0.06^{c*}	0.80± 0.01^{c*}	0.92± 0.05^{b}	0.94± 0.15^{b}	0.90± 0.08^{b}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

The control (+ve) rat group showed a significant decrease in the values of serum Hg and PCV (p< 0.01) compared with control (-ve) group. All treated groups showed non significant decrease in serum Hg and PCV compared with control (-ve) group but showed a significant increase in these parameters compared with control (+ve) rat group as represented in table (4).

Table (4): The Mean values ± SD of Hg and PCV of the experimental rat groups

Groups Variables	Control (-ve)	Control (+ve)	Cacao powder	Cacao extract	Choline	Cacao powder+ choline	Cacao extract+ choline
Hg (g/dl)	13.54± 2.11^{a}	8.25± 1.61^{c**}	11.31± 1.31^{ab}	11.99± 1.25^{ab}	10.88± 1.61^{ab}	11.14± 1.17^{ab}	12.11± 1.81^{ab}
PCV	38.25± 4.32a	29.13± 3.21^{c**}	34.14± 4.61^{ab}	34.35± 4.16^{ab}	33.21± 3.67^{ab}	32.41± 3.50^{ab}	35.62± 3.21^{ab}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Data in table (5) presented that control (+ve) rat group showed a significant increase in the values of serum cholesterol, triglyceride and LDLc (p<0.01&0.001) and a significant decrease in the values of serum HDLc (p<0.01) compared with control (-ve) group. The rat group which treated with cacao powder showed a significant increase in the values of serum cholesterol, LDLc (p<0.05) and triglyceride (p<0.01) compared with control (-ve) group. The rat groups which treated with cacao extract and choline showed a significant increase in the values of serum LDLc (p<0.05) compared with control (-ve) group.

All treated groups showed a significant decrease in cholesterol, LDLc and a significant increase in HDLc compared with control (+ve) rat group while rat groups which treated with choline, cacao powder with choline and cacao extract with choline showed a significant decrease in serum triglyceride compared with control (+ve) group.

Table (5) The Mean values ±SD of serum lipid patterns of the experimental rat groups.

Groups Variables	Control (-ve)	Control (+ve)	Cacao powder	Cacao extract	Choline	Cacao powder+ choline	Cacao extract+ choline
Cholesterol (mg/dl)	103.41± 10.61^{c}	155.61± 18.24^{a**}	120.11± 15.21^{b*}	110.14± 10.61^{bc}	112.21± 11.21^{bc}	105.21± 10.21^{bc}	106.11± 11.21^{bc}
Triglyceride (mg/dl)	75.11± 9.21^{c}	93.77± 11.21^{a**}	83.61± 10.14^{ab**}	80.21± 9.16^{bc}	82.11± 9.21^{bc}	79.21± 9.60^{c}	77.15± 8.11^{c}
HDLc (mg/dl)	31.11± 3.01^{a}	22.31± 2.96^{c**}	27.14± 2.91^{ab}	29.11± 2.69^{ab}	30.61± 3.16^{a}	33.21± 3.61^{a}	32.61± 3.11^{a}
LDLc (mg/dl)	57.28± 5.11^{c}	114.55± 11.13^{a***}	76.25± 9.12^{b*}	64.99± 6.35^{b*}	65.18± 6.25^{b*}	56.96± 5.41^{c}	58.07± 6.60^{c}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Data in table (6) illustrated that control (+ve) rat group and rat group which treated with cacao powder showed a significant increase in the values of (p<0.05, 0.01&0.001) compared with control (-ve) group. The rat group which treated with cacao extract showed a significant increase in the value of liver total lipids (p<0.01) and a significant decrease in the values of liver triglyceride and glycogen (p<0.05) compared with control (-ve) group. The rat group which treated with choline, cacao powder with liver cholesterol and total lipids (p<0.01&0.001) and a significant decrease in the values of liver triglyceride and glycogen choline and cacao extract with choline showed a significant decrease in the values of liver glycogen (p<0.05) compared with control (-ve) group.All treated rat groups showed a significant decrease in the values of liver cholesterol and total lipids and a significant increase in the values of liver triglyceride and glycogen compared with control (+ve) group

Table (6) The Mean values ± SD of liver cholesterol, total lipids, triglyceride and glycogen of the experimental rat groups.

