Research Paper

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Oral Loading of Coconut Oil Does Not Raise Homocysteine And Total Cholesterol and Other Metabolic And Hematological Parameters in Adult Female Rats

Medical Science

KEYWORDS : Homocysteine, Coconut Oil, Hematologic Parameters, Metabolic Parameters, Oral Gavage, Female Rats

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ABSTRACT

Objective: *Data on effect of administration of coconut oil on homocysteine level in blood and on various hematologic and metabolic parameters in animals or humans are scanty. Hence we attempted to evaluate the effect of oral administration of graded doses of this oil on homocysteine and on various hematologic and metabolic parameters in female rats.* ***Methods:*** *Adult female Sprague Dawley rats were divided into control study groups and given oral doses of 1 ml, 2 ml and 4 ml coconut oil twice per day respectively for a period of 30 days. Control group of rats received tap water. Oral loading of graded doses of coconut oil was done continuously for the study period. At the end of the period of study, the rats were lightly anaesthetized with ether and blood samples collected by puncturing the heart, for analysis of various parameters. Homocysteine levels in blood samples were analyzed using homocysteine reagent kit. Levels of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hg), platelets,lymphocytes and mean corpuscular hemoglobin concentration (MCHC) were determined using a Hematology Blood Analyzer. Metabolic parameters such as cholesterol, triglycerides, urea,uric acid,creatinine and protein were analyzed by specific analytical kits. Concentrations of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and total anti-oxidant activity (TAO) were assessed by specific analytical kits. Data were analyzed for possible statistical significance, using a SPSS data analytical package.* ***Results:*** *Oral feeding of coconut oil for 30 continuous days did not significantly alter homocysteine level even when oil was administered at a massive dose of 4 ml/day for 30 days and did not alter any of the hematologic parameters studied, compared to control animals even when animals were fed huge dose of the edible oil. Administration of coconut oil appeared to decrease WBC, Hg , platelet and lymphocyte blood concentrations in treated rats , but the difference was not statistically significant (ANOVATest; $p>0.05$).However, platelet concentration was significantly lower ($p<0.05$) in rats receiving coconut oil compared to control group rats. Coconut oil administration did not alter the concentrations of protein, cholesterol, urea, triglyceride s, uric acid and creatinine in treated groups of rats significantly (Student's t-test, $p>0.05$) compared to those of control rats. SOD,GPX and TAO levels in control and treated groups were significantly higher (ANOVA test ; $p>0.05$) than controls. Conclusions: We conclude that oral administration of coonut oil in adult female rats even in massive doses does not cause any significant alteration in homocysteine level and well as other hematologic and metabolic parameters. Detailed studies are currently underway to assess significance of these findings on human and community health.*

Introduction

Coconut oil has been widely used as a cooking medium in many asian and south east asian countries for many centuries. Considering its high content of saturated fatty acids [1], coconut oil is labelled as saturated edible oil . Research reports on the benefits or ill effects of its use in animals as well as humans are scanty. .Some researchers have implicated use of coconut oil to be associated with possible hypertensive and cardio-toxic effect ill-effects (2,3,4,5), but some others were unable to corroborate the above suspected noxious effects. Kumar [6] reported no increased risk of cardiovascular incidents in patients using this edible oil in adult men while Sirkar& Kansra[7] reported in fact a beneficial effect of coconut oil compared to another edible oil, sunflower oil in reducing risk from diabetes as well as cardiac problems in humans. Interestingly, Prior et al [8] reported no increased risk with intake of saturated fats such as coconut oil in causing ischemic cardiac attacks as well . Further an inverse relationship of ischemic stroke in men using greater amounts of saturated fats has been reported [9]. Another report concluded absence of increased association of cardio-vascular disease , using a low fat dietary regimen in randomly selected women population [10] Although the effect of virgin coconut oil on disposition of cholesterol and some fatty acids in rats and hematologic and metabolic parameters has been reported by many investigators including our research group [11,12] no detailed report, to our knowledge exists examining the effect of coconut oil on blood level of homocysteine, one of the crucial factors reported to be associated with increased heart attacks (13,14) in the human population In view of the contrasting findings relating to use of coconut oil in daily life as a cooking medium or food constituent in humans and considering the absence of any detailed or reported studies relating to long term use of coconut oil and effect on hematologic and metabolic parameters in pregnant women or pregnant animals, we thought it interesting to explore whether coconut oil administration per se in large quantities can alter one of the main cardio-toxic parameter, homocysteine in blood of treated animals.

