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Original Article

Beneficial effects of young coconut juice feeding on the lipid, renal and liver profiles, in ovariectomized rats: Preliminary novel findings

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Abstract

The purpose of this study was to determine the effects of feeding young coconut juice (YCJ), known to contain β-sitosterol, to ovariectomized rats, a model for postmenopausal women, on the lipid, renal and liver metabolism profiles. Four groups of female rats (6 in each group) were included in this study. These included sham-operated, ovariectomized (ovx), ovx receiving estradiol benzoate (EB) injections intraperitoneally, and ovx receiving YCJ orally. At the end of the third and the fourth week of study, the rats were sacrificed and their serum estradiol (E2) was analyzed. The uterus was removed along with the kidney and liver. The latter was paraffin processed for histopathological assessment. In contast with the 7 days treatments, all parameters of the 14 days treatment had improved. After 14 days of treatment, the circulating levels of BUN, creatinine, cholesterol, triglyceride, LDL, AST, ALT, ALP, total protein and albumin of ovx+YCJ group were not significantly different from the sham and ovx groups. Only the serum HDL level of the ovx+YCJ group was significantly higher than that of the sham group. The histopathological assessment of the liver and kidney showed no significant changes when compared with the control groups. Glycogen accumulation appeared in the cytoplasm of the hepatocytes particularly in the ovx+YCJ group but significant changes of the hepatocytes containing glycogen were not detected. No other abnormal features were seen in any of the four groups. The %age uterine/body weights indicated that YCJ feeding in ovx rats at 100 mL/kgBW for up to two weeks did not cause increased uterine weight. In summary, this study confirmed that feeding YCJ had beneficial effects on the serum lipid profile, and maintained liver and renal functions for up to 2 weeks after administration.

Keywords: young coconut juice, liver, kidney, estrogen, lipid

1. Introduction

Menopause is the permanent cessation of menstruation and leads to a marked decrease in estradiol levels. This decrease results in an unfavourable lipid profile that might have a deleterious effect on the cardiovascular system and accelerates neurodegenerative diseases (Baker *et al.,* 2003; Alkayed *et al.,* 2000). Atherosclerosis induced by the abnormalities of lipid metabolism after menopause is a major risk factor for cardiovascular disease. The major anti-artherosclerotic effect of estrogen is associated with its beneficial effects on lipid metabolism including increased high-density lipoprotein (HDL), decreased low-density lipoprotein (LDL) and cholesterol concentrations (Seed and

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Crook, 1999).

Hormone replacement therapy (HRT) is one treatment used to provide relief for postmenopausal women to relieve symptoms e.g. hot flushes, night sweats and sleep disturbances etc. However, health risks can exceed the benefits for any long-term treatment. An increased risk of heart disease, breast cancer, and stroke was found in long term HRT. Thus, alternative therapies using natural plant-derived products containing phytoestrogens might offer alternative options.

For example, Kapiotis and colleaques (Kapiotis *et al.,* 1997) found that genistein, a dietary-derived inhibitor of angiogenesis, prevented LDL oxidation and protected endothelial cells from damage by atherogenic LDL. Lucas and colleaques found that flaxseed, a rich source of the phytoestrogens, reduced the serum levels of both the low-density-(LDL-c) and high-density-lipoprotein cholesterol (HDL-c) and also the triglycerides (Lucas *et al.,* 2002).

Our previous studies have found that young coconut juice (YCJ) helped to prevent Alzeihmer pathologies, accelerate wound healing, and prevent osteoporosis in ovariectomized (ovx) and orchidectomized (orx) rats, a model for postmenopausal women and andropausal men, respectively (Radenahmad *et al.,* 2006, 2009, 2011, 2012; Yusuh *et al.,* 2010; Suwanpal *et al.,* 2011).

Recent studies have indicated that the juice from young coconuts had a significant cardioprotective effect in various male animals e.g. rats and quails, by lowering serum total cholesterol, LDL, very low-density lipoprotein (VLDL) and triglycerides (TGs). The effect of YCJ on the HDL level was however controversial (Zhonghua *et al.*, 1995; Anurug and Rajamohan, 2003; Sandhya and Rajamohan, 2006; Tangpong *et al.* 2008).

