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The effect of surfactant above and below the critical micelle concentration (CMC) and the mathematical models used to determine the kinetics of drug release from the matrix system
Hassan A. Alhmoud

Biochemical parameters and Histomorphometric cardiac evaluation among Wistar rats treated with chia seed (Salvia hispánica L.): Experimental model
Camila Saran da Silva, Giuliana Kanaguchi, Camila Ribeiro de Arruda Monteiro, David Feder, Ligia Ajaime Azzalis, Fábio Ferreira Perazzo, Laura Beatriz Mesiano Mafrino, Elisabete Ornelas, Paulo César Pires Rosa and Fernando Luiz Affonso Fonseca,

Combination of lauric acid and myristic acid prevents benign prostatic hyperplasia (BPH) symptoms in animal model
Anup A. Patil, Veeresh, B. and Adikrao Yadav

Evaluation of the extracts of Morinda lucida and Tridax procumbens for anti-trypanosomal activity in mice
Abubakar A., Okogun J. I., Gbodi T. A., Kabiru, Y. A, Makun, H. A. and Ogbadoyi E. O.,
Full Length Research Paper

The effect of surfactant above and below the critical micelle concentration (CMC) and the mathematical models used to determine the kinetics of drug release from the matrix system

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The liberation of propranolol HCl from a controlled release matrix, containing the hydrophilic polymer, sodium carboxymethylcellulose (NaCMC) and the hydrophobic polymer, Eudragit RL 100 (RL 100) as excipients, was studied. The influences of surface active agents on the dissolution rate of the drug were examined. Tablets were made by direct compression methods. The dissolution tests were performed by using the basket method. The incorporation of the cationic surfactants within the matrices increased the drug release until the critical micelle concentration (CMC). While, after the CMC, the increase in drug release was to a lesser extent. The incorporation of the anionic surfactants reduced the release rate of the drug from the matrices. At the CMC, the percent of drug release from the matrices were approximately the same with the matrices without the surfactants. While an increase in drug release was observed above the CMC of the anionic surfactants. The data obtained from in vitro drug release studies were plotted according to three kinetic models to study the release kinetic. These were zero order release, the first order release and the Higuchi equation. Zero order release of the drug was observed in all the formulations. The release mechanism was influenced considerably by the ratio of the excipients.

Key words: Propranolol HCl, controlled release, critical micelle concentration (CMC), dissolution, dissolution rate kinetics, surfactant.

INTRODUCTION

It has been established that excipients can influence or even alter considerably, the release rate of a drug from solid dosage forms. Matrices have been commonly used to enhance drugs dosage forms and consequently manage the drug release by embedding different types of matrices: hydrophobic matrix (Dredan et al., 1998), hydrophilic matrix (Nokhodchi et al., 1999), or a combination of both (Efentakis et al., 1990). The applied
method in the preparation of these matrices is the direct compression technique, which is rapid, cheap and needs less time, personnel and equipments. The effect of various surfactants on the release rate behavior of drug from ethylcellulose based matrices was discussed previously (Bolourtchian et al., 2005).

A controlled release matrix with propranolol hydrochloride as a model drug with a hydrophilic polymer, NaCMC and the hydrophobic polymer, Eudragit RL100 as excipients were prepared and investigated (Al-Hmoud, 2000a; Al-Hmoud, et al., 2014a, b). The effect of surfactants on the dissolution rate of drug from controlled release matrices has also been discussed in several studies (Efentakis et al., 1992; Nokhodki et al., 2002, 2008; Bolourtchian et al., 2005). The results of these studies varied, with many of the surfactants causing an increase in the dissolution of the drug, while others demonstrated a decrease, with some having no effect. This was dependent on several factors such as, their interaction with the other components of the matrix, surfactant charge and solubility, surfactant concentration, their wettability effect and their CMC value.

The purpose of this study was to investigate the effect of several surface active agents on the in vitro drug release above and below their CMC when incorporated in a solid matrix consisting of mixtures of Eudragit RL100 and NaCMC and the propranolol HCI as a model drug. In addition, this study compared the effect of different surfactants at the same concentrations and changes on the drug dissolution when they are incorporated within the matrices at different ratios. Also, how the solubility of each surfactant affects its CMC value is discussed.

The models used to describe the release mechanism of drug release and the kinetic assessment of the release data for all the formulations used in the study are:

A. The zero order release which describes the dissolution of the drug of many controlled release dosage forms such as, matrices, coated forms, and osmotic devices (Nnashimhan et al., 1999).
B. The first order release kinetic used in the dissolution of the pharmaceutical dosage forms which contain water soluble drugs that are released from the pores of the matrices (Nnashimhan et al., 1999).
C. Higuchi model can be used to describe the drug dissolution from the types of modified release pharmaceutical dosage forms such as transdermal and matrix tablets (Shoaib et al., 2006)

**MATERIALS AND METHODS**

For the preparation of the heterogeneous matrices, the following materials were employed: Propranolol HCl was provided by the Arab Pharmaceutical Manufacturing- Jordan (APM), sodium lauryl sulfate and magnesium stearate were purchased from BDH, cetrimide was purchased from Serva, Eudragit RL100 was provided by Rohm Pharma, sodium carboxymethyl cellulose was purchased from FMC, and sodium taurocholate and cetlypyridinium chloride were purchased from Fluka. All chemicals were of reagent grade.

**Preparation of tablets**

The acrylic resin Eudragit RL100 was powdered in a Ball mil and sieved through a 300-μm sieve and further blended with propranolol HCl and the other additives for 5 min in a blender. The different formulations prepared, containing various amounts of surfactants and their proportions, are shown in Table 1. The powder mixture was compressed to prepare tablets of 400 mg using the direct compression technique, an instrumental single punch tableting machine, (Korch-Erweka). The diameter and the thickness of the cylindrical tablets were 1 and 0.4 cm, respectively.

**Tablet hardness**

The hardness level of the tablets was about 9 kg and a schleuniger – 2 hardness tester was employed for its determination. A previous study indicated that the hardness of the tablets has no effect on the release rate of the drug (Al- Hmoud, 2002).

**In vitro dissolution test**

The United States pharmacopoeia (USP) basket method (Erweka, DT 6R, Heusenstamm, Germany) was used for all the in vitro dissolution studies. The test was performed at 37 ± 0.1°C with a rotation speed of 50 rpm using 900 ml of 0.1 N HCl, pH 1.2, as a dissolution medium.

Previous studies revealed that the buffer dissolution medium of a pH ≈ 7.4 increased the release rate of the drug, due to the eroding effect of this medium on the swollen matrices of NaCMC (Bavega et al., 1987; Al-Hmoud et al., 1991). In addition, it was observed from previous studies that an increase in the stirring speed increased the release rate of the drug due to the increase of agitation of the dissolution medium (Al-Hmoud et al., 1991).

**Assay**

Samples of 5 ml were withdrawn each hour; from the dissolution medium and replaced immediately with an equal volume of the respective dissolution medium maintained at 37 ± 0.1°C. Test samples were filtered through 0.45 μm filter, and assayed spectrophotometrically at 289 nm using a blank solution as a reference with a UV- Vis double-beam spectrophotometer (Systronic 2202). The mean of three determinations was used to calculate the drug release rate from each of the formulations (Al-Hmoud et al., 2014).

**Assessment of dissolution data**

The release data were assessed for the kinetics of release and dissolution using a suitable computer program

**Measurement of the surface tension**

The critical micelle concentrations of the surfactants used in the study were measured previously, (Al-Hmoud et al., 2014). These values were approximately as follows: cetrimide 0.5%, cetylpyridiniumchloride 0.75%, sodium taucholate 0.5% and sodium lauryl sulphate 0.75% by using the DuNouy tensiometer (Kruss).

**Kinetic and mechanism of drug release analysis**

The data obtained from the in vitro drug release studies were
Table 1. Composition of the different formulations matrices used in the study.

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
<th>F12</th>
<th>F13</th>
<th>F14</th>
<th>F15</th>
<th>F16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cet.</td>
<td>0</td>
<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CPC.</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ST</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>SLS</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Each tablet from formulations F0 weighs 400 mg and contains 20% (80 mg) of propranolol HCl, 1% of Mg stearate, 10% of Eudragit Rl 100, 69% of NaCMC, and the different concentrations of surfactants are as shown. The formulations from F1 to F4 contain 20% of propranolol HCl, 69% NaCMC, 10% Eudragit Rl 100, 1% Mg Stearate, and 0.25, 0.5, 0.75 and 1% of cetrimide, respectively. The formulations from F5 to F8 contain 20% of propranolol HCl, 69% NaCMC, 10% Eudragit Rl 100, 1% Mg Stearate and 0.25, 0.5, 0, 75 and 1% of cetyl pyridinium chloride respectively. The formulations from F9 to F12 contain 20% of propranolol HCl, 69% NaCMC, 10% Eudragit Rl 100, 1% Mg Stearate and 0.25, 0.5, 0, 75 and 1% of sodium taurcholate respectively. The formulations from F13 to F16 contain 20% of propranolol HCl, 69% NaCMC, 10% Eudragit Rl 100, 1% Mg Stearate and 0.5, 0.75, 1 and 2% of sodium lauryl sulfate, respectively.