Material and Methods

Sprague Dawley female rats weighing between 220-240 g were used for the study. Animals used for the study were bred in the Medical Faculty animal house and maintained in individual polypropylene cages in a room maintained at 25±1 degree centigrade room temperature, with alternating 12 hour light 12 hour dark cycle. Body weights of all rats were determined before the beginning of the study. 3 groups of 5 rats received 0.5 ml, 1 ml and 2 ml coconut oil(Parachute Brand, Mumbai, India) orally twice per day respectively for a continuous period of 30 days while the control group were given tap water during the study period. All groups of animals were allowed diet and water ad libitum during the study period. After 30 days of oil administration , all rats were weighed and lightly anesthetized by ether and sacrificed for collection of blood and tissue samples. Hematologic parameters such as RBC,WBC, platelets, Hg, lym-

phocytes and MCHC were determined in blood samples of control and study groups using a Hematology Analyzer(ERMA INC, PCE210, Japan) while the concentrations of metabolic parameters namely,protein, cholesterol,triglycerides,creatinine,urea and uric acid were assessed using specific analytical kits(Randox Labs,UK). Homocysteine level in blood of treated and control rats was done by specific Homocysteine Diagnostic Reagent Kit (Axis Labs, UK) Activity of antioxidant enzymes, superoxide dismutase(SOD), glutathione peroxidase (GPX) and total antioxidant activity (TAO) in various blood samples was determined spectrometrically, using a widely used specific analytical method (Randox Labs, UK)

Statistical Analysis

Data were expressed as Means+SD. Statistical analysis of data was done using SPSS statistical package, using Student's t-test or analysis of variance (ANOVA) where appropriate. Data were judged significant if probability <0.05

Results

Body weight of rats averaged 232, 242,236, and 230 gm before start of experiment in control, 1ml/day coconut oil, 2 ml/day coconut oil and 4 ml/day coconut oil groups of 5 rats respectively. After the 30 day study period, the corresponding weights averaged 225, 232, 235 and 222 gm in corresponding groups of rats. Coconut oil administration led to an apparent reduction in body weight during pregnancy ; however, ANOVA Test did not show any significant difference in body weights between control and treated groups.. Table I shows details of some hematological parameters, namely RBC,WBC,Platelets, Hg, lymphocytes and MCHC of the control and treated groups of rats after the 30 day study period. Although WBC and Hg appeared to be higher in rats receiving higher doses of coconut oil,Student's t-test did not show any significant difference (p>0.05) between control and treated groups. However, platelets were significantly lower (Student's t-test, p<0.05) in rats receiving 1ml and 2 coconut oil per day, compared to control although the lower platelet concentration in rats receiving 4 ml oil per day was not significantly different (p>0.05) than that of control group. Student's t-test showed no significant difference (p>0.05) on concentration of lymphocytes in rats receiving coconut oil compared to control rats

Total protein in blood of control animals averaged 5.72$\pm$0.32 g/dL while the level in one ml,2 ml and 4 ml oil-fed animals averaged 5.45$\pm$0.25, 5.52$\pm$0.21, 5.75$\pm$0.28 respectively. ANOVA Test showed no significant difference in total protein values between control and oil- fed animals. Urea level in blood of control animals averaged 38.90 $\pm$5.20 mg/dL in control group of rats while the level in one ml,2 ml and 4 ml oil-fed animals averaged 27.25$\pm$4.80, 29.50$\pm$5.50, 30.05 $\pm$5.75 mg/dL respectively. ANOVA Test showed that the decrease in urea level in treated animals was significantly different (p<0.05) compared to the control group. Level of uric acid in blood of control animals averaged 23.50 $\pm$0.65 in control group of rats while the level in one ml,2 ml and 4 ml oil-fed animals averaged 22.95$\pm$4.80, 21.90$\pm$0.68, and 22.05 $\pm$0.62 mg/L respectively. ANOVA Test showed that the decrease in uric acid level in treated animals was not significantly different (p>0.05) compared to the control group. Concentration of creatinine in blood of control animals averaged 77.20 $\pm$5.50 mg/dL in control group of rats while the creatinine level in one ml,2 ml and 4 ml oil-fed animals averaged 79.05$\pm$5.95, 76.90 $\pm$5.20, 78.05 $\pm$5.90 mg/dL respectively. ANOVA Test showed that the in creatinine level in blood of oil- treated animals was not significantly different (p>0.05) compared to the control group.