The present study was therefore carried out to investigate the effects of YCJ feeding on serum lipid levels, one of the biomarkers for atherosclerosis, in the ovx rat, a model used for postmenopausal women. Secondly, we examined the effects of YCJ feeding on renal functions by measuring serum BUN, creatinine levels and a histopathological assessment of renal tissue by measuring renal corpuscle areas. Thirdly, we investigated the effects of YCJ feeding on liver functions using the liver enzymes as biomarkers: AST, ALT, ALP; total protein and albumin. Since YCJ contains a high level of glucose (45%) and fructose (44%) (Santoso *et al.,* 1996), any excess of these consumed monosaccharides would be converted and accumulated as glycogen in the liver. Hence, a histopathological assessement using special staining, PAS (periodic acid Schiff), was carried out to establish the effects of YCJ feeding on glycogen accumulation in the hepatocytes of liver.

2. Materials and Methods

2.1 YCJ preparation

A large volume of young coconut juice (*Cocos nucifera* L., Arecaceae) was collected from Tungngai district, Hat Yai, Songkhla, Thailand, then dried, and the powder form was kept at -30°C until used. This powder was freshly reconstituted and prepared for oral intake every day. A complete description of YCJ, including its preparation and administration, has been provided in our previous publications (Radenahmad *et al.,* 2006).

2.2 Animals

All animals used were adult two-month female Wistar rats weighing approximately 230 g. The animals were housed in a controlled environment at 25±1°C on an illumination schedule of 12h light/12h dark. Rats had unrestricted access to standard pellet food and water. The study was approved by the Committee on Animal Care and carried out in accordance with the Guiding Principles for the Care and Use of Research Animals promulgated by Prince of Songkla University.

2.3 Experimental design

There were four groups (6 rats per group) included in this study. The first group consisted of ovx rats, the second group was the sham-operated rats, the third group was ovx rats injected intraperitoneally with exogenous estrogen (2.5 μg/kgBW of estradiol benzoate, EB) once a day everyday, and the fourth group included ovx rats that received YCJ (100mL/kgBW/day). The dose of EB and YCJ in this study was based on the one reported in our earlier study in which dose standardization and optimal administration had been set (Radenahmad *et al.,* 2006, 2009). The administration of EB and YCJ was started two week after ovariectomy was performed. Rats belonging to the first and second groups received deionizied water instead of EB and YCJ. The rats were force fed with deionized water, YCJ or those given an injection of EB for another 7 (called 7 day treatment) or 14 days (called 14 day treatment). At the end of the experimentation, animals were sacrificed and the liver and kidney were removed and processed into paraffin blocks. The uterus was also removed, weighed and compared among the four groups examined. Serum was collected for estradiol measurements using a chemiluminescent immunoassay (CIA).

2.4 Light microscopy

The kidneys of both sides and small blocks from the livers of six rats from each experimental group were fixed in 10% buffered formalin. After routine histological laboratory procedures, tissues were blocked in paraffin and sections of 5 μm were cut and stained with haematoxylin and eosin (H and E). Periodic Acid Schiff (PAS) was also applied for liver sections to detect glycogen accumulation in the hepatocytes.

2.5 Microscopic analysis by quantitative histomorphometry

Counting of all parameters was performed by two

independent observers. An eyepiece micrometer was mounted on a light microscope, and counting was made at x100 magnification. Readings from both observers were then added and the average was determined. Three sections from the biggest lobe of the liver and from the middle part of the kidney from each side were selected for this study. Histomorphometric analysis using image analysis and quantification of all parameters were performed using an Image Pro Plus program (DP11, Olympus SZX 12, Japan). For liver investigation, the data were expressed as numbers of hepatocytes containing glycogen per μm^2 from ten random areas of each section per rat. Cross sections were produced from the right kidney and longitudinal sections from the left side. The two perpendicular diameters across each renal corpuscle, reaching Bowman's capsule on each side, were measured. A total of 20 randomly chosen renal corpuscles were calculated in order to obtain the renal corpuscle areas (μm^2) per rat. The means ± SEM was used to compare the four groups.