Groups Variables	Control (-ve)	Control (+ve)	Cacao powder	Cacao extract	Choline	Cacao powder+ choline	Cacao extract+ choline
Cholesterol (mg/g)	4.11± 0.66^{c}	8.51± 1.21^{a***}	6.13± 1.25^{b**}	5.19± 1.11^{bc}	5.32± 1.20^{bc}	4.91± 1.34^{bc}	4.01± 1.31^{bc}
Total lipids (mg/g)	35.61± 4.11^{c}	62.67± 7.31^{a***}	45.67± 5.16^{b**}	40.13± 5.21^{b**}	38.21± 3.61^{bc}	35.21± 4.01^{c}	34.71± 3.21^{c}
Triglyceride (mg/g)	3.21± 0.21^{a}	1.96± 0.12^{c***}	2.77± 0.25^{b*}	2.96± 0.38$_{b*}$	3.01± 0.37^{a}	3.26± 0.33^{a}	3.61± 0.29^{a}
Glycogen (mg/100g)	6.91± 1.31^{a}	3.01± 0.55^{c***}	4.33± 0.76^{b*}	4.61± 0.68^{b*}	4.21± 0.77^{b*}	4.99± 0.63^{b*}	4.99± 0.63^{b*}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

Data in table (7) showed that control (+ve) rat group and rat group which treated with cacao powder showed a significant decrease in the values of liver GSH, GPX and SOD (p<0.05,0.001,&0.01) and a significant increase in the values of liver MDA (p<0.001&0.05) compared with control (-ve) group. The rat group which treated with cacao extract showed a significant decrease in the values of liver GPX and SOD (p<0.05) while rat group which treated with choline showed a significant decrease in the values of liver GPX and SOD (p<0.01) and a significant increase in the values of liver MDA (p<0.05) compared with control (-ve) group.

The rat group which treated with cacao powder with choline and cacao extract with choline showed a significant decrease in the value of liver SOD (p<0.05) compared with control (-ve) group.
.

Table (7) The Mean values ± SD of liver GSH, GPX, SOD and MDA of the experimental rat groups.

Groups Variables	Control (-ve)	Control (+ve)	Cacao powder	Cacao extract	Choline	Cacao powder+ choline	Cacao extract+ choline
GSH (µg /mg)	5.47± 1.12^{a}	2.35± 0.36^{c**}	3.96± 0.86^{b*}	4.11± 1.01^{a}	3.51± 0.77^{a}	4.81± 1.02^{a}	4.91± 0.99^{a}
GPX (µg /mg)	61.71± 8.21^{a}	27.14± 4.33^{d***}	43.65± 6.11^{b*}	49.32±5 .14^{b*}	32.41±3. 22^{cd**}	50.14± 6.11^{ab}	53.21± 5.99^{ab}
SOD (µg /mg)	57.11± 5.42^{a}	18.71±3. 20^{d***}	35.17± 3.71^{c**}	34.21±4 .51^{b*}	37.20±3. 21^{c**}	48.18± 4.36^{b*}	47.25± 5.11^{b*}
MDA (nmol/g)	42.17± 4.61^{c}	99.16± 8.21^{a***}	52.11± 5.16^{b*}	49.14±4 .13^{bc}	55.21±5. 61^{b*}	46.38± 4.88^{c}	45.11± 5.0^{3c}

Significant with control group * P<0.05 ** P<0.01 *** P<0.001
Mean values in each column having different superscript (a, b, c, d) are significant

4. Discussion

Previous studies have also shown that CCl4 has been extensively studied as a liver toxicant, and its metabolites such as trichloromethyl radical and trichloromethyl peroxy radical are involved in the pathogenesis of liver and kidney damage. CCl4 causes changes around the central vein in the liver and oxidative damages with the leakage of marker enzymes such as alanine and aspartate amino transferasein the serum (Lee et al., 2005). The highest levels of AST and ALT are found with disorders that cause the death of numerous liver cells. Generally, ALT and AST are good indicators for liver inflammation. Once inflammation is reduced, the progression of liver fibrosis can be held or even reversed (Abd El-Ghany 2006). Numerous studies indicated that the health promoting properties of cocoa powder were attributed mainly to their polyphenolic compounds and methylxanthines. Cocoa comprises mainly of procyanidins monomers, namely, catechin and epicatechin, dimer, trimer, tetramer, and up to tetradecamer. In addition, methylxanthines, namely, caffeine, theobromine, and theophylline, had also been identified in cocoa (Tomas-Barberan et al., 2007). Flavonoids in cacoa inhibit LDL oxidation and reduce thrombotic tendency in vitro and have potential protective effects on risk of cardiovascular disease (Wan et al., 2001). Oligomeric procyanidins from cocoa powder are the principal active components responsible for the hypocholesterolemic effect, and inhibit the intestinal absorption of cholesterol and bile acids through the decrease in micellar cholesterol solubility (Erdman et al .,2000). Cocoa intake enhanced total antioxidant capacity in all tissues. Moreover, SOD and catalase activities were also dose-dependently increased by cocoa (Emma et al., 2007). Copper in cocoa and chocolate significantly contributed to the human diet so cocoa could at least augment the antioxidant defense system through enhancement of SOD activity (Joo et al., 1995 and Vinson et al., 1999). Several studies have suggested that choline can synthesize it in small amounts but must be consumed in the diet to maintain health. The majority of the body's choline is found in specialized fat molecules known as phospholipids, the most common of which is called phosphatidylcholine or lecithin. Choline is essential for the production of the neurotransmitter acetylcholine which sends electrical impulses across synapses between nerve cells, and from motor neurons to muscle cells, causing the muscle cells to contract. It is also an important component of our cell membranes in the form of phosphatidylcholine and sphingomyelin. In addition, it is also required for the proper metabolism of fats (Zeisel 2000).Choline and compounds derived from choline serve a number of vital biological functions. Choline prevents trapped fats in the liver and prevents liver and kidney disorders. Also, it can prevent atherosclerosis by decreasing cholesterol. Choline is required to form the phosphatidylcholine portion of very low density lipoprotein (VLDL) particles which transport fat from the liver to the tissues. In choline deficiency, reduced blood VLDL particles synthesized and fat accumulates in the liver elevated blood levels of a liver enzyme called alanine aminotransferase (ALT) resulting in liver damage (Zeisel and Blusztajn1994 and Bidulescu et al .,2007). Choline keeps the liver healthy by helping to move fats from the liver to cells in the body. Choline makes phosphatidylcholine, which is crucial for making the fatty substance that is used to form cell membranes and phosphatidylcholine may in turn be used by the body as a source of choline (Canty and Zeisel 1994).