Figure 1. Drug release profile of propranolol HCl from tablets containing the same ratios of Eudragit Rl100/NaCMC and the different concentrations of the cationic surfactants (Cet. and CPC). F0: square, F1: circle (cet.0.25), F2: triangle-up (Cet, 0.5), F3: triangle down (cet, 0.75), F4: diamond (cet, 1%), F5: triangle-left (CPC0.25), F6: triangle-right (CPC0.5), F7: hexagonal (CPC0.75), F8: star (CPC0.1%).

Plotted according to two kinetic models to study the release kinetics. The zero order release model (equation 1), describes the concentration independent of drug release rate from the formulation, cumulative amount of drug release plotted versus time (Figures 1 and 2).

\[ C = k_{0}t \] (1)

Where \( k_{0} \) is the zero-order release constant expressed as units of concentration/time and \( t \) is the time in hours. The Higuchi's model (Equation 2) describes the release of drug based on Fickian diffusion as a square root of time-dependent process from swellable insoluble matrix. Cumulative percentage of drug released plotted versus square root of time is shown in Figures 3 and 4.

\[ Q = k_{h}t^{1/2} \] (2)

Where, \( k_{h} \) is the constant of Higuchi.

RESULTS AND DISCUSSION

Different concentrations of four surfactants are incorporated in the matrix tablets F0 (Propranolol/RL100/NaCMC/MgO), prepared previously (Al-hmoud, 2002) to investigate the effect that these surfactants on drug release above and below their CMC.

In the current study, four surfactants were used, two
Figure 2. Drug release profile of propranolol HCl from tablets containing the same ratios of Eudragit RL100/NaCMC and the different concentrations of the anionic surfactants used. F0: square, F9: circle (ST, 0.25), F10: triangle-up (ST, 0.75), F11: triangle down (ST, 0.75), F12: diamond (ST, 0.1%), F13: triangle-left (SLS, 0.5), F14: triangle-right (SLS, 0.75), F15: hexagonal (SLS, 1), F16: star (SLS, 2%).

Figure 3. Drug release profile of propranolol HCl from tablets containing the same ratios of Eudragit RL100/NaCMC and the different concentrations of the cationic surfactants used. F0: square, F1: circle (Cet, 0.25), F2: triangle-up (Cet, 0.5), F3: triangle down (Cet, 0.75), F4: diamond (Cet, 1%), F5: triangle-left (CPC0.25), F6: triangle-right (CPC0.5), F7: hexagonal (CPC0.75), F8: star (CPC 0.1%).

cationic: cetrimide (Cet) and cetylpyridinium chloride (CP), and two anionic: sodium lauryl sulfate (SLS) and sodium taurocholate (ST). The concentrations of these surfactants were above and below the CMC. The results revealed that, the incorporation of the cationic surfactants within the formulation increased the
release rate of the drug (Figures 1 and 3). The increase was proportional to the incorporated amount of the surfactant until the CMC of the surfactant was reached. The results also revealed that above the CMC, the increase of drug release was in a lesser extent despite doubling of the surfactant concentration within the formula F0. This might be due to 1- The formation of a stagnant layer around the tablets in the dissolution medium. Formula F2 shows that the incorporation of 0.5% (CMC of cetrimide) increased the release of propranolol HCl by 28%, while, doubling the concentration to 1% (formula F4) shows that the percent of drug release increased by 37% only (Figure 1). The formula F7 shows that the incorporation of 0.75% (CMC of CPC) increased the release of propranolol HCl by 26%, while in formula F8, when the concentration is 1%, the percent of drug release increased by 34% only (Figure 1). 2- The decrease in the wetting effect of the surfactant due to the relaxation of the hydrophilic polymer of the matrix which formed a net of gel that captured some of the surfactant inside (Al-Hmoud et al., 2014; Shargel and Yu, 1999).

Comparison of drug release from the matrices when embedding in 0.5% cetrimide and 0.5% cetylpyridinium chloride shows that the increase with cetrimide is 28%, while with cetylpyridinium chloride, it is 18%.

This was studied previously by Nokhodki et al. (2008).

This increase could be attributed to the differences in solubility of the two surfactants, which caused wide pores within the matrices and more drug release in the dissolution medium (Effentakis et al., 1991a; Effentakis, 1992b).

Moreover, the CMC of the more soluble surfactant is lower than that of the less soluble one, which may be due to the early formation of micelles. These results were observed in both the cationic and anionic surfactants.

The effect of the anionic surfactants show decrease in propranolol HCl release rate from the matrices until the CMC of the surfactant was reached. Following that, an increase in drug release was observed once the surfactant concentration was above the CMC. This increase in drug release may have been due to the formation of wide pores within the swollen matrices as a result of the high solubility of the surfactants. These pores permit the release of a large quantity of drug in the dissolution medium. It may have also been due to the formation of soluble micelles, which may facilitate the diffusion of the drug from the tablets into the dissolution medium.

The incorporation of the anionic surfactants SLS and ST within the matrices at concentrations below the CMC showed a decrease in the release rate of the drug (Figures 2 and 4), this decrease is 11% by incorporation of 0.25% of ST and about 25% by the incorporation of
0.5% SLS. This decrease could be attributed to the formation of weak complexes between the cationic propranolol HCl and the anionic surfactants, and these complexes might form tortuous channels within the tablets (Wells and Parrott, 1992). Above CMC, the results were not the same, and increase in the drug release rate was observed (Figure 2). This increase is about 20% with the incorporation of 1% ST (F11), and 12.5% with the incorporation of 1% SLS. This increase may be due to more than one reason.

1. The high concentration of the very soluble surfactant ST within the tablets (2 g of ST dissolved in 1 ml of water), and the freely soluble SLS in which 1 g was dissolved in 10 ml of water. These surfactants may facilitate the access of the dissolution medium to the formed pores within the tablets, lowering the contact angle of water on the tablets and increase their wettability, which leads to an increase in the release rate of the drug (Effentakis et al., 1991).

2. Formation of soluble micelles with a clear phase around the tablets, which increases the release rate of the drug (Al-Hmoud et al., 2014).

The kinetic assessment of the release data for all the formulations (from F0 to F16) with the different surfactant concentrations, and the estimated values of the correlation coefficient ($r^2$) (Table 2) appears to fit all the models used in the kinetic assessment of the release analysis. All these values ($r^2$) were of 0.980 and more which suggests that the release rate of drug was according to zero order kinetics in the specified time.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Zero order</th>
<th>First order</th>
<th>Square root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r^2$</td>
<td>Slope</td>
<td>Intercept</td>
</tr>
<tr>
<td>F0</td>
<td>0.997</td>
<td>5.881</td>
<td>8.286</td>
</tr>
<tr>
<td>F1</td>
<td>0.998</td>
<td>11.5</td>
<td>7.25</td>
</tr>
<tr>
<td>F2</td>
<td>0.998</td>
<td>7.2976</td>
<td>14.53571</td>
</tr>
<tr>
<td>F3</td>
<td>0.997</td>
<td>7.3214</td>
<td>17.42857</td>
</tr>
<tr>
<td>F4</td>
<td>0.995</td>
<td>7.5595</td>
<td>19.10714</td>
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<tr>
<td>F5</td>
<td>0.998</td>
<td>6.5714</td>
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<tr>
<td>F6</td>
<td>0.998</td>
<td>6.6666</td>
<td>12.75</td>
</tr>
<tr>
<td>F7</td>
<td>0.996</td>
<td>7.3928</td>
<td>13.35714</td>
</tr>
<tr>
<td>F8</td>
<td>0.995</td>
<td>7.9404</td>
<td>14.14286</td>
</tr>
<tr>
<td>F9</td>
<td>0.989</td>
<td>5</td>
<td>8.75</td>
</tr>
<tr>
<td>F10</td>
<td>0.996</td>
<td>5.9166</td>
<td>9.75</td>
</tr>
<tr>
<td>F11</td>
<td>0.996</td>
<td>6.0119</td>
<td>11.32143</td>
</tr>
<tr>
<td>F12</td>
<td>0.996</td>
<td>7.0357</td>
<td>12.96429</td>
</tr>
<tr>
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<td>0.994</td>
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<tr>
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<td>0.997</td>
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<tr>
<td>F15</td>
<td>0.996</td>
<td>6.6309</td>
<td>10.28571</td>
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<tr>
<td>F16</td>
<td>0.996</td>
<td>6.7738</td>
<td>12.64286</td>
</tr>
</tbody>
</table>

Conclusions

The results of the study revealed that the cationic surfactant showed an increase in the release rate of the propranolol HCl below and above the CMC of the surfactant but in different ratios. The percent of increase below the CMC was more than that above the CMC; these little increase may be due to the formation of a stagnant layer of solution around the tablets in the dissolution medium, which retard the drug diffusion from the matrix.

The anionic surfactants caused decrease of the drug release below the CMC of the surfactant. While, an increase of drug release was observed above the CMC of the surfactant, which might be due to the wide pores which formed within the swollen matrices as a result of the high solubility of the surfactants.

These pores permit the liberation of the drug in the dissolution medium; also, the formation of soluble micelles around the tablets may facilitate more dissolution and drug diffusion from the swellable tablets to the dissolution medium.

Conflict of Interests

The author have not declared any conflict of interests.

