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Comparison of the efficacy and safety of an oral combination of losartan, hydrochlorothiazide and simvastatin against separated components, in hypertensive and dyslipidemic patients

Emilio Exaire-Murad¹, Luis Fernando Paredes-Fernández¹, Jorge Antonio Aldrete-Velasco², Elvira Isabel Nuño-Carral³, Juan Gerardo Reyes García⁴, Francisco Javier Flores-Murrieta⁴,⁵, Miriam del Carmen Carrasco-Portugal⁶ and Noemí Santos-Caballero⁶*

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Hypertension is an important health problem that frequently requires multidrug treatment, whether due to monotherapy failure or in a concomitant manner. This situation has impulsed the development of some fixed dose combination products with the idea of improve the efficacy, the safety or the adherence of the treatment. In this study, the efficacy and safety of a fixed dose combination product composed by losartan, hydrochlorothiazide and simvastatin was compared with the mixture of losartan and hydrochlorothiazide plus simvastatin, in hypertensive and hypercholesterolemia patients. In this double-blinded, randomized and controlled clinical trial, one hundred and forty four (144) hypertensive and hypercholesterolemia patients received a daily capsule with losartan 50 mg, hydrochlorothiazide 12.5 mg and simvastatin 20 mg plus a tablet of placebo; or a daily tablet with losartan 50 mg and hydrochlorothiazide 12.5 mg plus a capsule of simvastatin 20 mg, during 81 days. Both treatments produced similar reductions on blood pressure and low-density lipoprotein-cholesterol levels, and more than 90% of treated patients achieved recommended values in these parameters. It is concluded that the fixed dose combination of losartan, hydrochlorothiazide and simvastatin, is as effective and safe as the fixed dose of losartan and hydrochlorothiazide plus simvastatin, in hypertensive and hypercholesterolemic patients.

Key words: Hydrochlorothiazide, hypercholesterolemia, hypertension, losartan, simvastatin.

INTRODUCTION

Hypertension is an important medical and public health problem, which approximately affects one billion people worldwide (Chobanian et al., 2003). The correlation between a high level of blood pressure with heart attack,
heart failure, stroke and kidney diseases is consistent (Anderson et al., 1991); on this way, the treatment of hypertension to <140/90 mmHg is associated with a decrease in cardiovascular disease complications (Hansson et al., 1998). Although, several drugs, including beta adrenergic blockers, angiotensin converting enzyme inhibitors, calcium channel blockers and angiotensin II receptor blockers are used for reducing blood pressure, the underused thiazide-type diuretics have been considered the basic drugs for the initial treatment of hypertension (Chalmers and Zanchetti, 1996; Psaty et al., 1997; 2003). Anyway, only 30% of hypertensive patients are adequately controlled with the administration of one drug, and two or more drugs are used to control the level of blood pressure in the rest of patients, either as separate prescriptions or in fixed-dose combinations (Sica, 2002).

The combination of losartan plus hydrochlorothiazide is currently approved in some countries as a second line therapy for hypertension, or, inclusive, as initial therapy in severe hypertension cases. On the other hand, the recommendation that a global anti-hypertensive therapy should include other measures including smoking cessation, management of diabetes, lipid lowering products, anti-platelet agents, exercise training, and weight reduction in obese patients (Chobanian et al., 2003), it has impulse the development of some fixed-dose combination products including antihypertensive with other drugs of a different therapeutic activity, which could improve the treatment adherence. In this study, we were interested in the preliminary evaluation of a fixed dose combination product composed by losartan, hydrochlorothiazide and simvastatin, in comparison with the mixture of losartan and hydrochlorothiazide plus simvastatin, in hypertensive and hypercholesterolemic patients.