2.6 Blood biochemistry and serum estradiol

Whole blood was allowed to clot and then centrifuged at 3000 g for 15 minutes to obtain serum and kept at -20°C until the determination of serum lipid, protein, renal and liver function markers. Serum levels of total cholesterol, triglycerides, HDL, LDL, AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), total protein, albumin, BUN and creatinine were determined using the Modular P800 Roche Diasnotic, Thailand and for estradiol (E2) using the chemiluminescent immunoassay (CIA) technique (ECLIA, Modular E 170C, Estradiol II 03000079 122, Roche, Germany).

2.7 Statistical analysis

The data were analyzed by calculating a mean value and the standard error of the mean (mean ± SEM). Statistical analysis was performed using the Kruskal-Wallis and the Mann-Whitney U-tests. Values of $p<0.05$ were considered statistically significant.

3. Results

3.1 Histopathological assessment of liver and kidney

Using periodic acid Schiff (PAS) staining of liver tissue, glycogen deposits in the cytoplasm of the hepatocytes were stained as pink clumps (Figure 1). No significant histopathological changes of the liver or kidney tissues were observed after 7 or 14 days of treatment with YCJ. Necrotic cells were not observed either in the liver or renal tissues (Figures 1 and 2). Comparisons among the four groups after either 7 or 14 days of treatment, also showed no statistically significant differences in the numbers of hepatocytes containing glycogen was measured (Figure 3A). However when the number of hepatocytes containing glycogen after

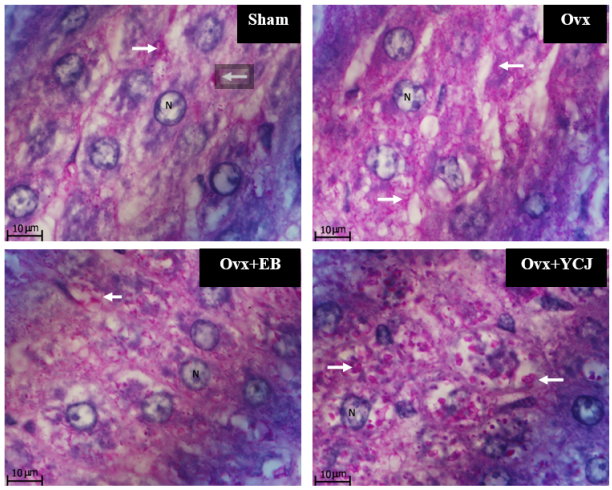

Figure 1. Histology of liver tissue:
Effects of YCJ feeding for 14 days compared with control groups (sham, ovx, ovx+EB) on the histopathology of the liver tissue. White arrows indicate the pink stained glycogen deposits in the cytoplasm of the hepatocytes. No necrotic cells or other histopathologies of the liver tissue were found. Periodic Acid Schiff (PAS) staining of liver tissue paraffin sections, 5 µm thick, x 40 magnification. N = nucleus of the hepatocytes.

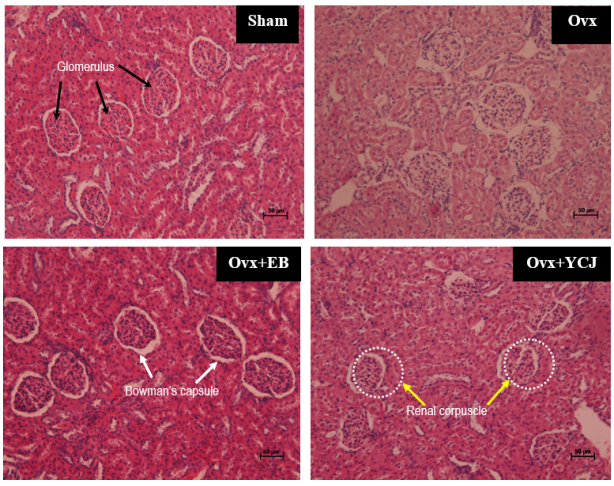

Figure 2. Histology of renal tissue:
Effects of YCJ feeding for 14 days compared with the control groups (sham, ovx, ovx+EB) on the histopathology of the renal tissue. No histopathologies of renal tissue were found. However, it was of interest that poor staining of the ovx group was observed. Haematoxylin-eosin staining of renal tissue paraffin sections, 5 µm thick, x 40 magnification. White circles indicate the renal corpuscles (yellow arrows) composd of a glomerulus (black arrows) and bowman's capsules (white arrows).