REFERENCES


Biochemical parameters and Histomorphometric cardiac evaluation among Wistar rats treated with chia seed (Salvia hispánica L.): Experimental model

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The advance of global economy affects developing countries that are facing a major public health problem. The prevalence of obesity triggers a series of non-communicable chronic diseases (NCDs). Several studies have suggested that the chia seed (Salvia hispánica L.) may be an alternative to combat NCDs, as it is rich in alpha-linolenic acid (ALA). This research is an experimental work in which 24 Wistar rats were divided into 4 groups (G1, G2, G3 and G4), out of which G1 was the control group (ad libitum + saline); G2 received a hyperlipidic and hyperglycemic diet (HHD); G3 was given HHD + 0.2 g ground chia and G4 was given HHD + 0.4 g of ground chia, for 4 weeks. The objective was to evaluate biochemical parameters (total cholesterol (TC) and lipoproteins, triglyceride (TG), glucose, homocysteine and high-sensitivity C-reactive protein (hsCRP) and histomorphometric of rats on a diet with chia seed, as well as to ascertain the safety of such treatment proposed evaluating its hepatotoxicity [aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyl transferase (GGT)]. There was no decrease, however, in dyslipidemia parameters (total cholesterol (TC), high-density lipoprotein (HDL), triglycerides) and glucose. The concomitant administration of chia to a hyperglycemic and hyperlipidemic diet was not able to alter these parameters, however with regard to histomorphometric analysis, there was a significant result (p < 0.08) with regard to the thickness of the right ventricle (RV), which leads to a possible cardioprotective effect.

Key words: Chia seed (Salvia hispánica L.), dyslipidemia, biochemical parameters.

INTRODUCTION

The progress of the world economy has been affecting developing countries which are facing a major public health problem, that is the prevalence of obesity, which has been a current issue in developed countries, and at
the moment is triggering a series of non communicable chronic diseases (NCDs) (Iauay and Kain, 2002; Popkin and Gordon-Larsen, 2004; Pinheiro et al., 2004). NCDs are currently responsible for the overload of the Unified Health System (SUS) being accountable for innumerable deaths caused by diabetes, dyslipidemia, hypertension, lung problems, cardiovascular disease (CVD) and metabolic syndrome (MS) (Coutinho et al., 2008; Franscischi et al., 2000; Silva et al., 2013). The excessive consumption of products rich in sugars, saturated and trans fats, as well as polyunsaturated n-6 is one of the hypothesis for the current obesity scenario, as these are associated with the increasing development of cardiovascular and inflammatory diseases. On the other hand, fatty acids of the n-3 series and eicosapentaenoic acid (EPA; C20: 5 n-3) to docosahexaenoic acid (DHA C22: 6 n-3) and alpha-linolenic acid (ALA: C18: 3 n-3), the latter coming from the vegetables, have proven to be effective as protectors of these factors (Pinheiro et al., 2004; Silva et al., 2013; Poudyal et al., 2012).

The chia seed (Salvia Hispânica L.) is regarded as a rich source of alpha-linolenic acid (ALA) in approximately 60% of its composition and 20% linoleic acid (LA). It is also rich in fiber, antioxidants and protein. Native from Mexico and Guatemala, chia seeds are currently subject of several studies for its possible protective role in dyslipemias, as results have shown they were able to reduce total cholesterol (TC) and raise high density lipoprotein (HDL) levels in animal model, in addition to being used in various experiments to a new alternative food as ALA enriched eggs and baked goods (Silva et al., 2013; Poudyal et al., 2012; Vázquez – Ovando et al., 2009; Martínez et al., 2012; Chicco et al., 2009; Ali et al., 2012).

This study aims at evaluating biochemical parameters (TC and lipoproteins, TG, glucose, homocysteine and hsCRP) and histomorphometric of rats on a diet enriched with chia seeds, and ascertain the safety of such treatment evaluating its hepatotoxicity [aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT)].

MATERIALS AND METHODS

Twenty four (24) male albino Wistar animals were used, weighing on average 300 g, from the Vivarium of Faculdade de Medicina do ABC (FMABC). During the experiment, the animals were kept in a photoperiodic cycle of 12 h light and 12 h dark temperature, controlled relative humidity and exhaustion (20 air changes per hour). The 24 animals were divided into 4 groups, with 6 animals in each group: G1 - control group, G2 - HHD group, G3 - HHD + chia seed (0.2 g) and G4 - HHD + chia seed (0.4 g). The Ethics Committee on Animal Experimentation from FMABC approved the study, under registration number 011/2012.

Diet composition

The control group (G1) was given 2.5 ml of saline, in addition to the ad libitum during the entire period of the experiment. The hyperlipidic and hyperglycemic diet (HHD) was composed of 52% (wt/wt) carbohydrate, 24% (wt/wt) fat and 25% (wt/vol) fructose. This mixture was administered by gavage for 2.5 ml. The animals (G2, G3 and G4) were subjected to this diet for four weeks. In the end, total cholesterol levels were checked and fractions of triglycerides and glucose determined. After characterizing the increase in fat diet parameters, G2 continued to receive only HHD, G3 received 0.2 g of grounded chia seed in addition to the HHD, as well as G4 obtained an increase in the ration of 0.4 g of grounded chia seed for another four weeks.

Tissue preparation

After eight weeks, the animals were sacrificed and blood samples were collected from the tail of the animals in dry tubes and kept under refrigeration until the time of analysis. Next, an incision was made in their chests in order to expose the heart of each animal. The hearts were removed and sectioned transversely at the level of papillary muscles. The apical fragments were fixed in formalin 10% buffered (pH 7.2) for 48 h. After the material fixation, a process of dehydration, diaphanization and paraffin inclusion commenced. Five (5) non-consecutive histological transverse sections of 6 microns in thickness of the fragments of the right and left ventricles from each animal were used. The sections were stained with hematoxylin-eosin method and examined under light microscopy.

Morphometric analysis

The Axiovision Zeiss software was used to capture the images used for morphometric studies. 20 micrographs/group with >30 zoom were captured to estimate the area of the ventricular cavity and the thickness of the ventricles and interventricular septum. To estimate the thickness (E), four measurements per frame obtained at 0, 90, 180 and 270° were used.

Biochemical parameters

Blood samples were collected in dry tubes and kept under refrigeration until the moment of analysis, including the determination of homocysteine (Hcy), alanine transaminase (ALT), aspartate transaminase (AST), gamma- glutamyltransferase (GGT), hsCRP. In addition to the total cholesterol checks and fractions, triglycerides and glucose were also determined. The measurement of plasma homocysteine was taken by enzyme immunoassay using Immulite 2000, automatic chemiluminescence equipment in the Clinical Analysis laboratory at FMABC. Prior to the beginning of the analysis in this system, adjustments were carried out according to the manufacturer’s recommendations. Glucose, total cholesterol,
Table 1. Biochemical parameters results of HHD within 4 weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC mg/dl</th>
<th>TG mg/dl</th>
<th>HDL mg/dl</th>
<th>Non cholesterol mg/dl</th>
<th>Glycemia mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (control)</td>
<td>78 ± 9.8</td>
<td>155 ± 45.2</td>
<td>26 ± 1.4</td>
<td>52 ± 11.3</td>
<td>201 ± 62.2</td>
</tr>
<tr>
<td>G2 (HHD)</td>
<td>65 ± 2.8</td>
<td>191 ± 16.2</td>
<td>23.5 ± 0.7</td>
<td>41 ± 3.5</td>
<td>313 ± 42.4</td>
</tr>
<tr>
<td>G3 (HHD)</td>
<td>68 ± 2.8</td>
<td>137 ± 135.0</td>
<td>28 ± 0</td>
<td>51 ± 2.8</td>
<td>267 ± 26.8</td>
</tr>
<tr>
<td>G4 (HHD)</td>
<td>122 ± 32.5</td>
<td>323 ± 36.7</td>
<td>22 ± 15.5</td>
<td>99.5 ± 48.0</td>
<td>393 ± 33.2</td>
</tr>
</tbody>
</table>

Table 2. Biochemical parameters of chia seed results after 4 weeks of treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC mg/dl</th>
<th>TG mg/dl</th>
<th>Non cholesterol HDL mg/dl</th>
<th>HDL mg/dl</th>
<th>Hcy mg/dl</th>
<th>hsCRP mg/dl</th>
<th>Glycemiaa mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (control)</td>
<td>70 ± 9.8</td>
<td>119 ± 6.3</td>
<td>42 ± 5.6</td>
<td>28 ± 4.2</td>
<td>20.5 ± 7.7</td>
<td>&lt;0.3</td>
<td>140 ± 89.8</td>
</tr>
<tr>
<td>G2 (HHD)</td>
<td>65 ± 5.6</td>
<td>188 ± 2.1</td>
<td>35.4 ± 2.1</td>
<td>31.4 ± 3.5</td>
<td>27 ± 6.4</td>
<td>&lt;0.3</td>
<td>280 ± 57.9</td>
</tr>
<tr>
<td>G3 (HHD + 0.2 Chia)</td>
<td>88 ± 6.3</td>
<td>135 ± 8.3</td>
<td>50.5 ± 3.5</td>
<td>38 ± 2.8</td>
<td>25 ± 0.7</td>
<td>&lt;0.3</td>
<td>277 ± 62.9</td>
</tr>
<tr>
<td>G4 (HHD + 0.4 Chia)</td>
<td>56 ± 20.5</td>
<td>187 ± 46.6</td>
<td>27 ± 14.1</td>
<td>29.5 ± 6.3</td>
<td>21 ± 14.1</td>
<td>&lt;0.3</td>
<td>550 ± 112.4</td>
</tr>
<tr>
<td>Value P</td>
<td>0.400</td>
<td>0.954</td>
<td>0.748</td>
<td>0.271</td>
<td>0.181</td>
<td>-</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

HDL-cholesterol, ALT, AST and GGT were performed by enzymatic-colorimetric method following the best practices in clinical analysis. The LDL-cholesterol and VLDL-cholesterol fractions were determined by Friedewald formula. hsCRP dosing was performed by competitive immunoassay method, by boiling in liquid phase, labeled with ligand. The following measurement was performed by chemiluminescence method by binding protein in vitro with immobilized anti-ligand detection system. All analyses were performed in the Clinical Analysis laboratory, at FMABC.

