Effects of the seeds of \textit{Aleurites moluccana} on the metabolic profile of Wistar rats

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Abstract

\textit{Aleurites moluccana} L. (Wild) is a native Euphorbiaceae from Indonesian and Malaysia but well adapted to the climatic conditions of the south and southeast of Brazil. It is popularly used for the treatment of asthma, hepatitis, ulcer, headache, fever, inflammation and exhibits anti-rheumatic effects. The objective of this study was to evaluate the effects of using the seeds of this plant in the biochemical and anthropometric profile, and visceral fat of Wistar rats. Male rats were divided into four groups: G1: control group treated with food and water ad libitum and propylene glycol orally, G2: control group treated with seed extract; G3: group treated with condensed milk and G4: group treated with condensed milk and seed extract). After 40 days we collect the blood samples to evaluation of glycaemia, cholesterol, triglycerides, HDL-c and Atherogenic Indices. Weight and visceral fat were also evaluated. Our results showed no interference in body weight, visceral fat, glycaemia, total cholesterol, LDL-c, HDL-c and triglycerides in the groups treated with the seeds but we found increase atherogenic indices. These results show that the seeds of \textit{A. molluccana} do not bring the health benefits that population may found when using the extract of the leaves. This indicates that the use of the seeds should be reviewed in order to avoid undesirable effects.

Keywords: \textit{A. molluccana}, body weight, visceral fat, glycaemia, lipids

1. Introduction

Diseases as diabetes, metabolic syndrome and cardiovascular diseases are among the leading causes of death in the modern world and are associated with changes in lifestyle and physical inactivity. Among other factors, hypertension, dyslipidemia, obesity and increased visceral fat contribute directly to the development of these diseases [1, 2]. In the other hand, the interest for improving quality of life and prevention of these diseases lead to the growing interest in evaluating plants that may be related to benefic metabolic or physiological effects when consumed as part of the regular diet [3-5]. Besides, the high costs of the allopathic drugs lead to an increase search for non-allopathic alternatives [6-8].

\textit{Aleurites moluccana} L. (Wild) is a plant from the Euphorbiaceae family and it is from Indonesian and Malaysia and well adapted to the climatic conditions of the south and southeast of Brazil. It was introduced in Brazil in the 1980s as an ornamental tree and is an important source of oil used in tanneries [9, 10]. Nowadays it is widely distributed in the south and southeast of the country. Popularly known as \textit{candlenut tree} or \textit{Indian walnut} it is used in folk medicine to treat asthma, hepatitis, ulcer, episodes of headache, pain, fever, inflammation and also as having anti-rheumatic, anti-bacterial and anti-viral effects [11-15].

Quintão et al. [13] performed phytochemical analyses with dichloromethane fraction from \textit{Aleurites moluccana} leaves and found the presence of triterpenes, a mixture of \textalpha{}-amyrone, glutinol, a mixture of \textalpha{}-amyrin, and friedelanol. Other phytochemical analysis with this plant have revealed the occurrence of n-hentriacontane, \textalpha{}-amyrin, \textbeta{}-amyrin, stigmasterol, \textbeta{}-sitosterol, triterpenes, steroids, coumarins, campesteryl and flavonoid glycosides (moretenone, moretenol, acetil aleuritic acid, moluc canin, swertisin, amyrin, stigmasterol, \textbeta{}-sitosterol, triterpenes, steroids, coumarins, campesteryl, stigmasteryl, and sitosterol) [16, 17].

There are only a few studies in the literature evaluating the effects of this plant but most of them are related to anti-inflammatory and antinociceptive properties, which is associated with
the presence of α and β amyrin and 2′, 3′-O-rhamnosylswertisin. This property may be related to the popular use of this plant in the treatment of headache [13-15, 17, 18]. We did not find studies associating the seeds with body weight and biochemical parameters. For this reason and as seeds are commonly used in Brazil to reduce body weight, the aim of this study was to evaluate the effects of the seeds of Aleurites moluccana L. in the biochemical and anthropometric profile of Wistar rats.