14 days of treatment were compared with those after the 7 day treatment in each group, there was a significant decrease at $p<0.05$ (Figure 3A).

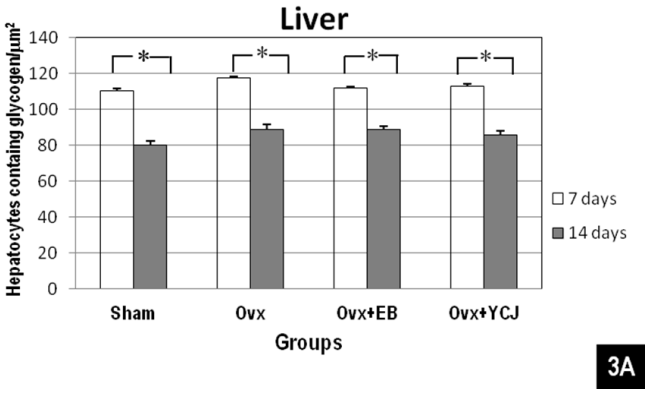

Figure 3A. Numbers of glycogen containing hepatocytes/µm^2 after 7 and 14 days of treatment in the four groups examined. Values are expressed as a mean ± SEM, n = 6 in each group. No statistically significant differences were observed between the controls (sham, ovx, ovx+EB) and the YCJ feeding (ovx+YCJ) groups. Nevertheless, when the number of hepatocytes containing glycogen after 7 and 14 days of treatments were compared, for each group statistically significant differences were observed: P = 0.02 for sham group, P = 0.01 for ovx and ovx+EB groups and P = 0.03 for ovx+YCJ group. Ovx = ovariectomized group; Ovx + EB = ovariectomized groups receiving estradiol benzoate; Ovx + YCJ = ovariectomized group receiving young coconut juice.

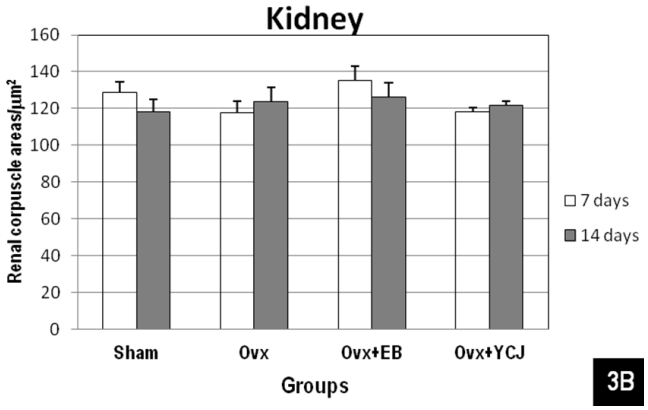

Figure 3B. Numbers of renal corpuscle areas/µm^2 after 7 and 14 days of treatment in the four groups examined. Values are expressed as a mean ± SEM, n = 6 in each group. No statistically significant differences between the controls (sham, ovx, ovx+EB) and YCJ feeding (ovx+ YCJ) groups were found. Ovx = ovariectomized group; Ovx + EB = ovariectomized group receiving estradiol benzoate; Ovx + YCJ = ovariectomized group receiving young coconut juice.

