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THE EFFECT OF CINNAMALDEHYDE ON HIGH FAT DIET INDUCED WISTAR RATS – A PRELIMINARY STUDY

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ABSTRACT: Anti-obesity effect of Cinnamaldehyde and Orlistat on High fat diet induced obese rats was investigated. 30 male wistar albino rats were divided into 5 groups: control group fed on normal standard diet; HFD group; Orlistat (HFD+ 50mg/kg body weight; CA (HFD+ 40mg/Kg body weight) and CA (HFD+ 80mg/Kg body weight) for 8 weeks. The anti obesity activity of CA and OR was estimated in terms of Food intake, body weight gain, feed efficiency ratio, lipid profile, AI, CRI and faecal lipids. Obesity induced group of rats showed significant (p< 0.001) increase in body weight, feed consumption, TG, TC, LDL-c, VLDL-c in serum, AI, CRI and decrease with HDL-c level (p< 0.001) compared with the normal group of rats. The BWG and FER were reduced in CA treated rats. CA treatment also resulted in significant (p< 0.001) decreases in serum TC, TG, LDL-c, AI and CRI and increased (p< 0.001) HDL-c concentrations in a dose dependent manner compared with untreated obese rats. The results were comparable with Orlistat, a standard antiobesity drug. 80 mg/kg body weight of CA was more effective in reducing the physiological changes, lipid profiles and histopathological changes that occurred in adipose tissue and heart to near normalcy. Significantly increased lipid levels were discharged in faeces during the supplementation of CA and OR. These preliminary results for the first time demonstrate that administration of CA can be beneficial for the suppression of obesity in HFD fed wistar rats and prevented the development of cardiovascular diseases.

INTRODUCTION: Obesity is the most common nutritional disorder in the Western Countries and among the higher income groups in the developing countries. Obesity is defined as an increase in total fat mass and it occurs when unilocular adipocytes show hyperplasia or hypertrophy following macrophage infiltration of fat tissue ¹. In recent years, the average consumer's diet has changed so that the intake level of carbohydrates has decreased, while the intake levels of meat and meat products have increased.



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As a result, lipid levels in the body have also increased, greatly raising the occurrence rate of cardiovascular diseases such as hyperlipidemia, arteriosclerosis and hypertension. In fact, these rank at the second position among the major factors causing death ². To aid in cholesterol reduction, there have recently been many attempts to use certain common compounds that are already well known in traditional medicine for having biological components that can reduce the lipid content in the body ³.

Since it is more beneficial to prevent dietary diseases than to cure them, and to change one's diet rather than take medicine, the diet of choice in modern societies should include food with functionality. The alarmingly increasing rate of unwanted weight gain and obesity in the past 20 years had become a worldwide concern.

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The World Health Organization estimates that there are currently more than 400 million obese and more than 1.6 billion overweight adults, a number that is expected to double by 2015 ⁴. At present, there are only two drugs approved by Food and Drug Administration (FDA) as anti-obesity drugs viz. Orlistat and Sibutramine. Their use is often associated with gastrointestinal or cardiovascular and central nervous system side effects, elevated blood pressure, dry mouth, constipation, headache and insomnia ⁵. Attempts to correct the metabolic disparity of obesity include the application of inhibitors of appetite (sibutramine), intestinal lipid uptake (Orlistat), and peroxisome proliferator activated receptor (PPAR)-α (fibrates). However, these drugs can produce adverse side effects.⁶. Thus, there is a need for the discovery and development of novel, safe and effective drugs for the control and treatment of obesity.

Cinnamaldeyde is a pungent compound in Cinnamon (*Cinnamomum verum*) and in the dried bark of cassia (C. cassia). Dried bark contains 1.0 – 3.4% essential oil which consists of 75 – 90% CA. CA has been reported to show vasodilation ⁷, inhibition of gastric motility ⁸, induction of adrenal catecholamine secretion ⁹, and improvement of type II diabetes ¹⁰. Cinnamaldehyde (CA), one of the agents in the Cinnamon bark, seems to be potent in terms of anti inflammatory and anticancer activity ¹¹. Cinnamaldehyde has been widely used as a component in perfumes, a fungicide, and a flavouring agent in foodstuffs such as chewing gum, ice cream, candy and beverages ¹². The oral LD₅₀ of Cinnamaldehyde for rat and mice varies from 3.4g/kg to >5.0g/kg ¹³.