References

1. Abd El-Ghany, M. A (2006): Nutraceutical effects of garlic, olive, parsley and mentha oils on CCL4 induced liver damage in rats. Egyptian J of Nutrition Vol XXI No .4:125-159.

2. Abell, L.L., Levy, B.B., Brodie, B.B. and Kendal, R. (1952): A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J .Biol .Chem , 357-366.
3. Allain, C.C., Richmond, N. and Rosechloy, P. (1974): Cholesterol enzymatic colorimetric test. Clin. Chem., 19 (20): 1350-470. Bartholomev, R.J and Delaney, A. (1966): Proc Aust. Assoc. Biochemists .1, 214.
4. Bergmeyer, H.U. and Harder, M. (1986): A calorimetric method of determination of serum glutamic oxalate and glutamic Pyurvic tranaminase. Clin. Bio Cham. 24: 481-488.
5. Beuchamp, C and Fridovich, J. (1971): Superoxide dismutase. Improved an assay applicable to acrylamide gels. Anal Biochem.44:276-287.
6. Bidulescu, A., Chambless, L.E., Siega-Riz, A.M., Zeisel, S.H. and Heiss, G. (2007): Usual choline and betaine dietary intake and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. BMC Cardiovasc Disord, 7:20.
7. Bonsens, K. E. and Taussky, D. H. (1984): Determination of serum Creatinine. J Ch Inv, 27, 648-660.
8. Buccolo, G. and David, H. (1973): Ouantitative determinarion of serum triglycerides by use enzymes. Clin. Chem., 19: 419-32.
9. Canty D. J. and Zeisel S. H. (1994): Lecithin and choline in human health and disease. Nutr. Rev, 52:327–339.
10. Chapman, D.G.; Gastilla, R. and Campbell, T.A. (1950): Evaluation of protein in food. I. A. Method for the determination of protein efficiency ratio. Can. J. Biochem. Physio. I (37) 679-686.
11. Coles, E. H (1974): Veterinary Clinical Pathology. Saunders Company, Philadelphia and London.
12. Drabkin, D.I. (1949): The standardization of hemoglobin measurements. Am. J. Med. Sci 21 (7): 710.
13. Emma , R., Mireia, U. , Francisco, J. , Ngels, F., Cristina, C., Cristena ,A, A., Maria, I. and Margarida, C.(2007): Cocoa-Enriched Diet Enhances Antioxidant Enzyme Activity and Modulates Lymphocyte Composition in Thymus from Young Rats. J. Agric. Food Chem., 55, 6431-6438.
14. Erdman, J.W., Wills, J. and Finley, D. (2000): Chocolate: Modern science investigates an ancient medicine. J Nutr, 130:2057–2126.
15. Folch, J., lees, M. and Stanley, G.H. (1957): A simple method for isolation and purification of total lipid from animal tissue. J Biol Chem 266: 497-509.
16. Fruchart, G.G. (1982): LDL-Cholesterol determination after separation of low density lipoprotein. Rev. Fr. Des. Laboratories, 103: 7:117.
17. Guyton, A.C. and Hall, J.E. (1996): The liver as an organ. In: Textbook of Medical Physiology, WB Saunders, Philadelphia.
18. Jaeschke, H., Gores, G.J., Cederbaum, A.I., Hinson, J.A., Pessayre, D., and Lemasters, J.J. (2002): Mechanisms of hepatotoxicity. Toxicol Sci , 65: 166–76.
19. Jendrassik, L. (1938): Colorimetric determination of bilirubin. Biochem., 97:72-81.
20. Joo, S., Kies, C. and Schnepf, M. (1995): Chocolate and chocolate-like products: impact on copper status of humans. J. Appl. Nutr., 47: 67–77.
21. Kind, P.R and King, E.J. (1954): Estimation of alkaline phosphatase activity by determination of hydrolyzed phenol with aminoantipyrene. J. Clin.Path.7, 322.
22. Kostener, C. M. (1977): Enzymatic determination of cholesterol high density lipoprotein fraction prepared by polyanion precipitation. J. Clin. Chem., 22:695.
23. Lecumberri, E., Mateos, R., Ramos, S., Alia, M., Ruperez, P.,Goya, L., Izquierdo-Pulido, M. and Bravo, L. (2006): Characterization of cocoa fiber and its effect on the antioxidant capacity of serum in rats. Nutr. Hosp, 21, 622-628.
24. Lee, G.P., Jeong, W.I., Jeong, D.H., Do, S.H., Kim, T.H. and Jeong, K.S. (2005): Diagnostic evaluation of carbon tetrachloride-induced rat hepatic cirrhosis model. Anticancer Res. Mar-Apr; 25(2A):1029-38.
25. Mc Inory, R.A. (1954): A micro heamatocrit for determining the packed cell and hemoglobin concentration on capillary blood. J. Clin. Path. (7): 32.
26. Reed, D.J. (1999): Glutathione: toxicological implications. Ann Rev Pharm Toxicol 30:603–631.
27. Reeves, P.G., Nielson, F.H. and Fahmy, G.C. (1993): Reports of the American Institute of Nutrition, adhoc wiling committee on reformulation of the AIN 93. Rodent diet. J. Nutri, 123, 1939-1951.
28. Rerup, E. and Lundquist, S. (1967): Precipitation and purification of liver glycogen in rats. Acta Pharmmic Tox, 25:47-51.
29. Steel, R.G.D. and Torrie, J.H. (1980): Analysis of covariance, In: Principles and Procedures of Statistics. A Biometrical Approach, McGraw-Hill, New York, 401-437.