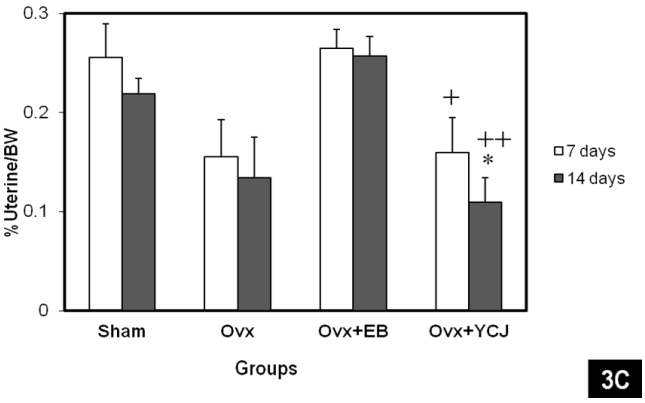

Figure 3C. The uterine weight was calculated as a percentage compared with the body weight (BW) (% Uterine/BW) in the four groups examined. Values are expressed as a mean ± SEM, n = 6 in each group. Ovx = ovariectomized group; Ovx + EB = ovariectomized group receiving estradiol benzoate; Ovx + YCJ = ovariectomized group receiving young coconut juice. *p<0.05 compared with the sham group. $^{+, ++}$p<0.05, p<0.01 compared with the Ovx+EB group respectively.

The observed areas of the renal corpuscles showed no significant changes when the control groups were compared with the experimental groups from either the 7 or 14 day treatment groups (Figure 3B).

3.2 Effects of YCJ on uterine weight

The %age uterine/body weight of the ovx+YCJ group after both 7 and 14 days were significantly lower than for the ovx+EB group at p<0.05 and p<0.01 respectively. Furthermore, the %age uterine/body weight of the ovx+YCJ group after 14 day treatment was significantly lower than for the sham group at p<0.05 (Figure 3C).

3.3 Blood biochemistry

After 7 days of treatment, the circulating levels of BUN, creatinine, triglyceride of the ovx+YCJ group were significantly higher than for the sham and ovx groups. Furthermore, serum triglyceride level of the ovx+EB group was significantly higher than for the ovx group. The AST and ALP levels of the ovx+ EB group were significantly lower

Table 1. Effects of 7 days of feeding with YCJ compared with the control groups (sham, ovx) on serum liver, kidney enzymes and lipid profiles of the ovx rats. Values are expressed as a mean ± SEM, n=6 in each group. Ovx = ovariectomized group; Ovx + EB = ovariectomized group receiving estradiol benzoate; Ovx+YCJ = ovariectomized group receiving young coconut juice. BUN = Blood urea nitrogen; HDL = High Density Lipoprotein cholesterol; LDL = Low Density Lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = Alkaline phosphatase; $^{a}p<0.05$, $^{aa}p<0.01$ compared with the sham group; $^{b}p<0.05$, $^{bb}p<0.01$ compared with the ovx group.

7 day treatment	Sham	Ovx	Ovx+EB	Ovx+YCJ
BUN(mg%)	25.52±1.33	22.47±0.52	25.10±1.51	25.35±1.02^{b}
Creatinine(mg%)	0.69±0.02	0.63±0.05	0.69±0.08	0.81±0.04^{a,b}
Cholesterol(mg%)	70.00±3.61	78.67±7.27	77.83±4.81	85.33±11.17
Triglyceride(mg%)	143.33±21.96	86.83±9.49	195.83±16.61^{bb}	155.17±15.99^{b}
HDL(mg%)	54.08±2.42	66.82±5.36	57.57±2.98	46.05±9.03
LDL(mg%)	12.13±2.92	11.43±2.24	9.88±2.31	13.25±3.28
AST(U/L)	388.00±59.28	219.67±37.82	188.60±20.66^{a}	193.33±38.50
ALT(U/L)	67.50±8.98	58.33±8.52	59.33±12.38	59.50±11.20
ALP(U/L)	105.17±18.69	116.83±12.98	72.83±5.59^{bb}	154.25±27.20
Total protein(g%)	7.72±0.19	7.02±0.17^{a}	7.60±0.16^{b}	7.68±0.29
Albumin(g%)	4.45±0.04	4.05±0.06^{aa}	4.68±0.15^{bb}	3.82±0.32

than for the sham and ovx groups respectively. The total protein and albumin levels of the ovx+ EB group were significant higher than for the ovx group (Table 1).