METHODOLOGY

Subjects

To evaluate the efficacy and safety of oral administration of a fixed-dose combination product composed by losartan, hydrochlorothiazide and simvastatin, in comparison with the mixture of losartan and hydrochlorothiazide plus simvastatin, one hundred and forty four hypercholesterolemic (LDL cholesterol ≥ 130 mg/dL) patients with either untreated grade 1 or 2 essential hypertension or uncontrolled on monotherapy (systolic blood pressure 140 to 179 mm Hg and diastolic blood pressure 90 to 109 mmHg), and 18 to 65 years old, were recruited to perform a phase III, double blinded, randomized and placebo-controlled clinical trial study. Subjects with a history of allergic reactions to thiazide diuretics, angiotensin II receptor blockers or statins, diabetes, pregnant, alcoholism, secondary hypertension, severe hypertension, unstable angina, acute myocardial infarction, hepatic disease or renal insufficiency, were excluded from the study. The protocol was carried out following the recommendations of the latest version of the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. All participants read the protocol, which was approved by the Institutional Research and Ethics Committee, and provided written informed consent for their participation in the study.

Study design

This randomized, double blind placebo-controlled study of 81 days was approved by The General Hospital “Dr. Enrique Cabrera” Review Board. After obtaining written informed consent, patients were instructed to withhold anti-hypertensive or anti-lipemic drug, and were randomly assigned to one of 2 treatments: a night daily tablet with losartan 50 mg and hydrochlorothiazide 12.5 mg and a capsule of simvastatin 20 mg, or a night daily capsule with losartan 50 mg, hydrochlorothiazide12.5 mg and simvastatin 20 mg, plus a tablet of placebo. All investigation products were provided by Landsteiner Scientific, S.A. de C.V., Mexico City. Sample size calculation was estimated to provide 80% power to detect treatment differences in absolute body weight loss with an alpha level of 0.05, and a mean difference of 12.0 mg/dL LDL-cholesterol with a standard deviation of 23.1 mg/dL LDL-cholesterol (Ronceros et al., 2012), being 58 patients, which is the minimum number of patients required in each group. Sample size calculation included an estimation of 25% patient withdrawals. Figure 1 shows the general outline of the trial phase. The patients were seen in six visits during this study of 81± 3 days (visit 1 of selection at day 0, visits 2 to 5 of treatment at days 1, 14, 44 and 74 days, and visit 6 of final revision at day 81, respectively). During all visits, general measurements, including weight, height, waist circumference, body mass index (BMI), respiratory frequency, temperature, heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained. In addition, during the visits 1 and 5 an electrocardiogram of 12 derivations was also performed, and a blood sample was taken for blood chemistry and hematic biometry assays. In each determination, patients upon arrival voided their bladder, wore only a clinical robe and a nude body weight was obtained on a calibrated scale. Height was determined with the patients placed with the heels together; and the buttocks, shoulders and head in contact with the stadiometer. Waist circumference was measured placing a tape in the midway between the top of hip bone and the bottom of ribs and wrapping around the waist at the mark level. Measurements of systolic and diastolic blood pressure were obtained using a mercury sphygmomanometer (mean of three measurements in the right arm in seated position), and blood samples for laboratory assays were obtained at approximately 8 AM after patients fasted overnight.

Data analysis

Baseline characteristics are summarized as number of patients and percentage (%) for categorical variables, and as mean ± standard deviation continuous variables. The analysis was performed on a per-protocol analysis. The underlying assumption of the statistical analysis was that all variables had a normal probability of distribution according to Shapiro-Wilk’s test. Values for baseline blood pressure and blood pressure reduction from baseline to 1, 14, 44, 74 and 81 days are presented as means of systolic and diastolic pressure ± standard deviation (mmHg).

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RESULTS

Demographic data

A comparison of the main demographic data is shown in Table 1. Patients of both groups resulted equilibrated regarding sex, age, weight, waist circumference, body mass index (BMI), respiratory frequency, temperature, heart rate, diastolic blood pressure (DBP) and systolic blood pressure (SBP) variables. There were no violations to the protocol that may have interfered with the study variables. Four patients were withdrawn from the study; in the losartan-hydrochlorothiazide plus simvastatin group (LH-S), one of them was withdrawn because of lack of treatment efficacy and two due to poor treatment adherence whereas in the losartan-simvastatin-hydrochlorothiazide group (LSH) one patient was withdrawn due to poor adherence treatment. 54.16% of population had a complete treatment adherence (100%), 42.36% had a very good adherence (90 to 99%), 0.69% had a good adherence (80 to 89%), and only 2.77% had a poor treatment adherence (< 80%).

