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Influence of Clove Tea (*Syzygium Aromaticum*) on Body Weight and Biochemical Parameters of Rats Subjected to Ethanol Consumption and Abstinence

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Abstract

Clove has a large number of therapeutic properties and its tea has been used by the general population as anti-inflammatory, antihyperglycemic, antimutagenic, bactericidal, and nematicidal, among others. Thus, the aim of this study was to assess the effect of clove on biochemical parameters of rats subjected to ethanol consumption and abstinence. The animals were subjected to ethanol intake before and after treatment with clove tea, according to their groups, as follows: GW received water (control); GE received 3% ethanol alone; GC received clove alone; GCE received clove for 7 consecutive days followed by 3% ethanol for 16 consecutive days; GEC received 3% ethanol for 16 consecutive days followed by clove for 7 consecutive days. Administration of clove tea alone or ethanol alone did not change serum parameters such as glucose, triglycerides, total cholesterol, total protein and albumin, and it did not alter weight gain although there was a significant difference in mean food consumption, which suggests that under such conditions clove tea or ethanol does not interfere with these parameters. On the other hand, administration of clove tea before or after ethanol administration led to significant changes in several parameters such as glucose, triglycerides, cholesterol, total protein and albumin. The obtained results thus suggest that prolonged ethanol intake should be avoided when clove tea is consumed daily.

Keywords: Clove; Ethanol; Glucose; Lipids; Proteins

Introduction

Clove and its properties

Syzygium aromaticum (synonym *Eugenia caryophyllata, Eugenia aromatica* and *Caryphyllus aromaticus*) is commonly known as clove and belongs to the family Myrtaceae, which covers around 3.000 tree and tropical and subtropical shrub species [1].

Commercial clove consists of the dried flower bud and has been used for years in cookery. Studies have attributed therapeutic properties to clove such as anti-inflammatory, antihyperglycemic, antimutagenic, bactericidal, nematicidal, insecticidal, antiviral, fungicide and allelopathic, as well as antiseptic and anesthetic to relieve toothache among other pains [2-12].

Such enormous variety of therapeutic properties is attributed to the presence of several chemical compounds already identified, including eugenol, eugenol acetate, caryophyllene, apigenin, eugenin, biflorin, oleanic acid, triterpene, benzaldehyde, plant waxes, ketones, chavicol, resins, tannins, gallic acid, kaempferol and quercetin [3,4,6,13-16]. Eugenol is a phenolic compound that characterizes the marked flavor and aroma of clove. In the leaves, it accounts for approximately 95% [17] and in the flower buds, it is the major compound, ranging from 70 to 85% of the total extracted oil [14]. Recently, Gülçin et al. [18] have reported that clove essential oil analyzed by several methods showed high antioxidant activity when compared to antioxidant compounds like BHA, BHT, α-tocopherol, trolox and DPPH.

Another interesting activity was shown by Prasad et al. [8], who noted that clove extract suppresses the expression of genes of the enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphate (G6-P) in hepatoma cells of H4IIE rats, showing effects similar to those of insulin, which suggests the possible use of clove in the treatment of diabetes. These enzymes are involved in the liver production of glucose, increasing its blood levels when activated and decreasing glucose production when inhibited by insulin. Recently, Shukri et al. [19] have investigated clove activity in diabetic rats. Those authors added 5% clove powder to the food of rats with diabetes induced by streptozotocin and noted that the blood level of glucose and the lipid peroxidation significantly decreased. These data suggested a possible effect of clove on gluconeogenesis and liver glucose, in addition to anti-insulin activity and anti-lipid peroxidation.

Ethanol and its effects

Differently from clove, moderate alcohol consumption induces lipid peroxidation in the liver of rats [20]. In the last years, several studies have shown that alcohol intake promotes the development of coronary heart diseases, alcoholic cardiomyopathy, systemic hypertension, arrhythmias, shock, arterial-thrombotic disease, and heart failures [21,22].

For humans, Avogaro & Cazzolato [23] showed that daily consumption of 180 g alcohol increased triglycerides and VLDL and decreased LDL but did not increase total cholesterol and HDL, suggesting a hyperlipidemic response to alcohol intake similar to that caused by high carbohydrate consumption.