After 14 days of treatment, all parameters were improved. The circulating levels of BUN, creatinine, cholesterol, triglycerides, LDL, AST, ALT, ALP, total protein and albumin of the ovx+YCJ group were not significantly different from the sham and ovx groups. In the ovx+YCJ group, the serum HDL was significantly higher than for the sham group. The serum triglycerides and albumin of the ovx+EB group were higher than for the sham and the ovx groups. When compared with the ovx group, the serum total protein of the ovx+EB group was higher. In contrast, the serum ALP of the ovx+EB group was lower than for the sham and the ovx groups. The serum cholesterol and HDL of the ovx group was higher than for the sham operated group (Table 2).

3.4 Serum estradiol

Serum E2 levels were highest in the ovx+EB groups after both 7 and 14 day treatments. The circulating E2 level was significantly ($p<0.01$) lower than in the ovx+YCJ group following 14 days of YCJ intake, when compared to the other groups. A similar trend was also seen after 7 days of treatment (Radenahmad *et al.,* 2012).

4. Discussion

Changes in lipid metabolism after menopause are characterized by an overall shift towards the more atherogenic lipid profile e.g. increased triglyceride and LDL cholesterol, decreased HDL cholesterol. These adverse changes may also contribute to the increased risk of coronary heart disease and neurodegenerative diseases (Baker *et al.,* 2003; Alkayed *et al.,* 2000).

The results from the present study have indicated that YCJ feeding has a significant beneficial effect on lipid metabolism in ovx rats. Firstly, it was found that after 7 day treatment the serum triglyceride levels of the ovx+YCJ and ovx+EB groups were significantly higher than the ovx group. After 14 days of treatment, the triglyceride level of the ovx+ YCJ group were not significantly different from the sham and ovx groups. In contrast, in our experimental conditions, estradiol treatment (ovx+EB group) had an unfavorable effect on increasing triglyceride levels after 7 and 14 days of treatment as it was significantly higher than for the sham and ovx groups. This is similar to the study by Rachon (2007) who found that triglyceride was slightly increased in the ovx rats treated with EB at 10 mg/kgBW compared to the control. The hypertriglyceridemic effect of estradiol has been well described by Turpin and Bruckert (1994) and Bottner and Wuttke (2006). Altogether, YCJ showed a beneficial effect on the lowering of triglycerides while EB, when used for HRT, increased the triglycerides.

Sandhya and Rajamohan (2008) showed that the decrease in the concentration of serum triglyceride in rats fed with coconut water may be due to the increased activity of a lipoprotein lipase in the heart and adipose tissue. This could increase the clearance of the triglyceride-rich lipoprotein, namely the chylomicrons, and VLDL. A decreased activity of the lipogenic enzymes e.g. malic enzyme, glucose-6-phospate dehydrogenase, isocitrate dehydrogenase in the liver all of

Table 2. Effects of 14 days of feeding with YCJ compared with the control groups (sham, ovx) on the serum liver, kidney enzymes and lipid profiles of ovx rats. Values are expressed as a mean ± SEM, n=6 in each group. Ovx = ovariectomized group; Ovx + EB = ovariectomized group receiving estradiol benzoate; Ovx+YCJ = ovariectomized group receiving young coconut juice. BUN = Blood urea nitrogen; HDL = High Density Lipoprotein cholesterol; LDL = Low Density Lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = Alkaline phosphatase; $^{a}p<0.05$, $^{aa}p<0.01$ compared with the sham group; $^{b}p<0.05$, $^{bb}p<0.01$ compared with the ovx group.