Anti-hypertensive effect

The time-course of mean SBP and DBP values are shown in Figure 2. Baseline SBP/DBP values for (LH-S) group corresponded to 147.5±6.0 / 95.8±3.77 mmHg and 148.2±6.0 / 96.0±3.21 mmHg for LSH group. Both treatments produced significant reductions of both SBP and DBP values in a gradual and similar manner, from the second to the sixth visit (P < 0.0001). There were no significant differences between treatments at any time. At the sixth visit, approximately after 12 weeks, 64/69 (92.8%) patients of LH-S group and 67/71 (94.3%) patients of LSH group achieved an adequate blood pressure control (SBP < 140 mm Hg / DBP < 90 mmHg).

Low-density lipoprotein cholesterol (LDL-C) lowering effect

The LDL-C level changes of patients at visits 1 and 6 are shown in Figure 3. All patients had a baseline LDL-C value lesser than 190 mg/dL. Patients of LH-S group had a reduction from 144.8 ± 24.8 mg/dL to 97.3 ± 24.5 (P < 0.0001), while patients of LSH group had a significant statistically reduction from 143.2 ± 24.1 to 101.2 ± 24.7 (P < 0.0001). On the same way, 61/69 patients of LH-S group vs 64/71 patients of LSH group achieved the recommended level of LDL-C < 130 mg/dL. Additionally, the mean atherogenic index LDL-C/HDL-C was reduced from 4.68±1.0 to 3.85 ± 0.94 in patients of LH-S group (P <0.0001) and from 4.67 ± 0.76 to 3.9 ± 0.85 in patients of

Likewise, values for baseline LDL-cholesterol and end LDL-cholesterol are presented as means ± standard deviation (mg/dL). Statistical analysis of the time-course of blood pressure and LDL-cholesterol values for both treatments was performed by repeated measures analysis of variance followed by the Tukey’s test. Blood pressure control in each treatment (<140/90 mmHg) was summarized as numbers of patients and percentages (%). Potential differences of demographic data between groups were assessed by the Student t-test or χ2-test. In addition, a paired t-Student test was used to assess significant changes in the laboratory parameters from baseline to 81 days. Patients with less than 80% of treatment adherence were not included in the analysis. Differences were considered statistically significant when P was <0.05. Data were analyzed using the statistical software statistical package for the social sciences (SPSS), version 20.0.

Table 1. Baseline characteristics of hypertensive and dyslipidemic patients eligible to receive a daily capsule of losartan / hydrochlorothiazide / simvastatin and a placebo tablet (LSH), or a tablet of losartan / hydrochlorothiazide and a capsule of simvastatin (LH-S).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LH (n=72)</th>
<th>LSH (n=72)</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)*</td>
<td>29/43</td>
<td>29/43</td>
<td>0.7340</td>
</tr>
<tr>
<td>Age (years)**</td>
<td>47.1±9.4</td>
<td>46.7±9.5</td>
<td>0.8377</td>
</tr>
<tr>
<td>Height (cm)**</td>
<td>162.7±8.2</td>
<td>163.2±9.3</td>
<td>0.7678</td>
</tr>
<tr>
<td>Weight (kg)**</td>
<td>76.3±12.3</td>
<td>78.9±16.1</td>
<td>0.2715</td>
</tr>
<tr>
<td>Body mass index (kg/m²)**</td>
<td>28.8±4.2</td>
<td>29.7±5.9</td>
<td>0.3240</td>
</tr>
<tr>
<td>Waist circumference (cm)**</td>
<td>94.7±11.6</td>
<td>96.0±11.8</td>
<td>0.4810</td>
</tr>
<tr>
<td>Heart rate (beats per minute)**</td>
<td>72.1±7.1</td>
<td>72.1±8.0</td>
<td>0.9913</td>
</tr>
<tr>
<td>Respiratory frequency (breaths per minute)**</td>
<td>19.5±2.2</td>
<td>19.7±2.4</td>
<td>0.6870</td>
</tr>
<tr>
<td>Temperature (°C)**</td>
<td>36.5±0.2</td>
<td>36.5±0.2</td>
<td>0.7338</td>
</tr>
<tr>
<td>Systolic arterial pressure (mmHg)**</td>
<td>147.6±5.5</td>
<td>148.3±6.1</td>
<td>0.4464</td>
</tr>
<tr>
<td>Diastolic arterial pressure (mmHg)**</td>
<td>96.1±4.1</td>
<td>96.6±3.4</td>
<td>0.4011</td>
</tr>
<tr>
<td>LDL-Cholesterol (mg/dL)**</td>
<td>144.9±24.8</td>
<td>143.3±24.2</td>
<td>0.6988</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. There were no significant differences between studied groups by *χ² or **t-Student test.
Figure 1. General outline of the study. LH-S = fixed dose combination of losartan and hydrochlorothiazide plus simvastatin. LSH = fixed dose combination of losartan, simvastatin and hydrochlorothiazide.