Statistical analysis

Data was expressed as average ± standard error of mean (SEM). After confirming that all continuous variables were normally distributed using the Kolmogorov-Smirnov test, the statistical differences between groups were subjected to analysis of variance (ANOVA). When another significance was detected, comparisons were performed by the post hoc Tukey test. P values less than 0.05 were considered statistically significant.

RESULTS

Twenty four (24) Wistar rats were divided into four groups G1, G2, G3 and G4, which in the first month received 2.5 ml of a HHD except G1 which served as a control group in order to elevate the parameters (total cholesterol, HDL, triglycerides and glucose) in order to analyze attenuation with the use of chia seed the following month. Table 1 shows the results of total cholesterol, HDL, triglycerides, non-HDL cholesterol and glucose levels, held within 4 weeks. It is possible to notice a considerable increase in blood glucose in all groups fed HHD, in contrast with the control group as well as the TG. Although there is variation in the average between the groups for the TC parameters, HDL and HDL non cholesterol, the results were satisfactory for the next phase of the study, in which G2 remained receiving the HHD, G3 received an addition of 0.2 g ground chia seed (plus HHD) and G4 received 0.4 g ground chia seed (plus HHD), for another month.

Table 2 shows the result of chia in the TC parameters, TG, non-HDL cholesterol, HDL cholesterol, homocysteine, hsCRP, glucose. Supplementation with chia seed for four weeks did not cause significant results (p) as shown in Table 2. Although the TC average at G4 has been shown lower than G1, the blood glucose increase was evident in all groups compared to the control group, since the TG average cholesterol non-HDL and HDL were approximate to the control group, demonstrating that the chia seed is ineffective to attenuate the effects of the evaluated parameters. Just as chia seed has not been able to reduce the cardiovascular risk, since no change in hsCRP and Hcy was observed throughout the work. None of the groups had hepatotoxic effect to the seed, the significant values (p) are shown in Table 3.

Morphometric analysis

The results (see Figure 1) of morphometric quantification of the thickness of the right ventricle (RV) is the only one which shows significant trend (p <0.08) when compared to the other groups. This may be related to a possible cardiovascular protective effect. Other parameters such
Table 3. Results about hepatotoxicity of chia seed.

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST mg/dl</th>
<th>ALT mg/dl</th>
<th>GGT U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (control)</td>
<td>73 ± 45.9</td>
<td>233 ± 6.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>G2 (HHD)</td>
<td>82 ± 41.0</td>
<td>167 ± 5.6</td>
<td>&lt;1</td>
</tr>
<tr>
<td>G3 (HHD + 0.2 Chia)</td>
<td>82 ± 6.3</td>
<td>213 ± 12.0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>G4 (HHD + 0.4 Chia)</td>
<td>60 ± 36.0</td>
<td>201 ± 0.7</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Value P</td>
<td>0.263</td>
<td>0.254</td>
<td>-</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study the chia seed was incapable of reducing the levels of TC, TG and increase HDL. Although in other studies these results have been satisfactory. Chicco et al. (2009) found that chia seed was able to prevent the onset of dyslipidemia and insulin resistance in rats (Chicco et al., 2009). Another study by Ayerza et al. (2007), also performed in rats, showed that only the seeds, either whole or ground, obtained results compared to the control group, and the whole seeds decreased TG levels, and ground seed increased HDL levels, the chia seed oil, on the other hand, did not show any significant results (Ayerza and Coates, 2007).

The use of chia seed along with a HHD may have influenced in a negative way to mitigate the parameters analyzed, since a balanced diet and physical exercise are widely advocated in order to minimize such factors (Pan and Storlien, 1993; Wang and Peng, 2011). The ALA-rich foods are currently the subject of several studies, as many studies have shown its relevance in the treatment of dyslipidemia and especially in the care of cardiovascular diseases (CVD), for being known as cardio-protective (Pan and Storlien, 1993; Wang and Peng, 2011).
The chia seed, when analyzed in the parameters of non-HDL cholesterol, hsCRP and Hcy, associated factors to quantify the risk of CVD, have not shown significant results (Neves et al., 2004; Silva et al., 2009). As in a study by Nieman et al. (2009), in which the chia seed was not successful in reducing the parameters evaluated for CVD, although there was a rise of plasma ALA, compared to placebo, this result was ineffective, the researchers used overweight individuals and supplemented their diets with 50 g/day chia seed for 12 weeks (Neiman et al., 2009).

However, in order to perform the histological analysis, we observed a promising outcome (p < 0.08) as the thickness of the RV, related to a possible cardioprotective effect, but the fact that chia seed also contains AL may have influenced for more significant results, since it competes with ALA for the same metabolic enzyme, raising the risk factors associated with CVD (Pan and Storlien, 1993; Vedtofte et al., 2011).

In literature, the relation of high fructose consumption, which can lead to diabetes or insulin resistance is consistently established. The same effect can be generated by the abuse of sweeteners, which are typically derived from fructose, a fact that was recently reported by Suez et al. (2014), to determine the effect of non-caloric sweeteners (NCS) in glucose homeostasis, they administered to a mixture of water with saccharin or aspartame or sucralose to rats, while the control group consumed pure water or water with sucrose or glucose, the control group remained at a similar tolerance curve, unlike the groups that received the NCS developed glucose intolerance, and the same occurred when the rats were given a diet high in fat to verify the effect on obesity, in both situations saccharin was the most efficient for the cause of insulin resistance (Suez et al., 2014; Barreiros et al., 2005).

The same mechanism that causes insulin resistance with the use of NCS, may have occurred in the concomitant administration of chia seed and fructose in this study, since the chia seed was not effective in reducing blood glucose parameters, which increased throughout the study, a fact that again confirms the hypothesis that a balanced diet is necessary and the practice of physical exercises with the use of chia seed, as previously mentioned (Marchon et al., 2015).

Finally, chia seed was ineffective to mitigate the biochemical parameters analyzed in this paper. Although it has shown an improvement in RV thickness, more experimental and clinical studies on the seed should be performed in order to analyze its function in dyslipidemias, since it has low cost and is a great source of ALA, serving as an option to existing products which in many cases are expensive to purchase. Moreover, it is not hepatotoxic, which is an advantage for the consumption of the same, when their effects are more accurate in the literature.

Conflicts of interests
The authors have not declared any conflict of interest.

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REFERENCES


Combination of lauric acid and myristic acid prevents benign prostatic hyperplasia (BPH) symptoms in animal model

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Benign and uncontrolled growth of prostate gland is known as benign prostatic hyperplasia (BPH). It is a common health issue that affects 8% of all men at the age of 40, 60% of men in their 70s, and 90% of those greater than 80 years of age. In this study, we investigated whether a combination of lauric and myristic acid improved BPH in a testosterone propionate (TP)-induced model of BPH in rats. BPH was induced in the rats with a subcutaneous injection of TP (3 mg/kg) and combination of different doses of lauric acid and myristic acids given every consecutive day for 4 weeks. Combination of lauric acid and myristic acid led to significant reductions in prostate weight and dihydrotestosterone levels in the serum and prostate. Therefore, combination of lauric acid and myristic acid was effective in reducing TP-induced BPH in a rat model, and may be useful for the clinical treatment of patients with BPH.

Key words: Testosterone propionate, benign prostatic hyperplasia, testosterone, dihydrotestosterone.

INTRODUCTION

Benign prostate hyperplasia (BPH) is a urological disorder caused by the noncancerous enlargement of the prostate as men age. As the prostate enlarges, it can constrict the urethra, inducing various symptoms including a weak urinary stream, incomplete bladder emptying, nocturia, dysuria and bladder outlet obstruction (Pais, 2010; Roehrborn, 2011). It is proliferative process of both stromal and epithelial elements of the prostate (McNeal, 1983). It is unclear what specific factors regulate the degree of hyperplasia, which ultimately dictates the size of the prostate gland, there are many consensus regarding the prostate size that qualifies for the diagnosis of benign prostatic enlargement (BPE). As men age, the caliber of the urinary stream diminishes (Girman et al., 1995). The diminution of the urinary stream was assumed to be attributable to bladder outlet obstruction (BOO) arising directly from the BPE (Lepor, 2000). Androgens may be involved in the epithelial
stroma interaction. In mature prostate, androgens are known to cause several changes in prostatic epithelium through androgen receptors located in the stroma. Immunocytochemical studies have shown that prostatic smooth muscle cells are uniformly androgen receptor positive. This fact indicates that smooth muscle located in prostatic stroma may be an important target for androgen action and able to regulate the expression of prostate growth factors (Niu et al., 2003).