30. Stephen, E., Akira,K., Erwin, G., Takehiko, U., Mathias, F.,Helmut, S., Ronald, G. and Gavin, E.(2002): Cocoa extract protects against early alcohol-induced liver injury in the rat. Archives of Biochemistry and Biophysics, 406: 40–46.
31. Tomas-Barberan, F. A., Cienfuegos-Jovellanos, E., Marin, A.;Muguerza, B., Gil Izquierdo, A., Cerdaa, B., Zafrilla, P., Morillas,J., Mulero, J., Ibarra, A., Pasamar, M., Ramoan, D., Espin, J. C.(2007):A new process to develop a cocoa powder with higher flavonoid monomer content and enhanced bioavailability in healthy humans. J. Agric. Food Chem., 55, 3926–3935.
32. Uchiyama, M. and Mihara, M. (1978): Determination of malondialdhyde precursor in tissues by thiobarbituric acid test. Anal. Biochem, 86 (1), 271-278.
33. Vinson, J. A., Proch, J .and Zubik, L. (1999): Phenol antioxidant quantity and quality in foods: cocoa, dark chocolate, and milk chocolate. J. Agric. Food Chem., 47, 4821–4824.
34. Wan, Y., Vinson, J. A., Terry, D., John, P., Sheryl, A. and Penny, M. (2001): Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. Am J Clin Nutr, 74:596–602.
35. Weichselbaum, T.F (1946): An accurate and rapid method for the determination of protein in small amount of blood serum and plasma .Am .J. Clin .Path. (16):40.
36. Weiss, C., Marker, H.S. and Lehrer, G.M. (1980): Sensitive fluorometric assays for glutathione peroxidase and reductase. Anal Biochem 106: 512–516.
37. WHO (1983): Protocol CG-04: Preparation of alcoholic extract for bioassay and phytochemical studies (APJF/IP, 1001A). Geneva, World Health Organization.
38. Young, A. and Pestaner, D.L. (1975): Determination of triglycerides in serum .Clin.Chem. 21:5-7.
39. Zeisel, S.H (2000): Choline: an essential nutrient for humans. Nutrition, 16:669-671.
40. Zeisel, S.H. (1999): Choline and phosphatidylcholine, In Shils M et al. (Eds). Nutrition in Health and Disease. Ninth Edition. Williams & Wilkins, Baltimore, 513-523.
41. Zeisel, S.H. and Blusztajn, J.K. (1994): Choline and human nutrition. Annual Review of Nutrition, 14: 269-296.

7/8/2011