McMonagle and Felig [24] studied volunteers with diabetes and showed that daily alcohol intake decreased the blood levels of glucose and increased insulin response during oral glucose overload. Recently, Hong et al. [25] have demonstrated that alcohol consumption significantly increases mammary tumors, as well as the sensitivity to

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insulin and the serum levels of leptin and estrogen in mice. These data suggest an action of alcohol on the organism causing several metabolic changes that still need to be studied.

Ethanol is a compound present in several alcoholic beverages such as beer, wine, vodka, liqueurs and "cachaça", which are frequently consumed by Brazilians in a slight, moderate or heavy manner. Excessive ethanol ingestion adds 9 kcal/g to the diet. Thus, addition of this compound to the diet may result in energy accumulation and consequently weight gain. However, a search in the literature using ethanol versus body weight lead us to several scientific studies from the 60's to the 90's reporting that ethanol does not interfere in weight gain [26-29].

There is no report in the literature about the effect of clove in association with ethanol administration. Thus, we studied the effect of clove on biochemical parameters after moderate alcohol intake, as well as on alcohol restriction.

Material and Methods

This study was submitted to the Ethics Committee on Animal Experimentation of the Institute of Biosciences of UNESP, Botucatu Campus, São Paulo State, Brazil, and was approved and certified by the Protocol no. 299-CEEA.

Obtaining and preparing clove tea

Clove was obtained in the local market in packages containing 1 kg of the respective product. The tea was prepared by following the method of Gazola et al. [30]; 65 mL water at 70° C was added to 5 grams of dry clove material and kept in infusion for 15 minutes. Then, the liquid was filtered off and used in the experiment. To obtain the dry weight, aliquots of clove tea were kept in an oven for solvent evaporation and estimation of the final concentration of clove was 26 mg/mL, which was subsequently used to calculate the concentrations to be administered to the animals.

Animals

Male Wistar rats (*Rattus norvegicus albinus*) were used, weighing 200 grams and were from the Central Animal Facility of UNESP/ Botucatu Campus. The animals were transferred to the Experimentation Room of the Department of Chemistry and Biochemistry of Institute of Biosciences of UNESP, Botucatu Campus, and were kept there during the whole experimental period. Rats were kept in collective cages (3 rats per cage) at controlled room temperature of 25°C and photoperiod of 12 h light/12 h dark (6:00/18:00) and received water and pet food Purina *ad libitum*.

Experimental groups

Thirty male Wistar rats were randomly allocated to five experimental groups and acclimatized for 15 days before the beginning of the experiment. The following groups of six animals each were established: GW, control group that received distilled water; GE, control group that received 3% ethanol; GC, control group that received clove tea; GCE, group that received clove tea for 7 consecutive days, followed by 3% ethanol for 16 consecutive days; GEC, group that received 3% ethanol for 16 consecutive days, followed by clove tea for 7 consecutive days.

During the experimental period, the animals were weighed once a week in a semi-analytical balance. The value of liquid and food consumed per day was measured during the 30 days.

Serum biochemical assays

Glucose, total protein, albumin, triglycerides and cholesterol in the serum were determined by means of colorimetric methods (Kits for diagnosis obtained from "CELM-Companhia Equipadora de Laboratórios Modernos", São Paulo, Brazil).

Statistical analysis

For these completely randomized experiments, Analysis of Variance was adopted and F statistics was considered significant when $p<0.05$. Pairs of means of controls and treatments were compared by the Tukey method, calculating the minimal significant difference for $\alpha=0.05$ [31].

Results

Initial and final weight, weight gain, food intake, and mean ingested volume obtained for each animal group. There was no significant difference between groups for final weight, weight gain and mean volume ingested by the animals; however, we noted a significant difference between groups in mean consumption of pet food per day. Compared to GW, groups GE, GC and GCE had a decrease in food consumption, i.e. administration of ethanol alone, clove alone or clove followed by ethanol induced a decrease in food consumption. This was not observed for GEC, which was subjected to alcohol restriction while receiving clove. On the other hand, compared to GE, groups GC, GCE and GEC had a significant increase in food consumption, suggesting that administration of ethanol alone during the experimental period induced a decrease in food consumption, whereas the use of clove before ethanol intake induced an increase in food consumption; similarly, the use of clove after ethanol intake increased the normal values. This effect of clove can be confirmed by comparing GC to GCE and GEC, which indicates that the values for GCE significantly decreased while those for GEC significantly increased, suggesting that this decrease is due to ethanol consumption after clove intake and the increase for GEC is due to clove intake, which suppresses the ethanol effect.