2. Materials and Methods

2.1 Ethical principles

This work was approved by the Animal Research Ethics Committee of the University of Marilia (UNIMAR) with registration number 71. The animals were treated according to the “Guide for the Care and Use of Experimental Animals” (that follows principles for the care of laboratory animals).

2.2 Preparation of A. moluccana extract

The seeds used in this study were obtained in local pharmacy at city of Marilia, São Paulo state, Brazil. These seeds were dried in an oven with air circulation at a temperature of 40°C for a period of seven days and subsequently they were crushed immediately before the use. The ethanolic extract was prepared with 100g seeds previously processed and submitted to maceration in 1000 mL of absolute ethanol for a period of 7 days. After this period the material was filtered. The extract was concentrated in a rotary evaporator at a temperature of 60°C. The extracts of A. moluccana were prepared at a concentration of 1:10 (A. moluccana: água) The extract was stored in amber glass vials of 30 mL and kept in the freezer for later use.

2.3 Preparation of the solution of condensed milk

Condensed milk is a product commonly used in Brazil to the preparation of candies and its formulation includes high percentage of sugar and fats. The solution used to the animals was prepared in a 1:1 proportion (volume of condensed milk/volume of water).

2.4 Experimental model

Forty eight male Wistar rats weighing approximately 250g were used. They were kept in the vivarium at UNIMAR (University of Marilia) under a dark/light cycle of 12 hours, room temperature of 22 ± 2°C, and relative air humidity of 60 ± 5%. The animals were divided randomly into 4 groups (n=12) and they were acclimatized to the laboratory conditions for 7 days. After that, we have started the experimental protocol and the animals were treated for 40 days, as follows:

- G1 received water ad libitum and 0.5mL of propylene glycol at a concentration of 50mg/mL (Control Group);
- G2 received condensed milk ad libitum by gavage route;
- G3 received water ad libitum and 0.5mL of A. moluccana extract by gavage route;
- G4 received condensed milk solution ad libitum and 0.5mL of A. moluccana by gavage route. Animals of all groups received food ad libitum during the treatment period.

2.5 Administration of A. moluccana

The administration of the plant extracts was done twice a day: in the early morning and late afternoon (according to the weight of animals) and the treatment lasted for 40 days. Animals received 1mL of A. moluccana extract.

2.6 Collection of blood samples and determination of the biochemical profile, Cardiovascular parameters

After treating animals for 40 days, they were anesthetized with Thiopental until complete sedation, after which blood samples were drawn to determine their biochemical profile: total cholesterol, LDL-c, HDL-c, triglycerides, and glycaemia. Cardiovascular risk parameters such as Atherogenic Coefficient (AC), Atherogenic Index (AI), Cardiac Risk Ratio 1 (CRR1), Cardiac Risk Ratio 2 (CRR1), and non-HDL-c levels were evaluated according to Ahmadvand et al. [19], Erejuwa et al. [20], and Ikewuchi et al. [21]; AC = (Total cholesterol – HDL-c)/HDL-c; AI = log (Triglycerides/HDL-c); CCR1 = Total cholesterol/HDL-c; CCR2 = LDL-c/HDL-c; Non-HDL-c = Total cholesterol - HDL-c.

2.7 Statistical analysis

The results were expressed as mean ± S.E.M. and evaluated by analysis of variance (ANOVA) complemented with Tukey test. The level of significance was set at p<0.05.

3. Results

Figure 1 shows that there are no significant differences for body glycaemia in the different groups.

Fig 1: Values of glycaemia (mean ± S.E.M.) in G1 (control group), G2 (condensed milk), G3 (A. moluccana) and G4 (condensed milk and A. moluccana). No significant differences were found between the groups.