Table II summarizes the levels of anti-oxidant enzymes, SOD&GPX as well as TAO levels in control and coconut oil-fed rats. Student's t-test showed that activities of anti-oxidant enzymes as well as total anti-oxidant activity were significantly higher (p<0.05) in oil-fed rat groups

Figure 1 summarises the values of Homocysteine in blood of control and coconut oil-fed rats. Homocysteine level averaged 6.90, 6.6, 5.95 and 5.72 umol/L in blood of control, and 1ml, 2ml, 4 ml coconut oil per day treated groups respectively. Though coconut oil treatment appeared to cause reduction of homocysteine level in treated rats, ANOVA Test did not show the decrease in homocysteine level in treated rats to be significantly different (p>0.05)

Concentration of total cholesterol in control and treated groups of rats are shown in Figure 2 .Total cholesterol averaged 72.20, 79.50 , 56.20 and 66.90 mg/dl in control, 1ml, 2ml, 4 ml coconut oil per day treated groups respectively. ANOVA Test showed that the reduction in cholesterol level in the case 2 ml and 4 ml coconut oil treated groups was significantly lower (p<0.05) than that of control group rats. Triglyceride values averaged 88.2, 82.1, 75.5 and 69.2 mg/dl in control, 1ml, 2ml, 4 ml coconut oil per day treated groups respectively (Fig.3) However only the lower triglyceride values obtained in the case of 4 ml oil-treated group was found to be significantly lower than that of the control group (ANOVA Test ; p>0.05)

Discussion

We were unable to show any harmful effect of coconut oil on level of homocysteine in blood of oil-treated female rats as well as on various hematologic and metabolic parameters, despite administration of relatively massive dose of coconut oil continuously for a period of 30 days and these findings are in accord with earlier reports from our loboratory (11,12) .Though coconut oil administration appeared to lead to reduction in body weight in treated rats, the difference was not statistically significant compared to controls. As reported in our earlier studies, even after receiving doses equivalent of about 300 ml, 700 ml and 1200 ml daily of the oil for an average human being, weighing 60 kg for a continuous duration of 30 days, the data indicating absence of any significance in hematological or metabolic parameters were surprising and totally unexpected too considering the saturated nature of coconut oil .

The clear beneficial effects reported by us in female rats of coconut oil administration, on cholesterol, triglycerides , urea levels and on antioxidant function (11) were evident in the present study as well . Other research groups [15,16] had concluded of no ill- effect of coconut oil administration on clotting factors and reported a beneficial effect in combating lipid peroxidation in treated animals. But since the researchers in that study administered the edible oil mixed with diet, it is not clear how much of oil was really consumed and absorbed by the treated animals during the period of study . However in this study , like in earlier studies (11,12) since coconut oil was administered orally twice daily and continuously for the designated study period , we were able to make sure that the animals received the exact amount or dose of the edible oil during the full course of study period.

It is generally hypothesized that since coconut oil is a mainly saturated oil , intake or feeding of this edible oil could lead to increased cholesterol and possibly increased triglyceride levels. However our data did not indicate such a deleterious effect on either cholesterol or triglyceride level. All the same, in a study on adult pregnant rats [12] administration of similar high doses of the edible oil had in fact resulted in lowered cholesterol levels in treated rats. It is likely that the altered hormonal and metabolic state of pregnancy condition had initiated a different metabolic response to the oil administration , in the case of cholesterol disposition in treated animals in that study . Similarly as in previous studies (11,12) it was surprising that even after massive administration of coconut oil daily for 30 days, triglyceride, urea, uric and creatinine levels did not increase to significant or abnormal levels, indicating that coconut oil administration per se does not cause any damaging effects on either the function of liver or the kidney or the heart in treated animals.

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The significantly lower blood urea level reported in this study are in agreement with our earlier study reported in adult female rats [11]. Despite receiving massive amounts of the oil for a period of 30 days, uric acid and creatinine levels in blood of control and coconut oil treated rats were not significantly different or elevated than those of control un-treated rats, implying that coconut oil did not cause any major defect in renal function in treated rats. Similarly total cholesterol was significantly lower in the group receiving 2 ml/oil per day and 4 ml oil per day, compared to control group while in the group receiving amount of 1 ml/day coconut oil the cholesterol level was not significantly different than the control rats receiving no coconut oil. This finding implies instead of causing hypercholesterolemia, higher consumption of coconut oil could in fact be beneficial in reducing cholesterol level and in reducing the risk of heart attacks, rather than increasing the risk as postulated by some other research groups.