The effects of Cinnamaldehyde as fungicide, insecticide and anticancer activity have been reported by various researchers. The purpose of the present study is to investigate the effect of Cinnamaldehyde on HFD induced obesity in Wistar rats. Further, the results have been compared with Orlistat, a standard anti-obesity drug. This goal could be achieved through anthropometrical parameters and testing the hypothesis, that may predict obesity. Adverse effects on lipid profile and histological changes in adipose tissue, heart and there by assess the effectiveness of CA in the treatment of obesity.

MATERIALS AND METHODS: Animal Model

An animal model that mimics the human counterpart is essential for preclinical evaluation of new treatment modalities for obesity. Wistar albino male rats weighing about 120-150g were used for the experiment. Animals were kept in animal house at an ambient temperature of 25°C and 45-55% relative humidity with 12 hours each of dark/light (day and night) cycles. Animals were fed pellet diet and water ad – libitum. CPCSEA guidelines for laboratory animal facility (IJP 2003; 35: 257- 274) were followed.

Experimental animals were handled according to the University and Institutional Legislation, regulation by the committee for the purpose of control and supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice, and Empowerment, Government of India (IAEC No. SU/BRULAC/RD/006/2013).

Administration Modality

All the rats were divided randomly into following 5 groups of 6 animals each: Group 1: (Control rats) rats were fed with normal or regular diet (procured from Tamil Nadu Veterinary and animal Sciences University, Kattupakkam, Chennai) for 8 weeks. Group 2: (Obesity induced rats) - rats were fed with high fat diet (HFD- Research Diet, D12492, USA) for 8 weeks. Group 3: (HFD + OR) - rats with HFD and Orlistat (50mg/kg body weight) simultaneously for 8 weeks orally. Group 4: (HFD + CA) - rats were fed with Cinnamaldehyde (40mg/Kg body weight) and HFD simultaneously for 8 weeks orally. Group 5: (HFD + CA) - rats were fed with Cinnamaldehyde (80mg/Kg body weight) and HFD simultaneously for 8 weeks orally. The Normal diet and HFD control rat were treated with vehicle (Corn Oil) only.

Measurement of food intake, body weight and FER

Body weight was measured throughout the experimental period and body weight gain (BWG) was calculated by the formula as, (final weight – Initial weight). Food intake was measured once per week at a fixed time. Fresh tap water was supplied every day at the same time. The food efficiency ratio (FER) was calculated as total body weight gain (g)/ total food intake amount (g) for 8 weeks.

Faecal Lipid Levels

Faeces were collected from each group during the last three days of the experimental period and dried in the oven at 70°C for 1 h. The lipid extraction was carried out with 2.0 ml of chloroform: methanol (2:1) for 30 min at 60°C. It was filtered and the volume was made to 4.0 ml with the extracting solvent. The faecal matter was re-suspended in 2.0 ml of the solvent and kept for 30 min at 60°C. After extraction it was filtered. The extracted solvent fractions were pooled together and evaporated to dryness. The lipid analysis was gravimetrically. The fecal lipid was calculated as % weight of fecal matter ¹⁴.

Assessment of Biochemical Parameters in Serum

Blood was collected from the retro-orbital plexus of overnight fasted rats using heparinised capillary tube after 8 weeks and serum was separated by centrifugation at 3000rpm for 15 mins. The rats were sacrificed and tissues (Adipose tissue & heart) were extirpated. Serum levels of TC ¹⁵, TG ¹⁶, LDL-c, HDL-c and VLDL-c ¹⁷. Atherogenic index (AI- LDL-c / HDL-c) was calculated using the formula of Abbot et al., ¹⁸ and Coronary risk index (CRI- TC / HDL-c) was obtained by the method of Alladi et al., ¹⁹.

Histopathological Analysis

Adipose Tissue and Heart were removed from the animals and fixed in buffered solution of 10% formalin, washed, dehydrated, cleared and embedded in paraffin. Then, the specimens were processed into 5 μ m sections for light microscopic examination using hematoxylin and eosin stain.

Statistical Analysis

The data were expressed as mean \pm SD. All statistical analysis was performed using SPSS 20.0 statistical software (IBM, USA). Significant differences among the treatment groups were analysed by variance (One way ANOVA) followed by least significant difference (LSD) test. Results were considered to be statistically significant at P values < 0.05.