Figure 2. Mean of plasmatic concentrations of low density lipoprotein – cholesterol (LDL-C) ± standard deviation, in hypertensive and dyslipidemic patients treated with a tablet of a fixed dose combination of losartan and hydrochlorothiazide plus a capsule of simvastatin (LH+S, closed circles) or a capsule with a fixed dose combination of losartan, simvastatin and hydrochlorothiazide and a tablet of placebo (LSH, open circles). *Significantly different from baseline values (P<0.0001), as was determined by two-way analysis of variance with repeated measures, followed by the Tukey’s test.

LSH group (P <0.0001).

Adverse events

The frequency of adverse event was similar for both groups. Twenty four patients reported adverse events; 14 from LH-S group and 10 from LSH group. The most frequent adverse events were mild and consisted of tonsillitis, headache and abdominal pain (4, 1, and 0, for LH-S group and 2, 4, 2 for LSH group respectively). After 81 days treatment, both treatments significantly improved total cholesterol, LDL-cholesterol and atherogenic index, as well as creatinine and uric acid levels, and lactate dehydrogenase activity. There were no other clinically significant changes in the laboratory parameters. More
Figure 3. Mean of systolic blood pressure (SBP, panel A) and diastolic blood pressure (DBP, Panel B) values ± standard deviation, in hypertensive and dyslipidemic patients treated with a tablet of a fixed dose combination of losartan and hydrochlorothiazide plus a capsule of simvastatin (LH-S, closed circles) or a capsule with a fixed dose combination of losartan, simvastatin and hydrochlorothiazide plus a tablet of placebo (LSH, open circles). *Significantly different from baseline values (P<0.0001), as was determined by two-way analysis of variance with repeated measures, followed by the Tukey’s test.

details are shown in the Table 2.