Fig 2: Values of cholesterol (mean ± S.E.M.) in G1 (control group), G2 (condensed milk), G3 (A. moluccana) and G4 (condensed milk and A. moluccana). ***p<0.0001; ns: not significant, according to Tukey Test.
In Figure 2 it is possible to see that levels of cholesterol increase in the groups treated with condensed milk (G2 and G4). *A. moluccana* seed promoted significant increase in these levels in the animals treated with condensed milk (G4). We do not find differences among G1 and G3 and among G2 and G4.

The levels of HDL-c are significantly different only when comparing G1 with G4 and G3 and G4 (Figure 3).

![Fig 3: Values of HDL-c (mean ± S.E.M.) in G1 (control group), G2 (condensed milk), G3 (*A. moluccana*) and G4 (condensed milk and *A. moluccana*). ns: not significant; **P<0.001, according to Tukey Test.](image)

Figure 4 shows that LDL-c levels increase significantly in the groups treated with condensed milk independently of the use of the seed.

![Fig 4: Values of LDL-c (mean ± S.E.M.) in G1 (control group), G2 (condensed milk), G3 (*A. moluccana*) and G4 (condensed milk and *A. moluccana*). ns: not significant; ***P<0.0001, according to Tukey Test.](image)

The levels of triglycerides are significantly higher in the groups treated with condensed milk, but the seed did not interfere in the groups treated with regular or hypercaloric diet (Figure 5).

![Fig 5: Values of triglycerides (mean ± S.E.M.) in G1 (control group), G2 (condensed milk), G3 (*A. moluccana*) and G4 (condensed milk and *A. moluccana*). ns: not significant; **P<0.001 according to Tukey Test.](image)

Figure 6 shows no significant modifications in the weight increase.

![Fig 6: Values of body weight increase (mean ± S.E.M.) in G1 (control group), G2 (condensed milk), G3 (*A. moluccana*) and G4 (condensed milk and *A. moluccana*).](image)

In Figure 7 we observe that visceral fat values are higher in the groups treated with hypercaloric diet but do not suffer interference with the use of *A. moluccana* seeds.

![Fig 7: Values of visceral fat (mean ± S.E.M.) in G1 (control group), G2 (condensed milk), G3 (*A. moluccana*) and G4 (condensed milk and *A. moluccana*). ns: not significant; *P<0.05; ***P<0.0001, according to Tukey Test.](image)
Atherogenic index increased in the groups treated with condensed milk and with the seeds (G2, G3 and G4) when comparing with the control group (G1). Atherogenic coefficient, Cardiac Risk Ratio and non-HDL-c are significantly higher in groups treated with the seeds of *A. mollucana* (Table 1).

### Table 1: Atherogenic parameters and non-HDL-c values found in G1 (control group), G2 (condensed milk), G3 (*A. mollucana*) and G4 (condensed milk and *A. mollucana*).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI</td>
<td>0.51±0.13</td>
<td>0.76±0.18</td>
<td>1.29±0.19***</td>
<td>1.28±0.12***</td>
</tr>
<tr>
<td>AC</td>
<td>3.19±0.67</td>
<td>4.06±1.05</td>
<td>6.9±1.73***</td>
<td>5.4±1.64***</td>
</tr>
<tr>
<td>CRR1</td>
<td>4.91±0.67</td>
<td>5.06±1.05</td>
<td>7.82±1.73***</td>
<td>6.36±1.61***</td>
</tr>
<tr>
<td>Non-HDL-c</td>
<td>47.00±7.66</td>
<td>62.10±11.80</td>
<td>110.70±28.24***</td>
<td>135.10±21.67***</td>
</tr>
</tbody>
</table>

AI: Atherogenic Index; AC: Atherogenic coefficient; CRR1: Cardiac Risk Ratio 1. **P = 0.001; ***P = 0.0001, according to Dunnett Test.