RESULTS:

Body Weight, Food consumption and FER

Animals in all experimental groups were apparently showing no pathological signs or abnormalities during the entire experimental period. Table 1 shows the effects of CA and Orlistat on body weight gain, food intake and feed efficiency in HFD induced group of rats treated for 8 weeks. The body weight of the normal group of rats in the regular diet gradually increased as the rats grew during the 8 weeks period. By contrast the body weight of animals on the HFD induced group showed rapid increase during experimental period.

TABLE 1: EFFECTS OF CINNAMALDEHYDE ON BODY WEIGHT, BODY WEIGHT GAIN, FEED INTAKE AND FOOD EFFICIENCY RATIO (FER) IN EXPERIMENTAL RATS.

Treatment after 8 Weeks	Group I	Group II	Group III	Group IV	Group V
Initial Weight (g)	144.83±1.83	143.33±2.58	143.66±3.14	144.78±3.65	143.88±4.37
Final Weight (g)	186.45±3.34	$302.66\pm4.08^{a^*}$	$213.06\pm2.21^{a*b*}$	220.75±3.40 a*b*c*	202.5±2.73 a*b*c*
Weight Gain (g)	41.61±2.87	159.33±4.76 a*	$69.4\pm3.04^{a*b*}$	75.96±3.52 a*b*c@	58.61±3.73 a*b*c*
Feed Intake (g)	876.83±6.61	1119±5.83 a*	985.35±5.30 a*b*	1050.33±6.05 a*b*c*	950.75±4.30 a*b*c*
FER	0.047 ± 0.003	$0.142\pm0.004^{a*}$	$0.070\pm0.003^{a*b*}$	$0.072\pm0.003^{\ a*b*c-NS}$	$0.061\pm0.003^{\ a*b*c*}$

Values are expressed as mean \pm SD (n=6). Significant at *P* values < 0.05[@], 0.01[#], and 0.001*. a- Value compared with G1, b-Value compared with G2, c- Value compared with G3.

Body weight was compared with the HFD induced group, the CA and Orlistat groups had 27.06, 33.09 and 29.60 fold, lower in the final body weights (p<0.001), respectively. Body weight is the predominant physical parameter in obesity in which the weight of an individual is increased to a great extent 20 . After feeding the rats with High fat diet for 8 weeks, the body weight significantly

increased due to the accumulation of fat in the body. Weight gain in the regular diet group and HFD induced group during the 8 weeks period were 41.61 ± 2.87 and 159.33 ± 4.76 (p<0.001) respectively. CA dosage of 40 mg/ Kg body weight and 80 mg/ Kg body weight seemed to inhibit rapid weight gain, compared to the HFD group of rats by 52.32 and 63.21 fold (p<0.001), respectively and

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increased 82.55 and 40.85 (p<0.001) fold as compared to normal group of rats.

Feed efficiency ratio was calculated by weight gain divided by total feed consumption during the 8 weeks period. As shown in **Table 1**, weight gain of the HFD group of rats was actually due to the increased feed intake. However, the body weight of the CA fed rats was significantly reduced, despite a decreased in food consumption compared to the obesity rats. Feed efficiency of the CA treated group was 0.072 ± 0.003 and 0.061 ± 0.003 (p< 0.001) which is lower than that for the HFD

groups, indicating that CA has the potential to control body weight gain despite decreased food intake.

Decrease in the body weight gain increased with the dosage of CA. On the other hand body weight and weight gain of the Orlistat fed group was reduced, similar to that of the CA treated group of rats. The rats treated with standard drug Orlistat though showed reduction in the body weight gain and FER and it was, 69.4 ± 3.04 and 0.070 ± 0.003 (p< 0.001), as compared with the HFD induced group.

TABLE 2: EFFECT OF CINNAMALDEYDE ON SERUM LIPID PROFILE, ATHEROGENIC INDEX (AI), CORONARY RISK INDEX (CRI) AND FAECAL LIPID ANALYSIS (FLA).