DISCUSSION

Interest in using fixed-dose combination therapy has been particularly increased in hypertension, including those cases of patients with concurrent medical problems such as hyperlipidemia, diabetes and renal disease. The combination therapy looks for the additive or synergistic effect of two or more drugs with different mechanisms of action, mainly in the case of the fixed-dose combinations
Table 2. Baseline and final metabolic parameters measured in hypertensive and dyslipidemic patients that received a daily capsule of losartan / hydrochlorothiazide / simvastatin and a placebo tablet (LSH), or a tablet of losartan / hydrochlorothiazide and a capsule of simvastatin (LH-S) during 81 days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LHS</th>
<th>P-value</th>
<th>LSH</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td></td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>87.6±10.89</td>
<td>0.1582</td>
<td>87.14±12.11</td>
<td>0.0977</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>212.30±30.08</td>
<td>&lt;0.0001</td>
<td>212.17±25.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol-LDL (mg/dL)</td>
<td>144.85±24.83</td>
<td>&lt;0.0001</td>
<td>143.26±24.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol-HDL (mg/dL)</td>
<td>47.39±12.05</td>
<td>0.2402</td>
<td>46.57±9.54</td>
<td>0.6483</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>163.03±56.56</td>
<td>0.0076</td>
<td>173.29±53.32</td>
<td>0.1463</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>4.68±1.00</td>
<td>&lt;0.0001</td>
<td>4.67±0.76</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>29.68±7.48</td>
<td>0.6844</td>
<td>28.29±6.62</td>
<td>0.5363</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.33±1.22</td>
<td>0.0004</td>
<td>5.35±1.43</td>
<td>0.0011</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.86±0.16</td>
<td>&lt;0.0001</td>
<td>0.88±0.15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lactate dehydrogenase (UI/L)</td>
<td>185.74±32.51</td>
<td>0.0004</td>
<td>187.08±32.68</td>
<td>0.0001</td>
</tr>
<tr>
<td>Alkaline phosphatase (UI/L)</td>
<td>89.78±26.14</td>
<td>0.0074</td>
<td>94.79±24.47</td>
<td>0.9707</td>
</tr>
<tr>
<td>Aspartate transaminase (UI/L)</td>
<td>24.64±11.78</td>
<td>0.2950</td>
<td>27.86±18.92</td>
<td>0.1257</td>
</tr>
<tr>
<td>Alanine transaminase (UI/L)</td>
<td>31.35±21.53</td>
<td>0.5896</td>
<td>38.07±40.27</td>
<td>0.1809</td>
</tr>
<tr>
<td>Creatine phosphokinase (UI/L)</td>
<td>116.57±57.32</td>
<td>0.1495</td>
<td>123.26±77.73</td>
<td>0.4590</td>
</tr>
<tr>
<td>K⁺ (mEq/L)</td>
<td>4.43±0.41</td>
<td>0.1573</td>
<td>4.47±0.39</td>
<td>0.6121</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. Differences were considered statistically significant when P < 0.05 by paired Student test.

(Sica, 2002; Barrios et al., 2008). In this preliminary study, it was found that daily administration of losartan (antagonist of the AT₁ receptor) 50 mg, hydrochlorothiazide (blocker of Na⁺/Cl⁻ co-transporter) 12.5 mg and simvastatin (inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase) 20 mg to hypercholesterolemic and hypertensive patients, either as a fixed-dose combination of losartan, simvastatin and hydrochlorothiazide plus placebo, or as a fixed dose combination of losartan and hydrochlorothiazide plus simvastatin, was able to reduce both the blood pressure and the LDL-C level in a similar way. This study agree with previous observations about the efficacy of the combination of losartan 50 mg and hydrochlorothiazide 12.5 mg daily, which produced an excellent or good antihypertensive response, as initial therapy, in 78% of patients (MacKay et al., 1996).

Additionally, the result of the study are in line with previous studies where the combination of losartan 50 mg with hydrochlorothiazide 12.5 or 25 mg once daily resulted consistently more effective than losartan (25, 50 or 100 mg) or hydrochlorothiazide (6.25 mg, 12.5 mg or 25 mg) alone in the treatment of mild to severe essential hypertension (Soffer et al., 1995; McKay et al., 1996; Ruilope et al., 1996; Owens et al., 2000). In the same way, the fixed high-dose combination of 100 mg losartan and 25 mg hydrochlorothiazide once daily was found to have sustained antihypertensive effectiveness over a 24 hour period, in patients with severe (n=9; 180/110 mmHg or higher) essential hypertension (Coca et al., 2002). These results shows that the multidrug therapy in hypertension with drugs with complementary mechanism of action increase the efficacy of each drug against either as monotherapy. Actually, in some countries the combination of losartan and hydrochlorothiazide is approved for the treatment of hypertension as a second line therapy, or as initial therapy for severe hypertension.