Although the etiology of benign prostatic hyperplasia is not completely elucidated, it involves hormonal changes in the aging man. The development and growth of prostate gland depends on androgen stimulation, mainly through dihydrotestosterone (DHT), an active metabolite formed due to enzymatic conversion of testosterone by 5α-reductase.

Production and accumulation of DHT in the prostate increases with ageing which results in encouraging cell growth and induction of hyperplasia (Carson and Ritterman, 2003; Bartsch et al., 2000). Benign prostatic hyperplasia also involves augmented adrenergic tone in prostate smooth muscles, regulated through α1-adrenoceptors (Michel et al., 1998).

Conventionally used drugs like 5α-reductase inhibitors (finasteride and dutasteride), α-adrenoceptors antagonists (alfuzosin, doxazosin, tamsulosin, terazosin) are used to treat benign prostatic hyperplasia, but they possess various side effects like impotence, decreased libido, ejaculation disorder, gynaecomastia, dizziness, upper respiratory tract infection, headache, fatigue and chest pain (Patel and Chapple, 2008). Along with conventional therapy, some alternative therapies are also available to treat prostatic hyperplasia. Various in vitro studies have reported that fatty acids inhibit the enzymes activity (Liang and Liao, 1992). Coconut oil (Arruzaba et al., 2006), lauric and myristic acid (Veeresh Babu et al., 2010) have proven to be effective benign prostatic hyperplasia mainly due to 5α-reductase inhibitory activities, which is due to their high content of lauric acid and myristic acid (mainly lauric).

However, there is no evidence for efficacy combination of lauric and myristic acid on testosterone induced benign prostatic hyperplasia whether oral dose with combination of lauric/myristic acid could prevent testosterone induced hyperplasia in rats.

**MATERIALS AND METHODS**

**Animals**

Male Wister rats weighing 180 to 220 g were procured from an institutional animal facility centre. They were housed individually in clean and transparent polypropylene cages maintained at room temperature with 12 h light/dark cycle and had free access to food and water. After 7 days of acclimatization, they were randomly distributed into experimental groups. All the experimental procedure was carried out in accordance with Committee for the Purpose of Control and Supervision of Experimental on Animal (CPCSEA) guidelines.

**Chemicals**

Lauric acid and myristic acid (obtained from Sigma-Aldrich Pvt. Ltd). Finasteride (was obtained from FINAST, Dr.Reddy’s Lab).

Testosterone Propionate (was Courtesy of Genesis pharmaceuticals, japan).

ELISA Kit for measurement of testosterone, hydrotestosterone, and Alanine transaminase (ALT), aspartate transaminase (AST) Kits were (purchased from Transasia Bio chemical Ltd Daman).

**Experimental BPH model and drug administration**

Lauric acid and myristic acid were suspended in distilled water using Tween 80 and administer orally. Testosterone propionate was diluted with distilled water using Tween 80 and injected subcutaneously. Experimental groups were divided into 5 groups.

Group I (normal control group) did not receive any treatment.

Testosterone treated groups were randomly divided into four groups (n = 6): Group II (positive control) testosterone propionate (TP, 3 mg/kg body weight); Group III, which received finasteride (10 mg/kg body weight) administered orally and TP (3 mg/kg body weight), Group IV: Lauric acid and myristic acid (180 and 70 mg/kg) administered orally and TP (3 mg/kg body weight) injected subcutaneously. Group V: Lauric + myristic acid (360 and 140 mg/kg) administered orally and TP (3 mg/kg body weight). All rats were treated once a day for four weeks.

**Body and prostate weight – ratio of prostate weight to body weight and percentage of inhibition**

Animal were sacrificed after weighing by sodium pentobarbital and prostates were removed and weighted immediately. Then, prostate weight to body weight ratio were calculated. Further percentage of inhibition was calculated as follows:

\[
\text{100 - (treated group - negative control) / (positive control - negative control} \times 100
\]

**Blood and tissue samples**

After a treatment period of 4 weeks and an overnight fast, the rats were anesthetized with sodium pentobarbital (100 mg/kg body weight, i.p.). Blood samples were collected and centrifuged at 2000 \( \times g \) for 10 min. The prostate was collected from each rat and weighed. All prostatic specimens from each group were fixed with 10% buffered formalin for 24 h.

**Measurement of DHT and testosterone in prostate and serum**

The prostate tissue was homogenized in lysis buffer containing protease inhibitors (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 0.1% SDS, 1 mM EGTA, 100 μg/mL PMSF, 10 μg/ml pepstatin A, and 100 μM Na2VO4). The homogenates were centrifuged at 12,000 \( \times g \) for 25 min at 4°C, and the protein concentrations in the supernatant fractions were determined using Bradford reagent. DHT and testosterone levels in the serum and
Table 1. Effect of combination of lauric and myristic acids on prostate enlargement in testosterone treated rats.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Group</th>
<th>PW (g)</th>
<th>% inhibition</th>
<th>PW/BW ratio (× 10^-3)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>1.55±0.10</td>
<td>-</td>
<td>2.55±0.01358</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>3.65±0.55</td>
<td>-</td>
<td>3.98±0.0845</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>1.98±0.28*</td>
<td>79.71</td>
<td>2.01±0.01096*</td>
<td>82.11</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>2.40±0.26*</td>
<td>59.93</td>
<td>2.06±0.00587*</td>
<td>62.12</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>2.10±0.24*</td>
<td>73.93</td>
<td>2.25±0.00975*</td>
<td>78.69</td>
</tr>
</tbody>
</table>

PW: Prostate weight, BW: body weight, Group I: negative control; Group II: Positive control; Group III: Finasteride (10 mg/kg); Group IV: Lauric + myristic acid (180 and 70 mg/kg); Group V: Lauric + myristic acid (360 and 140 mg/kg). Values are expressed as mean± S.E.M. Statistical analysis is done by one way ANOVA followed by Dunnett's multiple comparisons test.

Prostate weights were measured with ELISA kits according to manufacturer's instructions (Transasia Bio chemical Ltd Daman).

Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

ALT and AST levels were determined to assess liver function using commercial kits (Transasia Bio chemical Ltd Daman) and an auto analyzer.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis is done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test.

RESULTS

Evaluation of prostate enlargement

Prostate weights

Significant enlargement of prostate weights was found by testosterone treatment when compared with negative control. But significant reduction in elevation of prostate weights was found in group-V in testosterone treated rats. Percentage inhibition was 73.93% (Table 1).

Prostate weights to body weight ratio

In Group II, significant elevation in prostate weights/body weight ratio was noted when compared with Group I rats. But significant reduction in elevation of prostate weight/body weight ratio was observed by Group IV and V. Percentage inhibition was 62.12 and 78.69%, respectively when compared with Group I (Table 1).

Measurement of DHT and testosterone in serum

There was no significant difference in testosterone levels in serum found between all groups except for the group II (Table 2). DHT levels in serum, in group II, increased statistically more than Group I (8.79 ± 1.20 ng/ml). Conversely, in Group III (finasteride) (14.45 ± 2.36 ng/ml) markedly decreased the DHT levels in serum compared with the Group II. Group IV (10.56 ± 2.36 ng/ml) also significantly decreased the DHT levels in serum as well as the result of finasteride group (Table 2).

Measurement of DHT in prostate

As shown in Graph 1, the DHT level of group II (20.4 ± 7.87 ng/ml) significantly increased more than the normal...
Graph 1. Group I: negative control; Group II: Positive control; Group III: Finasteride (10 mg/kg); Group IV: Lauric + myristic acid (180 and 70 mg/kg); Group V: Lauric + myristic acid (360 and 140 mg/kg).

Graph 2. Group I: negative control; Group II: Positive control; Group III: Finasteride (10 mg/kg); Group IV: Lauric + myristic acid (180 and 70 mg/kg); Group V: Lauric + myristic acid (360 and 140 mg/kg).

Measurement of testosterone in prostate

As shown in Graph 2, the testosterone level of group II (209.04 ± 6.87 pg/ml) significantly increased more than the normal control (Group I) (135.07 ± 0.50 pg/ml). In contrast, the finasteride Group III (172.02 ± 3.77 pg/ml) significantly increased the testosterone level in prostate control (Group I) (10.4 ± 0.60 ng/ml). In contrast, the finasteride group (11.36 ± 6.77 ng/ml) significantly decreased the DHT level in prostate compared with the Group II. The group V (14.25 ± 9.70 ng/ml) also observed the significant reduction in the DHT level of prostate in comparison with Group II, which was similar to the result of finasteride group.
compared with the Group I. The group V (174.23 ± 8.70 pg/ml) also observed the significant reduction in the testosterone level of prostate in comparison with the Group II, which was similar to the result of finasteride Group III.

Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

Treatments did not affect the activities of the serum toxicity marker enzymes, ALT and AST, indicating normal liver function (Figure 1).