4. Discussion

In Brazil, seeds of *A. mollucana* are commonly used to reduce body weight and cholesterol levels but our results contradict these findings once they show that these seeds did not decrease visceral fat and weight gain. We also observed that the use of hypercaloric diet may increase glycaemia and lipid levels but the use of the seeds did not interfere in these parameters. Furthermore, the use of the seeds increased several parameters that indicate cardiovascular risk (Figures 1-7 and Table 1).

Pedrosa et al. [17] studied the effects of methanol extract of *A. mollucana* leaves in rats and showed it can exhibit reduction in the body weight and lipid profile of high-fat-diet fed rats. They observed that the use of the extract reversed the increased levels of cholesterol, LDL-c, and triglycerides and increased the levels of HDL-c. Authors postulated that the action of this plant was due to the inhibition of endogenous cholesterol biosynthesis, reduction of lipid absorption in the intestine, enhancement of cholesterol degradation, or interference with lipoprotein distribution. Ado et al. [22] studied the effects of *A. mollucana* leaves and showed it possesses 100% of anti-lipase activity indicating that it may has potential as an anti-obesity agent. Authors attribute these pharmacological activities possibly to the presence of secondary metabolites such as saponins, polyphenols, terpenes, tannins, flavonoids and alkaloids that may be active as inhibitors of pancreatic lipase. *A. mollucana* present bioactive diterpenoids, 3,4-secopodocarpane trinorditerpenoids, moluccanic acids, moluccanic acid methyl ester, and 6,7-dehydromoluccanic acids [23]. Our findings do not show similar results with the seeds. High values for AI, AC, CCR1 and non-HDL-c increase the risk of oxidative stress and inflammatory processes. G3 and G4 showed the higher values for these parameters, indicating that the use of the seeds increase risk for developing chronic degenerative diseases as diabetes and cardiovascular issues [20–21].

Atherogenic indices may play an important role in predicting cardiovascular disease risk. The higher the results, the higher the risk of developing cardiovascular pathologies and vice versa [21,24]. The increase in the values of these indices found in our work (Table 1) may represent that the seeds of *A. mollucana* should not be used once they can bring undesirable effects (contrary to the use of the leaves). Other parameter used in our work was non-HDL-c values. Many authors have shown that it is a better predictor of cardiovascular disease risk than the LDL-c once it encompasses all of the atherogenic apolipoprotein B – containing lipoproteins as LDL-c, VLDL-c, IDL-c (intermediate-density lipoprotein cholesterol), chylomicrons, and their triglyceride-rich remnants). Furthermore, literature shows that this parameter, together with low HDL-c levels may be associated with higher plaque burden and smaller lumen volume in the vessels [25-27]. Our results for this parameter show that the use of the seeds may bring deleterious effects, as well as we found for the atherogenic indices.

Cardiovascular disease is one of the main cause of death worldwide. Atherogenic lipoproteins are able to accelerate several risk factors such as hypercholesterolemia, inflammation and oxidative conditions. Dyslipidemia has been associated with the main triggers in atherosclerosis development once the high values are more vulnerable of suffering oxidation. This process, besides oxidation of LDL-c, increases endothelial dysfunction leading to the entrance of these molecules in the intima resulting in macrophage foam cells, and after atherosclerotic plaques [25,28,29]. Several plants are related to the decrease of these risk factors and, thus, contribute with vascular health. As pointed before, *A. mollucana* leaves may also have this potential but unfortunately, our results show that the seeds of this plant are not associated with heart protection.

5. Conclusion

Our results show that the seeds of *A. mollucana* do not bring the health benefits that population may found when using the extract of the leaves. This indicates that the use of the seeds should be reviewed in order to avoid undesirable effects.

6. Authors' contributions

ELG, ACA and SMB carried out the conception and design of the study, treated the animals and drafted the manuscript. PCSB and DPC performed the statistical analysis. MSSS, FAD, ALM, RAP prepared the extracts of the plants and treated the animals. LCCU, MP and VHM performed the laboratorial analysis. All authors read and approved the final manuscript.

7. References

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