On the other hand, based on the idea that angiotensin II is very potent endogenous vasoconstrictor, whereas LDL induces up-regulation of the AT1 receptor (Nickenig et al., 1997). A comparative study was performed in patients with hypertension and hypercholesterolemia, who received simvastatin 20 mg or losartan 20 mg alone or in combination, in this study, individual drugs produced the expected anti-hypertensive or hypocholesterolemic effect, but the mixture significantly improved the effect of individual drugs on endothelial function and inflammatory markers, such as plasma malondialdehyde, C-reactive protein and monocyte chemoattractant protein-1 levels, which suggest that the fixed combination of simvastatin-losartan could reduce cardiovascular events in hypercholesterolemic and hypertensive patients more than monotherapy with either drug alone (Kwang et al., 2004). Although, the current study focused on the preliminary comparison of a fixed-dose combination of losartan, hydrochlorothiazide and simvastatin versus the mixture of a fixed-dose combination of losartan and hydrochlorothiazide plus simvastatin, remarkable levels of effectiveness in terms of an adequate blood pressure control and a recommended level of LDL-C, in more than 90% of hypertensive and hypercholesterolemic patients were found.
One of the objectives of a fixed-dose combination is the attenuation of adverse events induced by single agents, both clinic and metabolic. In this study, the frequency of adverse event was similar for both groups being tonsillitis, headache and abdominal pain the more frequent adverse effects. All of them were mild and transient, and they were consistent with those seen with losartan, hydrochlorothiazide or simvastatin as monotherapy. Moreover, there were no new adverse events due to the combination. Both treatments were well-tolerated since both treatments improved significantly lipid profile, creatinine and uric acid levels, and there were no other clinically significant changes in the laboratory parameters. Tolerability and safety of the fixed-dose combination of losartan, hydrochlorothiazide and simvastatin or the mixture of a fixed-dose combination of losartan and hydrochlorothiazide plus simvastatin could be explained on the following basis: there is no clinically significant pharmacokinetic interaction between losartan and hydrochlorothiazide (McCrea et al., 1995); there seems no identified significant pharmacokinetic interactions between simvastatin and losartan, which is mainly metabolized by CYP2C6 as irbesartan (Marino and Vachharajani, 2001; the combination of losartan plus simvastatin improve the anti-atherosclerotic effect of each drug as monotherapy (Nomura et al., 2004); losartan may attenuate some of the deleterious metabolic effects of hydrochlorothiazide because losartan may counterbalance the augment of uric acid (Soffer et al., 1995, MacKay et al., 1996) and glucose intolerance (Ruilope et al., 1996) induced by hydrochlorothiazide; while hydrochlorothiazide may reduce the potassium-increased levels induced by losartan (Soffer et al., 1995; Palmer, 2008). In line with the results, a randomized double-blind placebo-controlled crossover trial of a Polypill (containing amlodipine 2.5 mg, losartan 25 mg, hydrochlorothiazide 12.5 mg and simvastatin 40 mg) was performed in individuals aged > 50 years old without a history of cardiovascular disease, the study showed that the fixed-dose combination was effective, safety and well-tolerated (Wald et al., 2012). In general, the use of fixed-dose combinations show a better patient compliance due to simplification of the regimen, reduction of the pill burden and a potential reduction of cost, due to a fixed-dose combination is often less expensive than buying each drug individually and reduces costs associated with cardiovascular events and productivity loss due reduces their incidence. In fact, it is well known that patient compliance is inversely related to the number of drugs being administered. To overcome this problem, several dual and triple-drug, fixed-dose combinations have been developed and marketed, which are easier to administer, and they have been shown to increase patient compliance and adherence to treatment (Ram, 2013). In this regard, there is evidence that show the proven cost-effectiveness and patient compliance of a fixed-dose combination of a statin-like drug plus an anti-hypertensive drug (Delgado-Montero and Zamorano, 2012) or an AT1-receptor antagonist plus a thiazide diuretic (Coca, 2008). Moreover, the fixed-dose losartan/hydrochlorothiazide is more cost-effective than candesartan/amlodipine (Shimosawa et al., 2007), and similar to other fixed-dose AT1-receptor antagonists/hydrochlorothiazide combinations (Ekman et al., 2008). Currently, a study demonstrating the cost-effectiveness of the current fixed-dose combination is lacking, but 97.2 and 98% patients had good treatment adherence in this study and in the study of Wald et al. (2012), respectively, since they took more than 80% of their allocated pills.