Biochemical assay of glucose

Comparison between groups indicated that the serum level of glucose remained unaltered, except for GEC which showed a significant increase relative to the remaining groups. This means that administration of ethanol followed by clove increased the serum levels of glucose.

Biochemical assay of triglycerides

The triglyceride level obtained for each group. Our results demonstrated that the triglyceride level remained unaltered for groups treated with ethanol alone (GE) or clove tea alone (GC), compared to the group that received only water (GW). However, the groups that received clove followed by alcohol (GCE) or ethanol followed by clove tea (GEC) had a significant increase compared to the control groups or to the groups that received ethanol or clove tea.

Biochemical assay of cholesterol

Our statistical analyses of serum levels of cholesterol did not indicate significant difference when treated groups were compared to control groups.

Biochemical assay of protein

The serum level of total protein did not change significantly when

GW was compared to GE and GC but showed a significant difference when animals that received clove followed by ethanol were compared to those that received either clove or ethanol.

Biochemical assay of albumin

The serum levels of albumin showed a significant increase when the group treated with ethanol alone was compared to the group that received clove followed by ethanol.

Discussion

Little is known about the effect of clove tea on health. Thus, we investigated its effect on biochemical parameters relevant to the development and prevention of good health.

In Brazil, the use of popular plants as tea, infusion and dry extract in capsules is common as an easy manner to lose weight [32]. Clove tea is also popularly reported to induce weight loss. However, studies for clove and weight loss, or *Syzygium aromaticum* and weight loss were not found in the scientific literature. Thus, we investigated whether clove tea has the property to reduce body weight. Our results showed that the group of rats that ingested 200 mg/kg/day of clove for 23 consecutive days and the group that ingested clove tea for 7 days followed by ethanol intake did not have difference in weight gain evolution but had a significant decrease in the daily consumption of food compared to control GW. However, analysis of weight gain evolution indicated it was similar among groups and typical of the growth of animals. A decrease in weight was thus expected as a consequence of the reduced food consumption. However, the same metabolic behavior relative to weight gain and lower calorie intake was also reported by Haraguchi et al. [33]. The latter authors demonstrated that male adult rats subjected to hypercholesterolemiant diet had higher weight gain although they had consumed a significantly lower quantity of food; they also showed that food intake is commonly found to be inversely proportional to energetic density in rats. Thus, we suggest that under these conditions clove does not induce weight loss. On the other hand, group GEC which received ethanol for 16 days and clove tea for 7 days did not show a significant difference in weight gain and food consumption compared to GW but had significantly decreased values compared to GE, GC and GCE. We must emphasize that GEC underwent alcohol restriction since we interrupted ethanol administration on the sixteenth (16^{th}) day, substituting it for clove until the twenty-third (23^{rd}) day. An experiment involving alcohol restriction was carried out by Richardson et al. [29], indicating that animals subjected to daily ethanol intake for 7 days had lower food consumption compared to the control, but when subjected to alcohol restriction the levels were similar to those of the control, which suggests that the animals undergo a transition in the quantity of consumed food when introduced to ethanol, recovering the normal values after a period of abstinence. Therefore, we suggest that the significant decrease in food consumption for group GCE was due to its introduction to ethanol. In respect to the normal values of food consumption for GEC, we suggest that the latter behaved similarly to the group of alcohol restriction described by Richardson et al. [29], which was introduced to ethanol and then underwent restriction while receiving clove tea. Thus, we suggest that clove tea does not influence weight gain or loss under these conditions.

We also investigated whether the administration of clove alone or alcohol restriction changes serum biochemical parameters which are important for the cell metabolism such as glucose, triglycerides, cholesterol, proteins, and albumin. As to glucose levels, our results showed a significant increase for GEC compared to GW, GE and GC. This result can be an effect of the alcohol restriction to which these animals were subjected and can be explained based on the findings of Villega et al. [34], who demonstrated that individuals subjected to alcohol restriction have an increase in the blood glucose rate which is not observed for individuals with occasional, moderate and high consumption, who remain with unaltered glucose levels. Considering the clove effect, Shukri et al. [19] showed that clove does not change the plasma glucose levels in normal rats. We suggest that this significant increase in glucose levels for GEC may be attributed to the alcohol restriction (abstinence) to which these animals were subjected.