14 day treatment	Sham	Ovx	Ovx+EB	Ovx+YCJ
BUN(mg%)	26.13±1.37	27.87±1.35	27.27±1.66	23.65±1.32
Creatinine(mg%)	0.68±0.02	0.64±0.04	0.67±0.04	0.67±0.03
Cholesterol(mg%)	61.33±2.08	71.67±2.44^{a}	70.33±4.02	71.00±3.83
Triglyceride(mg%)	137.17±11.57	113.17±17.84	233.00±25.23^{a,b}	112.83±8.89
HDL(mg%)	47.05±1.32	57.57±3.20^{a}	48.58±1.73	63.12±3.04^{aa}
LDL(mg%)	5.98±1.77	9.17±2.27	6.10±5.14	6.73±0.89
AST(U/L)	277.25±26.84	233.50±8.62	358.00±38.95	301.67±27.71
ALT(U/L)	87.17±13.81	75.33±15.04	78.67±7.43	65.83±3.96
ALP(U/L)	77.67±6.73	127.80±14.21	51.67±8.63^{a,bb}	102.80±11.39
Total protein(g%)	7.70±0.14	7.42±0.17	8.20±0.19^{b}	7.22±0.17
Albumin(g%)	4.52±0.09	4.05±0.30	5.20±0.16^{aa,bb}	4.18±0.22

which are involved in producing NADPH for fatty acid synthesis also is in line with a decrease of synthetic triglycerides in rats fed with coconut water (Sandhya and Rajamohan, 2008).

Secondly, compared with the sham group, the serum cholesterol levels in the ovx group after 14 days of treatment were significantly higher, while that after 7 day treatment were not. It was of interest that cholesterol levels in the ovx+EB and ovx+YCJ groups were not significantly different from the sham group after either 7 or 14 days of treatment. This could be explained by Sandhya and Rajamohan (2008) who found that the lipid lowering effect in rats fed with supplements of coconut water resulted from various mechanisms such as inhibition of intestinal absorption of dietary lipids, inhibition of the *de novo* synthesis or from increased catabolism of cholesterol and fatty acids in the liver. Also the observed the lower level of serum cholesterol was not due to a decrease of cholesterol biosynthesis but may be caused by a greater increase in the rate of degradation of cholesterol to bile acid rather than to an increased rate of synthesis (Sandhya and Rajamohan, 2008).

Sandhya and Rajamohan (2006) also showed that YCJ contained several biologically active compounds that could influence lipid levels. These included a free amino acid L-arginine, ascorbic acid, and minerals such as calcium, magnesium, and potassium. Among these, L-arginine could play a major role as YCJ contained about 30 mg% or 150 mg% constituents of L-arginine in tender (six months maturity, the same as we used in the present study) and mature (10 months maturity) coconut juice respectively. There have been several reports that L-arginine has both significant hypolipidemic and anti-atherogenic effects (Ryzhenkov *et al.,* 1984; Hadzieva *et al.,* 2000; Miguez *et al.,* 2004) that is mediated via the formation of nitric oxide (NO) (Wang *et al.,* 1994; Cooke *et al.,* 1992). NO is one of the major endothelium-derived vasoactive mediators, that has been characterized as an "endogenous anti-atherosclerotic molecule". Experiments are being conducted to see if the hypolipidemic and anti-atherogenic effects of YCJ at lower doses of 100 mL/kgBW were still effective over a longer period e.g. 10 weeks, in the ovx rat model of osteoporosis.

The HDL level of the ovx+YCJ group after 7 days of treatment was not significantly different from that of the sham or ovx group but after 14 days of treatment it was significantly higher ($p<0.01$) than the sham group. This study has provided similar results, with some values that were even better when compared to previous studies using YCJ. For example, Tangpong (2008) found that overweight male rats orally force fed with YCJ at a dose of 5 mL/kgBW/day for 4 weeks had reduced serum cholesterol, triglycerides, LDL cholesterol levels but not HDL-cholesterol. In the present study we showed that feeding with a higher dose of YCJ at a dose of 100 mL/kgBW/day for only 2 weeks improved the lipid profile by increasing the HDL levels while a dose of 5 mL/kgBW/day (Tangpong *et al.,* 2008) did not. Hence these results clearly show that the higher the concentration of YCJ, the higher the HDL level. In addition, the duration of the YCJ feeding might also be an important factor for lowering serum lipids and increasing HDL.