Cholesterol levels in rats receiving one ml coconut oil/day and 4 ml oil per day were not significantly different, compared to the control group. However in rats receiving 2 ml of coconut oil per day during the study period, cholesterol level was significantly lower than the level reported in control rats. The absence of hypercholesterolemia and triglyceridemia in coconut oil treated rats reported in this study are in agreement with report of Feoli et al [17] who further reported that LDL from rats receiving coconut oil in their food were found to be more resistant to peroxidation compared to control animals receiving soybean oil , implying a therapeutic effect of coconut oil in preventing noxious peroxidation and possible atherosclerosis in rats receiving the former compared to the latter. Interestingly, another research group [18] reported hypolipidemic as well as antiperoxidative effect of proteins derived from coconut oil in experimentally- induced hypercholesterolemic rats fed with this edible oil in diet. Beneficial effect of coconut oil in lowering level of circulating lipoprotein as well as the effect on lipoprotein disposition was attributed by the investigators , to the presence of biologically active polyphenol compounds in the oil [14,15].

In this study platelet count in rats receiving massive amounts of coconut oil was significantly lower than that of control rats receiving no oil and such an effect was observed in the case of pregnant rats receiving coconut oil during the pregnancy period (12) as well .This effect of coconut oil on platelet level reduction in blood of treated rats could be considered as another beneficial parameter capable of preventing formation of clots or plaques in lining of coronary or other blood vessels in the human body. The absence of abnormal or bad effects of coconut oil observed in this study on homocysteine level as other hemataologic and metabolic parameters in conjunction with data reported by our research group in earlier studies [12, 13] are strongly indicative of the premise that instead of being labelled as a bad edible oil, coconut oil might instead qualify to be one of the best edible oil available for human consumption, in terms of various health benefits for the general public.

Present study has shown that coconut oil administration increases activity of antioxidant enzymes SOD and GPX in oil-treated rats, receiving higher doses of the edible oil. These findings are in agreement with our previous reports (11,12) and the protective antioxidant function of coconut oil reported by other investigators . Thus, coconut oil, by reducing oxidation of LDL moiety mediated through reactive oxygen could play a beneficial role in preventing formation of plaques and could in fact be anti-atherogenic, contrary to the report of some others [4,5]who wrongly implicated coconut oil to be atherogenic and noxious for the myocardial function. Report of Kumar [6] and Sircar& Kansra [7] however did not observe any association between increased cardiac abnormality and use of coconut oil and the present study as well as previous reports from our laboratory [11,12] appear to corroborate their observations. The higher anti-oxidant protective function of coconut oil could be attributed to the presence of high concentration of phenolic dependent antioxidants in this oil [19]. It is possible that presence of vitamin E reported by some research groups, as one of the constituents of this edible oil could also confer antioxidant protection to the consumers of coconut oil.

Verallo-Rowell et [19] an and Agueiro&Verallo-Rowell [20] have reported beneficial effect of coconut oil in fighting skin infections and in maintaining integrity of the skin [18] in humans though the mechanism of action of this oil on skin-protective action has not been adequately elucidated. It needs to be emphasized that data obtained in animals cannot be extrapolated directly to humans and contrary to mistaken beliefs, our studies have shown that use of coconut oil per se is not harmful to the health of the consumer even in massive amounts. However we refrain from speculating whether coconut oil intake in pregnant women could elicit the same response relating to the hematologic and metabolic parameters investigated in this study on non-pregnant rats as well as in earlier [11] studies. Studies involving larger animal populations are currently in progress in our laboratory to corroborate our findings.

TABLE I . HEMATOLOGICAL PARAMETERS IN CONTROL AND TREATED RATS

	RBC (*10^6/µL)	WBC (*10^6/µL)	PLATELETS (*10^3/µL)	Hb (g/dL)	MCHC (g/dL)	LYMPHOCYTES (*10^3/µL)
CONTROL	7.2 ± 0.18	5.28 ± 0.32	852 ± 28.2	13.05 ± 0.21	28.2 ± 0.2	3.2 ± 0.09
GROUP I	7.52 ± 0.12	5.38 ± 0.42	402 ± 40.9	13.95 ± 0.15	28.82 ± 0.15	3.75 ± 0.22
GROUP II	7.69 ±0.19	7.90 ± 0.35	482 ± 42.0	14.05 ± 0.35	29.25 ± 0.25	4.72 ± 0.52
GROUP III	7.49 ± 0.23	6.82 ± 0.31	602 ± 40.2	14.27 ± 0.32	29.05 ± 0.21	4.05 ± 0.21