Subsequently, we investigated the serum levels of triglycerides and cholesterol, which are associated with triglyceridemia. The latter is related to several diseases such as diabetes, atherosclerosis and myocardial infarction. Thus, the use of natural products to prevent triglyceridemia has been widely investigated. In our experiments, clove itself did not change triglyceride levels compared to control or when administered previously to ethanol. However, this result was not obtained when clove was administered during alcohol restriction. The groups of animals subjected to daily ingestion of 3% ethanol for 30 days did not show a significant difference compared to animals treated only with water (GW) or only with clove (GC). Visual analysis, however, showed an increase in triglyceride levels compared to GW and GC, suggesting that at the biological level there was an increase in serum triglycerides for these animals. On the other hand, there is controversy about the exact effect of ethanol on triglyceride levels, and scientific papers can be found in the literature showing increase [23, 35,36], decrease [37] or absence of ethanol effect on triglycerides [38]. However, GCE and GEC had increased triglyceride levels compared to GW, GE and GC. These results indicate that administration of ethanol followed by clove or administration of clove followed by ethanol increases the serum levels of triglycerides, which was not observed when ethanol or clove was administered alone, suggesting that this effect is only potentiated when there is administration of one after the other. However, there is the need of further experiments to define the exact effect of clove tea and ethanol, which will be carried out soon.

We did not find reports in the literature about the effect of clove on the serum levels of cholesterol in rats. In regard to ethanol, the available data are still confusing since we found reports of ethanol decreasing [39] or not changing [40] the serum levels of cholesterol in humans and in rats, while Wilson et al. [41] and Hashimoto et al. [42] showed that cholesterol accumulated in the pancreas and the liver when ethanol was ingested. Our results lead us to suggest that clove tea does not change the serum levels of cholesterol.

The change in total protein levels of animals is common in certain situations. A high level of total protein may be due to the presence of a paraprotein (abnormal plasma protein) and dehydration, whereas reduced protein levels occur in hepatic disease, nephrotic syndrome, and malnutrition [43]. The serum levels of total protein showed a significant difference when ethanol intake was followed by clove administration. In this case, there was an increase in the serum levels of total protein which was not observed when rats received clove alone, ethanol alone, or clove followed by ethanol, suggesting that the increase in total protein levels was due to the administration of clove. Although the statistical analysis did not show significant difference when the graph was visually analyzed, we could note an increase in the protein levels for animals that ingested clove alone (GC) and a slight decrease for animals that received ethanol alone. However, analyzing the animals that received ethanol followed by clove, we found values

similar to those obtained when clove or ethanol was administered alone, suggesting that ethanol minimized the effects of clove in increasing the plasma level of total protein. In association with the statistical analysis which suggested that the use of clove followed by ethanol intake increases the serum levels of protein, which in turn is not observed when ethanol intake is followed by clove consumption, these results indicate that clove administration to animals increases the serum levels of total protein when administered previously to ethanol.

Serum albumin constitutes approximately 60% plasma proteins [44]. Thus, we investigated whether the increase in total protein for GCE is related to the increase in albumin level. This group had a significant albumin increase when it received clove followed by ethanol, compared to the group that received ethanol alone. Therefore, we suggest that increased serum levels of total protein are due to increased serum levels of albumin. Analysis of the serum level of albumin is used to help diagnose dehydration when it is high, and response to acute phase, chronic inflammation, malnutrition and inherited deficiency when it is reduced [45]. On the other hand, it is too early to suggest any diagnosis based on the serum levels of protein and albumin under the conditions analyzed in these experiments; moreover, further experiments are needed to assess the exact role of clove and alcohol on the level of total protein and albumin.

Conclusion

In Brazil is usual the unrestricted consumption of tea as a natural product to revert or prevent metabolic changes. Our results imply that the clove tea consumption has no benefic properties over the metabolic changes caused by alcohol intake, thus his use has to be made with caution, when consumed with alcohol drinks.

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