Treatment after 8 Weeks	Group I	Group II	Group III	Group IV	Group V
Total Cholesterol (mg/dl)	85.35±1.00	183.33±1.36 a*	99±0.89 ^{a*b*}	103.16±1.12 a*b*c*	96.73±1.13 a*b*c#
Triglyceride (mg/dl)	76.06±0.9	162.93±0.86 a*	110.1±0.86 a*b*	127.16±0.93 a*b*c*	106.13±0.91 a*b*c*
HDL-c (mg/dl)	34.23±0.22	20.3±0.23 a*	$28.33\pm0.27^{\ a*b*}$	25.26±0.22 a*b*c*	30.3±0.26 a*b*c*
LDL-c (mg/dl)	23.33±0.25	55.36±0.18 a*	32.46±0.13 a*b*	39.5±0.26 a*b*c*	28.43±0.28 ^{a*b*c*}
VLDL-c (mg/dl)	21.33±0.37	41.56±0.36 a*	28.56±0.36 a*b*	25.96±0.50 a*b*c*	21.6±0.23 ^{a-NSb*c*}
AI	0.67±0.005	2.72±0.02 a*	1.14±0.01 ^{a*b*}	1.56±0.01 a*b*c*	0.93±0.005 a*b*c*
CRI	2.49±0.01	9.03±0.08 a*	$3.49{\pm}0.03^{~a*b*}$	$4.08\pm0.04^{~a*b*c*}$	3.19±0.01 a*b*c*
FLA (%)	3.81±0.06	5.35±0.11 a*	6.82±0.06 a*b*	6.51±0.09 a*b*c*	$7.34\pm0.08^{\ a*b*c*}$

Values are expressed as mean \pm SD (n=6). Significant at P values $< 0.05^{\circ}$, 0.01° , and 0.001° . a- Values compared with G1, b-Values compared with G2, c- Values compared with G3.

Serum Lipid Levels

The changes of serum lipid components, AI and CRI are shown in **Table 2**. The TC, TG, LDL-c and VLDL-c increased significantly in the obese group in comparison with normal group of rats (**Table 2**). In groups fed with the CA, the lipid levels in serum significantly decreased with increased in dose.

Orlistat treated group of rats also showed significant decrease (p< 0.001), as compared to HFD group of rats. In CA at 40 and 80 mg/ Kg body weight, the TC, TG, LDL-c, VLDL-c showed significant decrease (p< 0.001) as compared to the HFD group of animals. In particular, the lipid levels showed reduction to a level similar to that of normal group. HDL-c levels for the HFD group

was significantly lower (p< 0.001) than that for the normal group. The HDL-c level is increased by the feeding of CA and Orlistat, (p< 0.001) when compared to the HFD group of rats.

AI and CRI ratios were increased in HFD fed rats and this has an effect on cardiovascular diseases 21 . Higher the value, higher the risk of developing cardio vascular diseases. Intake of HFD leads to significant rise of AI and CRI in obese rats when compared to normal group of rats as shown in **Table 2**. But treatment with CA and Orlistat resulted in significant reduction (p< 0.001) of AI and CRI when compared to obese rats indicating protective role of them against atherogenesis and cardio vascular diseases.

Faecal Lipid Analysis

Fecal lipid levels were determined in the all group of rats as shown in **Table 2**. Total lipid levels in faeces were significantly (p< 0.001) higher in the HFD induced group than the normal group. The fecal total lipid levels in the groups supplemented with CA and Orlistat was significantly (p< 0.001) increased as compared to the HFD induced group. Therefore, it seems that the reducing the lipid levels in the serum has a stronger relevance with the increase of fecal lipid levels.

Histopathology of Adipose Tissue and Heart

Figure 1 shows the histopathology of heart. The heart of the normal group of animals Shows normal myocardium, blood vessels and pericardial tissue (Fig 1a). The heart of the CA (80mg/ Kg BW) and Orlistat treated group of animals did not show any alteration in the architecture of the heart (Fig 1e and c). HFD group and CA (40mg/ Kg Bodyweight) shows normal myocardial tissue with increased pericardial fat and blood vessels appear normal (Fig 1d). Furthermore, histolological examination of heart revealed that the HFD group showed significant changes, when compared to normal group of rats (Fig 1b).

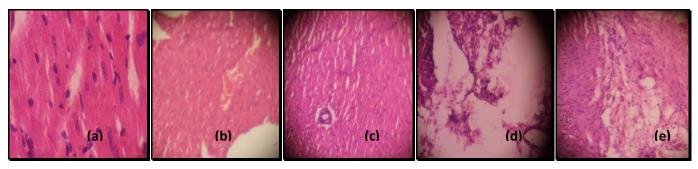


FIGURE 1: Histopathology of Heart. (a) ND, normal diet group- G1; (b) High fat diet (HFD) induced obese group- G2; (c) HFD+ Orlistat (OR)- G3; (d) HFD+CA 40- G4; (e) HFD+CA 80- G5.