Another possibility that could explain the lowering effect of the lipid parameters by YCJ were the results from feeding phytosterols, as well as soy and flavone. Using gas

chromatography-mass spectrometry our team has recently confirmed that YCJ contains sitosterol, stigmasterol, campesterol (Rujiralai and Sitaruno, 2009) and β-sitosterol (Ratanaburee *et al.* 2014). Kritchevsky found that a diet rich in β-sitosterol reduced the total liver cholesterol in rats (Kritchevsky *et al.,* 1999) and Heinemann found that phytosterol lowered the plasma cholesterol in hypercholesterolemic patients (Heinemann *et al.,* 1991).

We found that after 14 days of treatment, serum BUN and creatinine levels of the ovx+YCJ group were not significantly different from the sham and the ovx groups while after 7 days of treatment, both parameters were significantly higher than either the sham or the ovx group. Our findings are consistent with the results of Manosroi *et al.* (2004) using *P. mirifica* and *B. superba*. Our study showed that YCJ was safe to feed rats for up to a period of 2 weeks. In addition, we are the first group who carried out the kidney safety tests by analyzing serum BUN and creatinine levels after YCJ treatment.

We also found that after either 7 or 14 days of treatment, serum AST, ALT, ALP, total protein and albumin levels of the ovx+YCJ group were not significantly different from the sham and ovx groups. Our findings are similar to the results of Manosroi *et al.* (2004) using *P. mirifica* and *B. superb*. They found that all those parameters produced no significant differences among all the groups. Furthermore, our study has shown that in the ovx+EB group after either 7 or 14 days of treatment, serum AST, ALT, ALP, total protein and albumin levels were significantly different from the sham or ovx group. This indicated that YCJ was safe compared with EB, often used for HRT.

Histopathological studies revealed that glycogen did accumulate in the hepatocytes of liver particularly in the ovx+YCJ group (Figure 1). However, when the number of hepatocytes containing glycogen were counted and compared, there were no significant differences among all 4 groups. This is a similar result to that of Sandhya and Rajamohan (2006) who found that fatty accumulation in the liver was much less in cholesterol-fed rats supplemented with coconut water compared with the control group. In the present study, by gross observation, no fat was observed to accumulate in the liver, even though we used a very high dose of 100 mL/kgBW of YCJ compared with Sandhya and Rajamohan (2006), who used a lower dose of YCJ (40 mL/kgBW). That might be because their rats were treated over a longer period (90 days), than ours (1 and 2 weeks). Nevertheless, a further study by Sandhya and Rajamohan in 2008 found that rats fed a fat-high cholesterol diet lead to increased activities of serum AST, ALT, ALP while supplementation with coconut water leads to a decreased activity of these enzymes. This agrees with the present study as seen when serum analysis was tested as mentioned above and when the number of hepatocytes containing glycogen after 7 and 14 days of treatments were compared, for each group, it was found that hepatocyte numbers after 14 days of treatment were significantly lower than that of 7 day treatment. That means that the longer the rats were forced fed with YCJ, the lower the glycogen accumulated in hepatocytes. These results indicate that fatty infiltration and degeneration of liver cells caused by fat-cholesterol feeding were significantly reduced by coconut water.

We also found that the %age uterine/body weight of ovx rats force fed with YCJ either for 7 days or 14 days was lower than for the ovx+EB group. Furthermore, the %age uterine/body weight of the ovx+YCJ group after 14 days of treatment was significant lower than for the sham group. Since the uterus is one of the target organs of estrogen, uterine weight could be used as a primary marker of estrogen activity. This result indicates that the estrogen effects of YCJ do not cause increased uterine weight in the ovx rats fed with YCJ.

5. Conclusions

In summary, we believe we are the first group to study the effects of YCJ on the lipid profiles, of kidney and liver functions in "female" rats. We have clearly demonstrated the benefits of the effects of YCJ on improving the lipid profile by increasing the HDL cholesterol, producing no changes of total cholesterol, LDL cholesterol, or triglyceride; and causing no harm to the liver and renal functions. These encouraging findings have exciting clinical implications and indicate the importance of conducting a clinical trial on the effects of the use of YCJ to treat postmenopausal women to help them maintain a good quality of life.

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