DISCUSSION

The present study examined the effects of combination of lauric and myristic acid in a testosterone induced rat model of BPH and compared them with the effects of finasteride, which is currently used to treat BPH. Treatment with lauric and myristic acid for 4 weeks significantly inhibited the development of testosterone induced prostatic hyperplasia, which was evident in the reduction in the elevated prostate weight/body weight ratio, reduced DHT levels in the prostate and serum.

The increased prostate weight is used as one of crucial markers of BPH according to previous study (Pais, 2010). BPH is characterized by stromal and epithelial cells hyperplasia, resulting in prostate enlargement. In previous studies, animals with BPH had a significant increase in prostate weight compared with normal control animals, whereas those of animals treated with finasteride or others herbal remedies for the management of BPH had meaningfully reduced compared with BPH animals (Bisson et al., 2007; Pais, 2010). For these reason, many studies have evaluated the inhibitory effects of various materials on the development of BPH by measuring prostate weight (Jang et al., 2010; Veeresh Babu et al., 2010). In the present study, the animals with BPH showed significantly increased prostate weight compared with the normal control animals; however, combination of lauric and myristic acid animals showed the significant reduction in these measures compared with the BPH animals.

The two main classes of drugs used as BPH treatments are inhibitors of α1-adrenoceptor inhibitors, which inhibit smooth muscle cell contraction (Furuya et al., 1982), and inhibitors of Type II 5 α-reductase, an enzyme responsible for the conversion of testosterone to the more potent androgen dihydrotestosterone (DHT) (Griffiths and Denis, 2000). Steroid 5 α-reductase converts testosterone to DHT, an active form of androgen, in the prostate. It has been noticed in earlier study that increased production of DHT results in the development of prostatic hyperplasia (Pais, 2010). Because DHT has a 10 times higher affinity for the androgen receptor than testosterone, DHT easily binds to androgen receptor, which stimulates the transcription of growth factors that are mitogenic for the epithelial and stromal cells for prostate (Carson and Rittmaster, 2003). The importance of DHT in prostatic hyperplasia was demonstrated by previous studies in which an inhibitor of 5α-reductase was administered to experimental animals with BPH (Roehrborn, 2011). For instance, at clinical doses, finasteride is selective for 5α-reductase and
achieves about 70% suppression of serum DHT and 68 to 86% suppression of intraprostatic DHT (Span et al., 1999). These findings are in agreement with results of present study. Moreover in this study, combination of lauric and myristic acid reduced the DHT levels in the prostate and serum relative to those in rats with testosterone-induced BPH. Thus, these results indicate that combination of lauric and myristic acid inhibits the development of BPH via down-regulation of DHT.

Interestingly, although testosterone level in finasteride-treated group was observed no significant difference, it was decreased compared with the BPH animals. Before performing the present study, it was expected to have an increase in testosterone level as observed in previous studies (Pais, 2010; Roehrborn, 2011). However, this finding is different from previous studies and unexpected. Many researchers have conducted the studies on relationship between drug treatment and testosterone level in BPH condition. In most previous studies, administration of finasteride showed that the testosterone level is increased compared with that of BPH animals in many studies due to inhibition of the transformation testosterone to DHT. On the other hand, some previous studies showed that the testosterone level is similar to that of BPH animals following drug treatment as well as the result of the present study (Gasco et al., 2007). However, we did not find the clear reason why testosterone level is not changed in present study.

Conclusion

Based on the results of present study, it is concluded that oral administration of combination lauric acid and myristic acid in a BPH rat model significantly decreased the prostate weight, prostatic epithelial hyperplasia, and DHT levels in the serum and prostate. These results indicate that combination of lauric acid and myristic acid may effectively inhibit the development of BPH and may be useful for treatment of BPH patients. However further study is required to explore the effects these to fatty acids on 5α-reductase inhibitor activities and underlying major molecular mechanisms of their action.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Evaluation of the extracts of Morinda lucida and Tridax procumbens for anti-trypanosomal activity in mice

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A major problem besetting the chemotherapy of African Trypanosomiasis is parasite resistance to the few available drugs, and a known strategy is to use combination of drugs to overcome parasite drug resistance. An attempt has been made in this study to explore the potential of Tridax procumbens and Morinda lucida individually as single therapy and as combination therapy in the treatment of trypanosomiasis. The effective dosage of the T. procumbens and M. lucida extracts were combined at ratio 1:1, 1:2 and 2:1(w/w) respectively. All treatments were by intraperitoneal and commenced after the establishment of infection. The methanol extract of M. lucida stem bark and leaves gave significant mean survival of 7.0 ± 3.3 and 9.7 ± 3.7 days respectively when compared to the untreated control (P<0.05). The ethyl acetate and methanol extracts of T. procumbens gave a mean survival of 11.7 ± 5.4 and 14.3 ± 10.2 days respectively (P<0.05). The combination of Tridax procumbens and leaves of Morinda lucida methanol extracts at 1:2 gave significant mean survival of 10±2.2 days (P<0.05) at 200 mg/kg body weight, while the least was when combined in ratio 1:1. Phytochemical screening revealed the presence of steroids, saponins, tannins, alkaloids, flavonoids, phenols and carbohydrate in the crude methanol extract and phenols, flavonoids and steroids in the crude ethyl acetate extract of T. procumbens while M. lucida showed the presence of alkaloids, tannins and saponins. The methanol extracts of the two plants exhibited significant anti-trypanosomal activity and when combined, the plant extracts showed synergy in their activity. The plant extracts therefore have the potential for use as antitrypanosomal phytomedicine which could provide antitrypanosomal drug leads.

Key words: Tridax procumbens, Morinda lucida, antitrypanosomal activity, combination therapy, Rubiaceae, Asteraceae.

INTRODUCTION

In tropical Africa, protozoan parasites cause several diseases of social and economic importance. One of the most devastating is trypanosomiasis which is caused by infection with trypanosomes. Trypanosomes are transmitted by tsetse flies to people, domestic livestock and wildlife. The disease constrains agricultural
development on over a third of the African continent by causing livestock production losses due to poor weight gains, stunted growth, poor milk production, reproductive failure and finally death (Olu kunle et al., 2010)

*Morinda lucida* Benth (Rubiaceae) is a medium-sized tree about 15 m tall (Yinusa et al., 2005). It is known as Sangogo or Bondoukou alongua in Cote d’Ivoire; Twi, kon kro ma or Ewe amake in Ghana; Ewe amake or atak ake in Togo and Oruwo in South Western Nigeria (Adeneye and Agbaje, 2008). In many countries different parts of the plants are used in different ways. Cold decoction of the plant leaves is used for the treatment of fever in Cameroon; the bitter water decoction of the plant bark, root and leaf are used as bitter tonic and as astringent for dysentery, abdominal colic and intestinal worm infestation (Adeneye and Agbaje, 2008). Oliver-Bever (1986) reported the use of weak decoction of the stem bark in the treatment of jaundice. There are documented in vitro antimalarial activity of *M. lucida* leaf extract Koumaglo et al. (1992) and stem bark extract (Bello et al., 2009) against *Plasmodium falciparum* and antimalarial activity of *M. lucida* against *Plasmodium berghei berghei* in mice (Obih et al., 1985). Methanolic extract of *M. lucida* leaf extract have been reported to possess trypanocidal activity (Asuzu and Chine me, 1990) and aortic vasorelaxant effect (Ettarh and Emeka, 2004). Oliver-Bever (1986) documented the use of a weak decoction of the stem bark to treat severe jaundice.

*Tridax procumbens* (Asteraceae) is known for several potential therapeutic activities like antiviral, antibiotic efficacies, wound healing activity, insecticidal and anti-inflammatory activity (Suseela et al., 2002). Some reports from tribal areas in India state that the leaf juice can be used to cure fresh wounds, to stop bleeding. The plants has been extensively used in Ayurvedic system of medicine for various ailments and is shown to possess significant antiinflammatory, hepatoprotective, wound healing and antimicrobial properties (Diwan et al., 1989; Pathak et al., 1991; Saraf et al., 1991; Udupa et al., 1991; Taddei and Rosas, 2000).

The entire plant is used by indigenous people in Guatemala for the treatment of protozoal infections (malaria, leishmaniasis, vaginitis, dysentery) and gastrointestinal disorders (colic/stomach pains, gastritis/enterocolitis) (Caceres et al., 1998; Berger et al., 1998). Ethnobotanically, in Guatemala the whole plant of *Tridax procumbens* is used by the population for topical applications to treat chronic ulcers caused by leishmaniasis (Caceres et al., 1998).

The present study is designed to evaluate antityrpanosomal activities of *M. lucida* (leaf and stem bark) extract and *T. Procumbens* singly and in combination using various solvents.

**MATERIALS AND METHODS**

**Parasites (Trypanosoma brucei brucei)**

A stabilate of *Trypanosoma brucei brucei*, a parasite originally isolated from cattle in Lafia, Nasarawa State and kept in liquid nitrogen at the Nigerian Institute for Trypanosomiasis Research Vom, Plateau State was used. It was maintained in rats by serial passaging.

**Plant materials**

The *T. procumbens* were collected in Kaduna Vom of Plateau state and Bida and Minna of Niger state while *M. lucida* was from Osogbo in Osun state of Nigeria. The aerial part of *T. procumbens* were collected in the months of May and June. Similarly, the leaves, flowers as well as the local names of *M. lucida* were collected in March at Oba ile, Olurunda Local Government Area, Osogbo, Osun State, Nigeria.