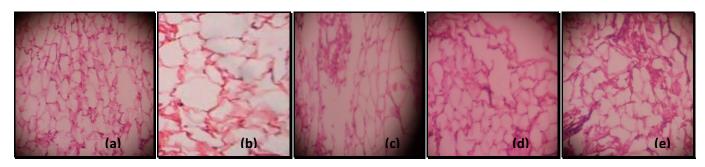


FIGURE 2: Histopathology of Adipose Tissue. (a) ND, normal diet group- G1; (b) High fat diet (HFD) induced obese group- G2; (c) HFD+ Orlistat (OR)- G3; (d) HFD+CA 40- G4; (e) HFD+CA 80- G5.

The size of adipose tissues including subcutaneous, retroperitoneal, epididymal and gonadal adipose tissue in the HFD induced group was significantly larger than those of the normal group, as shown in **Fig 2 a and b**. The adipose tissue of HFD rats shows increased deposition of adipocytes and increased peri adrenal fat (**Fig 2b**). Group 3 and 4 rats show deposition of adipocytes. Group 5 group shows normal appearance similar to that of normal group of rats (**Fig 2e**). These morphological results strongly suggest that the feeding with CA (80mg/Kg Body weight) inhibits the adipose tissue expansion through the process of hypertrophy rather than hyperplasia.

DISCUSSION: The most important finding of this study was that Cinnamaldehyde significantly act as an anti-obesity agent in rats fed with high fat diet. Model of high fat diet induced obesity in Wistar rats has many features common with human obesity ²².

Several reviews have appeared in the literature about this compound, and this may reflect the popularity of the subject and its common use as spice (Cinnamon) and a medicinal plant. Reducing body weight and body fat are important in preventing obesity ²³. We have also reported that Cinnamaldehyde has potential antioxidant capacity²⁴.

Tetrahydrolipstatin (Orlistat) accelerates gastric emptying, reduces pyloric pressure and enhances duodenal motility ²⁵. CA and Orlistat treatment of HFD induced rats showed decreased body weight gain and inhibited lipid profile in the serum samples. Changes in biochemical parameters, especially in lipid profile, are the predominant changes caused in obese people. In obesity, the levels of TC, TG, LDL and VLDL increases and the level of HDL decreases. Elevated serum levels of TG, cholesterol and LDL are the major risk the premature development factors for cardiovascular diseases like atherosclerosis, hypertension, coronary heart disease, etc., Increase in plasma lipid levels, mainly TC, TG and LDL, along with decrease in HDL are known to cause hyperlipidaemia which is the reason for initiation and progression of atherosclerosis impasse ²⁶.

Normally hepatocyte initiate synthesis of triglycerides and cholesterol during states of increased free fatty acid flux to the liver (e.g., after the fatty meal or in the situation of increased lipolysis) but due to anti-hyperlipidemic drug, there may be inability of hepatocytes to increase cholesterol synthesis and decrease hepatocyte cholesterol concentration by increasing the conversion of cholesterol to bile acids in liver ²⁷.

Sibutramine, Orlistat and Rimonabant had several serious adverse effects in clinic, including gastrointestinal adverse effect and significant unfavourable effects on cardiovascular system. As a result, much safer therapeutic is necessary. In addition, there has been a large increase in the use of complementary treatments such as herbal remedies in the treatment of these diseases over the past decade ²⁸. Adipocytes play an important role in lipid homeostasis and energy balance by relating to triglyceride storage and free fatty acids release. Adipocyte differentiation and the amount of fat accumulation are associated with the occurrence and development of obesity ²⁹.

Thus, these results show that CA has potent antiobesity and anti hyperlipidemic effects in *in-vivo*. Obesity is characterized by an increase in fat cell numbers, fat cell size, or combination of the two. Histological examination of adipose tissue showed that HFD group had dramatically increased adipose size in fat pad and pericardial fat accumulation in the heart, whereas CA administration decreased these changes. The morphology of the adipose tissues and heart indicated that CA inhibited the accumulation of TG and adipose tissue expansion by modulating hypertrophy rather than hyperplasia.

CONCLUSION: In conclusion, the results of the present investigation demonstrates that CA has an anti-obesity effect, which is evidenced decreased lipid levels in serum and by the improvement of histological alterations in the adipose tissue and heart of obese rats. These results present be treated as initial evidence that CA may be useful for the treatment of obesity by enhancing the anti-lipidemic defense mechanism. However further studies need to be carried out to investigate anti-obesity the mechanism of effects Cinnamaldehyde.

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