Means+ SEM of 5 Rats in Each group. Group ! = One ml Coconut Oil/Day ; Group II = 2 ml Coconut Oil/Day ; Group III= 4 ml Coconut Oil/Day ; RBC=Red Blood Cell ; WBC=White Blood Cell ; Hb=Hemoglobin ; MCHC =Mean Corpuscular Hemoglobin Concentration

TABLE II. CONCENTRATIONS OF ANTI-OXIDANT ENZYMES IN CONTROL AND OIL-FED RATS

	SOD (Units/ml)	GPX (Units/L)	TAO (mmol/L)
CONTROL	182 ± 08	421 ± 15	82 ± 08
GROUP I	172 ± 11	436 ± 18	89 ± 09
GROUP II	192 ± 12	492 ± 19	95 ± 11
GROUP III	208 ± 09	501 ± 16	105 ± 06

Means ± SEM OF 5 Rats in Each Group. SOD= Superoxide Dismutase; GPX = Glutathione Peroxidase; TAO = Total Anti-Oxidant Activity.Group I= 1ml Coconut oil/day; Group II= 2ml coconut oil/day; Group III= 4ml coconut oil/day

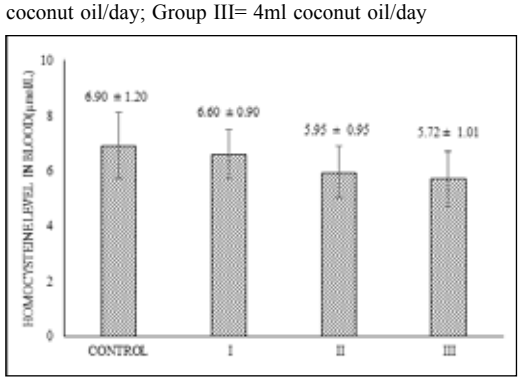

Figure 1 : Homocysteine Level in Blood of Control and Coconut

Oil-fed Rats. Means+SEM of Rats in Each group. Group I = One ml Coconut Oil/Day ; Group II = 2 ml Coconut Oil/Day; Group III= 4 ml Coconut Oil/Day ; Statistical Significance was assessed-by ANOVA Test

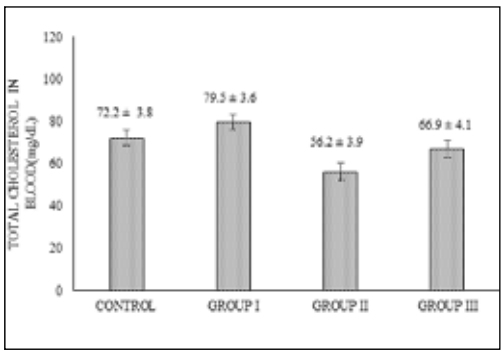

Figure 2 : Level of Total Cholesterol in Blood of Control and Coconut Oil-fed Rats. Means+SEM of Rats in Each group. Group I = One ml Coconut Oil/Day ; Group II = 2 ml Coconut Oil/Day; Group III= 4 ml Coconut Oil/Day ; Statistical Significance was assessed by ANOVA Test

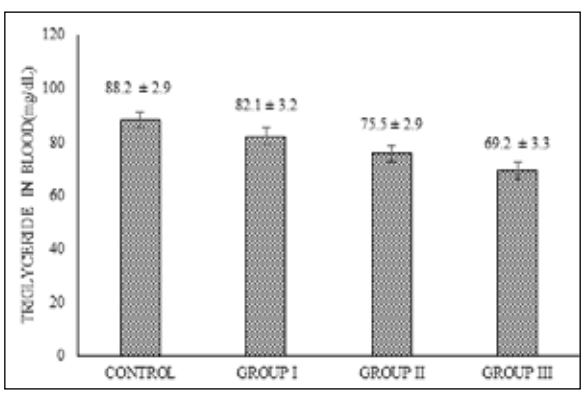

Figure 3 : Level of Triglycerides in Blood of Control and Coconut Oil-fed Rats. Means+SEM of Rats in Each group. Group I = One ml Coconut Oil/Day ; Group II = 2 ml Coconut Oil/Day; Group III= 4 ml Coconut Oil/Day ; Statistical Significance was assessed by ANOVA Test

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