**Identification of plants materials**

All the plant materials were identified at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. The Plant, *Tridax procumbens* with voucher number NIPRD/H/6155 and *Morinda lucida* with voucher number NIPRD/H/6289 were deposited at the herbarium of NIPRD, Idu, Abuja.

**Preparation of plant materials**

About 1 kg each of the *T. procumbens* and *Morinda lucida* (stem and leaves) were freshly obtained washed with running tap water and dried at room temperature to a constant weight. The dried plant samples were grinded into powder form using mortar and pestle. The powdered samples were stored in clean polythene bags until required for use.

**Preparation of crude extracts**

The extraction and screening carried out using the method described by Ogbadoyi et al. (2007). In this method, fifty grams (50 g) of the dried powdered samples of each of the leaves, stem of the *M. lucida* and whole plant of *T. procumbens* were extracted sequentially under reflux with 400 ml of hexane, ethyl acetate, methanol and water in that order for 2 h in each case. Extracts were filtered hot using muslin cloth and solvent was removed using rotatory evaporator for organic solvents and freeze-drier for water extracts. The dried extracts were finally transferred into sterile sample bottles for storage at refrigerated temperature until when required for use. The residue was dried after each extraction for the next extraction process.

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Table 1. Phytochemical constituents of *T. procumbens* crude methanol and ethyl acetate extracts.

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<tr>
<th>Phytochemical</th>
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<tr>
<td>Carbohydrates</td>
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<tr>
<td>Steroids</td>
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<td>Saponins</td>
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<td>Flavonoids</td>
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<tr>
<td>Alkaloids</td>
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<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
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</tr>
</tbody>
</table>

++, Highly present; +, fairly present; _, absent.

Phytochemical analysis

The crude methanol and ethyl acetate extracts of *T. procumbens* was subjected to phytochemical analysis using standard analytical methods described by Sofowora (1979) (Table 1).

Infection of animals

Blood from heavily infected donor mouse was obtained by cardiac puncture and collected with EDTA coated syringe to avoid clotting. The blood was immediately diluted with physiological saline to give 1.0 × 10^7 parasites per ml to obtain inoculums. Healthy mice were then infected intraperitoneally with 0.1 ml of the inoculum containing about 10^7 trypanosomes (Herbert and Lumsden, 1976).

Administration of extracts

Crude extracts dissolved in (physiological buffered saline PBS) for aqueous extract or in little Dimethyl sulphoxide (DMSO) and made up with PBS for organic solvent extract in varied concentrations were administered on infected animals via the intraperitoneal route. Parasitaemia in the blood of infected animals was monitored daily by obtaining blood from the tail end of mice and observing the wet smear under light microscope set at 40X magnification for parasite per field. The number of parasites per ml of blood is then estimated using "Rapid Matching Method" described by Herbert and Lumsden (1976).

Initial screening of the extracts for antitrypanosomal activities

In *T. procumbens*, the ethyl acetate, methanol and aqueous extracts of the whole plants were subjected to the screening against *T. b. brucei* infected mice. For each extract, there are 7 groups (A - G) of 3 mice each. Groups A - F were inoculated with *T. b. brucei* and with the appearance of parasitaemia, animals in Groups A - D were each treated intraperitoneally with the extract at 100, 200, 300 and 400 mg/kg body weight respectively for 14 consecutive days respectively. Mice in Group E were treated once intraperitoneally with beneril at 3.5 mg/kg body weight while Groups F was untreated and Group G was neither infected nor treated and served as control. Similarly in *M. lucida*, the hexane, ethyl acetate, methanol and aqueous extracts of the stem bark and leaves were screened for antitrypanosomal activity against *T. b. brucei* in mice. For each extract of leaves and stem bark, there are 7 groups (A - G) of 3 mice each. Groups A - F were inoculated with the parasite and with the appearance of parasitaemia, animals in Groups A - D were each treated intraperitoneally with the extract at 100, 200, 300 and 400 mg/kg body weight respectively for 14 consecutive days respectively. Mice in Group E were treated once intraperitoneally with beneril at 3.5 mg/kg body weight while Groups F was untreated and Group G was neither infected nor treated and served as control.

Confirmatory screening with effective doses of *T. procumbens* and *M. lucida*

In order to ascertain the efficacy and reproducibility of the doses that demonstrated appreciable antitrypanosomal activities in the initial screening, eight groups of mice (A - H) each containing three mice, were set up. Group A, B and C were administered with methanolic leaves, methanolic stem bark and ethyl acetate extracts of *M. lucida* at 400, 200 and 100 mg/kg body weight respectively. Groups D, E and F were administered with ethyl acetate, methanol and aqueous extracts of *Tridax procumbens* at 200, 300 and 300 mg/kg body weight respectively. All the administration was through intraperitoneal route for 14 consecutive days. Groups G was infected untreated while Group H was neither infected nor treated and served as controls. Parasitemia was monitored three times weekly.

Combination therapy

The possibility of synergetic action of different extracts in varying combinations was investigated using a modified method of Gerardo et al. (2007). To establish this, different extracts that gave highest antitrypanosomal activity in the initial screening were combined in varying ratios and was screened against *T. b. brucei* infected mice. Thus, *T. procumbens* ethyl acetate extract at 200 mg/kg body weight and *M. lucida* methanolic leaves extract at 400 mg/kg body weight were combined in ratios 1:1, 2:1 and 1:2. Each ratio was used treat 3 groups of *T. b. brucei* infected mice comprising of 3 animals each at 200, 300 and 400 mg/kg body weight respectively. In all cases, parasitaemia was monitored daily and means (±SD), maximum / minimum survival was calculated.

RESULTS

Weight of extracts

When 100 g of the crude *M. lucida* stem bark was extracted, hexane gave 0.37 g; ethyl acetate = 0.42 g, methanol = 1.19 g and aqueous = 5.14 g extracts. Similarly, 100 g of crude *M. lucida* leaf extract gave 1.27 g with hexane, 5.12 g for ethyl acetate, absolute methanol 3.92 g and aqueous = 10.62 g extracts. The weight of *T. procumbens* extracts using 100 g of the crude is as follows: Ethyl acetate = 3.31 g; Methanol = 4.5 g and Aqueous = 7.12 g.

Screening of *M. lucida* leaf extract

The result for the screening of leaf extract of *Morinda lucida* is presented in Figures 1 to 4. The parasitaemia
Figure 1. Effect of different doses of *Morinda lucida* hexane leaves extract on *T. b. brucei* infected mice.

Figure 2. Effect of different doses of *M. lucida* ethyl acetate leaves extract on *T. b. brucei* infected mice.

was lowered with methanol extract (Figure 3) having a means prolongation of life by 9.7 days at 400 mg/kg body weight (Table 2). Other solvent extracts also extend the life of treated groups and the least was the hexane
Figure 3. Effect of different doses of *M. lucida* Methanolic leaves extract on *T. b. brucei* infected mice.

Figure 4. Effect of different doses of *M. lucida* aqueous leaves extract on *T. b. brucei* infected mice.

Screening of *M. lucida* stem bark extract

The result for screening stem extract of *M. lucida* was presented on Figures 5 to 8. The methanol extract at a dose of 200 mg/kg recorded the longest mean life prolongation by 7.0 days beyond the infected untreated control. The active principle may be less shielded and hence the desire effect was observed at low dosage. The least was the animals treated with ethyl acetate extract (Table 3). In all, the parasitaemia kept fluctuating until the animals died of acute infection.

Screening of *T. procumbens* extracts

Treatment with the *T. procumbens* at all the dose levels resulted to lowering of the parasitaemia leading to prolongation of life (Figures 9 to 11). Ethyl acetate and methanol extract have the best trypanostatic effect resulting to significant means prolongation life by 11.7 and 14.3 days respectively (*P*<0.05). Aqueous extract
Table 2. Summary of screening *M. lucida* leaves extract.

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Dose (mg/Kg)</th>
<th>Survival range (Days)</th>
<th>Survival beyond control (Days)</th>
<th>Means survival (±SD)</th>
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<tr>
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Figure 5. Effect of different doses of *M. lucida* hexane stem bark extract on *T. b. brucei* infected mice.

has the least effect (Table 4).

Confirmatory test

Confirmatory test for the initial screening with the extracts at different doses and a subsequent repeated screening showed that 400 mg/kg body weight of methanol extract of *M. lucida* leaves and 200 mg/kg body weight of *T. procumbens* ethyl acetate extracts gave consistent antitrypanosomal activities. The means prolongation of life was almost the same with the result of the initial
screening. However, the methanol stem bark extract of *M. Lucida* and methanol extract of *T. procumbens* at 200 mg/kg body weight could not reproduce the result of the initial screening (Table 5). The control animals that were untreated died one week post infection.

**Combination of extracts**

The combination of *T. procumbens* and *M. lucida* at 1:2 gave significant means survival of 10 days (P<0.05) at 200 mg/kg body weight, while the least was when combined in ratio 1:1 (Figures 12 to 14). It is possible that there is less interference of other compounds with active principle in the extract, hence the observed effect at low dosage.

**DISCUSSION**

As a medicinal plant, all the various solvent extract of the dried leaves, in order of increasing polarity, has shown
some level of sporadic antitypansomal activity. The most promising activity was recorded with groups treated with more polar methanolic leaves extract. In methanol extract of *M. lucida* leaf extract, the highest dose level of 400 mg/kg body weight recorded highest means survival of 9.7 days with maximum life prolongation of 14 days.

Table 3. Summary of screening *M. lucida* stem bark extract (Table 2).

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<th>Dose (mg/Kg)</th>
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<th>Survival beyond Control (Days)</th>
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</tr>
<tr>
<td>Infected, untreated</td>
<td>4-5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
This was possible because there was suppression of parasitaemia which was more in group treated at 400 mg/kg body weight. The ethyl acetate treated group was the second best in terms of mean survival and parasitaemia. The ethyl acetate extract is more polar than hexane extract and less than methanol. The effect of hexane and aqueous extract on the parasitaemia and prolongation of life beyond the control are minimal (P>0.05).

The stem bark extract showed a similar activity with leaves extract although to a lesser extent. Therefore, the antitrypanosomal property of leaves methanol extract supersede that of the stem bark methanol extract both in term of mean survival and prolongation of life beyond the untreated control.

The transient trypanocidal activity of methanol extracts
of Morinda lucida leaves and stem bark is in agreement with the earlier studies by Asuzu and Chineme (1990). They reported the suppression of the T. b. brucei infected animal when treated with 50% methanol dry leaves extract and 1000 mg/kg produced the highest suppression. In this study, the extracted leaves of M. lucida were in absolute methanol and the dose level that gave highest suppression of parasite was 400 mg/kg body weight. Furthermore, Asuzu and Chineme (1990) obtained the best trypanocidal activity when treatment with M. lucida extract commenced simultaneously with trypanosome inoculation. In this study the best anti-trypansomal activity was observed when treatment commenced at 24 h post parasite inoculation. Therefore

![Graph showing effect of different doses of T. procumbens aqueous extract on T. b. brucei infected mice.](image)

**Figure 11.** Effect of different doses of *T. procumbens* aqueous extract on *T. b. brucei* infected mice.

**Table 4.** Summary of screening *T. procumbens* extracts (Table 2).

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Dose (mg/Kg)</th>
<th>Survival range (Days)</th>
<th>Survival beyond Control (Days).</th>
<th>Means survival (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>100</td>
<td>8-10</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>9-22</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>7-9</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>6-16</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6-17</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6-10</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Methanol</td>
<td>300</td>
<td>7-30</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>5-15</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Infected, untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>100</td>
<td>8-10</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>7-12</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
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<td>300</td>
<td>7-21</td>
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<td>15</td>
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<tr>
<td></td>
<td>400</td>
<td>8-16</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Infected untreated</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Abubakar et al.

Table 5. Summary of screening combined T. procumbens and M. lucida methanolic extracts.

<table>
<thead>
<tr>
<th>Combination type</th>
<th>Dosage (Mg/Kg)</th>
<th>Survival range</th>
<th>Survival beyond control (Days)</th>
<th>Means survival (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>TP:ML(1:1)</td>
<td>200</td>
<td>9-11</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>13</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>9-11</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>TP:ML (1:2)</td>
<td>200</td>
<td>13-19</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>9-14</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>9-11</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>TP:ML (2:1)</td>
<td>200</td>
<td>13-15</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>12-17</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>8-13</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Infected, Untreated</td>
<td>5-6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TP, T. procumbens; ML, M. lucida.

Figure 12. Effect of different doses of combined T. procumbens and M. lucida methanol extract (1:1) on T. b. brucei infected mice.

the efficacy of treatment is dependent on the number of parasite inoculated and time the treatment commenced. This studies and many other investigators (Asuzu and Chineme, 1990; Aguiyi et al., 1999; Onyekwelu and Okwuasaba, 2006; Ogbadoyi et al., 2011) all observed that the trypanocidal activity of the extracts from the various plants was not sustained and parasites were not cleared from the peripheral circulation. The phytochemicals present in M. lucida was earlier reported to be alkaloids, saponins, tannins. Doughari (2012) reported that plant-derived alkaloids in clinical use include the analgesics, the muscle relaxant, the antibiotics, the anticancer agent, the antiarrythmic, the pupil dilator, and the sedative.

Tannin rich medicinal plants are used as healing agents in a number of diseases. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinorrhoea and diarrhea (Doughari, 2012). Extracts of T. procumbens has shown antitrypanosomal potential against T. b. brucei infected mice. Both the prolongation of life and suppression of parasitaemia in infected animals was possible probably because T. procumbens was earlier reported to have immunomodulatory activity which suggest its therapeutic
Figure 13. Effect of different doses of combined *T. procumbens* and *M. lucida* methanol extract (1:2) on *T. b. brucei* infected mice.

Figure 14. Effect of different doses of combined *T. procumbens* and *M. lucida* methanol extracts (2:1) on *T. b. brucei* infected mice.

usefulness (Tiwari et al., 2004). *T. procumbens* has been demonstrated to stimulate both humoral as well as cell mediated immune system vis-a-vis assists in genesis of improved antibody response against specific clinical antigen (Tiwari et al., 2004). Since trypanosome infection causes immunosuppression, any herbal preparation that
is immune boosting may have a significant effect on trypanosomes. Infection with trypanosomes has been known to impair the immune system of the host, cause anaemia, weight loss, reproductive disorders and death of animals if not treated (Olukenle et al., 2010). Consequently in the presence of antigen (parasites) in the blood circulation, the administration of T. procumbens therefore may have activated the lymphocytes to increase the effectiveness of antigen clearance by phagocytosis or to secrete various immune effector molecules.

This resulted to low parasitaemia and prolongation of life beyond the untreated control of all this plant extract investigated. The immunostimulative effect of T. procumbens may have occurred in all the extract and methanolic extract recorded highest means survival followed by ethyl acetate, while aqueous extract was the least. Furthermore, the presence of various phytochemicals particularly, flavonoid in this plant could be responsible for the observation recorded. The phytochemical analysis of the methanol and ethyl acetate extract of the plant revealed the presence of flavonoid in high amount in ethyl acetate than methanol extract. An important effect of flavonoids is the scavenging of oxygen-derived free radicals. In vitro experimental systems also showed that flavonoids possess anti-inflammatory, anti allergic, antiviral, and anticarcinogenic properties (Middleton, 1998). Any anticarcinogenic plant could serve as a good source of trypanocide since trypanocide currently in use to treat sleeping sickness are known to have some level of anticancer activities (Barrett and Barrett, 2000).

All the extracts with antitrypanosomal activities are polar and most likely the active compound could therefore be polar in nature. In addition to its reported immuno-modulatory effect, T. procumbens Linn (compositae) is also employed as indigenous medicine for a variety of ailments, including jaundice (Saraf et al., 1991). The plant has been extensively used in traditional medicine as anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and dysentery (Ali et al., 2001). Moreover, it also possesses wound healing activity and promotes hair growth (Saraf et al., 1991). Tridax procumbens is also dispensed as ‘Bhringraj’, which is well known Ayurvedic medicine for liver disorders (Pathak et al., 1991). Antioxidant properties (Ravikumar et al., 2005), have also been reported. In Africa, Nigeria in particular, most of herbal preparations used for the treatment of illnesses is usually a combination of two or more herbs. Combinations of medicinal herbs in medicinal prescriptions may not only affect a balance of active components, but also undergo a mutual synergy which improves efficacy, safety, and minimizes side-effects. The combination that gave highest mean survival was T. procumbens and M. lucida (1:2) treated at 200 mg/kg body weight. When compared with the activity of all the single extracts, the combination at 200 mg/kg body weight gave the best antitrypanosomal effect by having higher prolongation of life. From the result of phytochemical analysis, M. lucida has high amount of alkaloid while T. procumbens is rich in flavonoids. This implies that the synergistic effect of alkaloids present in M. lucida and antinflammatory, antiallergic, antiviral, and anticarcinogenic properties of flavonoids present in T. procumbens could be responsible for the observed activity. Another advantage of this combination studies is that the dose level that gave highest activity was lower than those that gave activity singly. Dosage reductions of each drug combined may reduce the overall toxicity while maintaining good efficacy (Gerardo et al., 2007).

A major problem besetting the chemotherapy of African Trypanosomiasis is parasite resistance to the few available drugs (DeKoning, 2001). One major benefit of combination therapies therefore is that they reduce development of drug resistance, since a pathogen is less likely to have resistance to multiple drugs simultaneously. Drugs that has different mode of action can be combined to achieve desirable effect. In these studies an attempt was made to explore the potentials of combination therapy using T. procumbens and M. lucida leaves extract in different combinations to treat T.b.brucel-infected mice. The only combination that has antitrypanosomal activity from the infected mice was the combination of the methanol extracts of the leaves of the two plants (Figures 12 to 14). Though the mean survival rate was not very high as compared to when treated singly, but there was prolongation of life particularly at 200 mg/kg. This also provided evidence that the combination of plants has some efficacy as practise in tradmedicine. Sometimes combination chemotherapy is used not to cure but to reduce severe symptoms and prolong life.

Conclusion

M. lucida and T. procumbens have trypanostatic effect and prolonged the life of T. b. brucei infected, treated animals. There is synergy in combination of the 2 plants extracts which result in significant prolongation of life than when used singly.

Conflict of Interests

Authors have not declared any conflict of interest.

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