On the other hand, the fixed-dose combinations have also disadvantages as the loss of dose flexibility or the difficulty in identifying the active ingredient responsible for adverse reaction in the fixed-dose combination. In the former disadvantage, the fixed-dose of losartan 50 mg/hydrochlorothiazide 12.5 mg/simvastatin 20 mg could be only increased twice daily in patients unable to achieve LDL-C goal and blood pressure control according to recommended dose for hypertension and dyslipidemia by Food and Drug Administration (Hsu et al., 1995; Ripley and Hirsch, 2010; Palmer, 2011). However, for those who has blood pressure control but not LDL-C goal or vice versa, should consider alternative therapy. With respect to the difficulty in identifying the active ingredient responsible of an adverse reaction, the three drugs that belong to the fixed-dose losartan/hydrochlorothiazide/simvastatin combination are well tolerated and its more common adverse events are mild to moderate and transient, so they do not have clinic relevance.

However, it has been reported that some uncommon adverse events with each drug should be taken in account for the control patient. Losartan induces uricosuric and hyperkalemic effects (Ripley and Hirsch, 2010), while thiazide diuretics as hydrochlorothiazide can provoke hyperglycaemia and diabetes, as well as hypokalemia and hyperuricaemia (Palmer, 2011). Simvastatin, as other statins, has been associated with rare cases of severe myopathy and rhabdomyolysis, which is accompanied by elevations in creatine kinase, as well as renal failure (Alonso et al., 2005). In this fixed-dose combination, some adverse events are counterbalanced as earlier stated, but patients should be monitored for creatine kinase activity, as well as for creatinine, uric acid, glucose and K+ levels. Finally, this problem might be alleviated by starting the medications individually and monitoring for reactions, and then switching to a fixed-dose combination when no problems have been observed.

**Conclusion**

The results show that fixed dose combination of losartan,
hydrochlorothiazide and simvastatin is as effective and safe as the fixed-dose combination of losartan and hydrochlorothiazide, plus simvastatin, in the treatment of mild and moderate essential hypertension and hypercholesterolemia. This combination could be used as a substitute therapy in the treatment of hypertensive and hypercholesterolemic patients already treated with the association of losartan, hydrochlorothiazide and simvastatin at the same doses, in whom a better compliance is desirable.

AKNOWLEDGMENTS

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Conflict of interest

The authors declare that they have no conflicts of interest.

REFERENCES


Full Length Research Paper

Genotoxicity and anti-genotoxicity of aqueous extracts of herbal recipes containing *Luffa cylindrica* (L), *Nymphaea lotus* (L) and *Spondias mombin* (L) using the *Allium cepa* (L) assay

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Management of diseases with medicinal plant is an ancient practice that has improved in recent years. Extracts of *Luffa cylindrica* (Linn), *Nymphaea lotus* (Linn) and *Spondias mombin* (Linn) are used for traditional management of cancer in Nigeria. Four recipes prepared from the combinations of two or three of these plants; *L. cylindrica, N. lotus* and *S. mombin* (LNS), *N. lotus* and *S. mombin* (NS), *N. lotus* and *L. cylindrica* (NL), and *S. mombin* and *L. cylindrica* (SL), were evaluated for genotoxicity and anti-genotoxicity using the *Allium cepa* chromosome aberration and root growth inhibition assay. Five concentrations (1, 2.5, 5, 10 and 20%) of each recipe were considered. There was a concentration-dependent inhibition of root growth and reduction of mitotic index in each of the recipe compared with the negative control. All the recipes induced chromosomal aberrations but not significant (p˂0.05) at tested concentrations. The extract of LNS reduced the frequency of chromosomal anomalies induced by lead nitrate. These show the potential of tested extracts to induce and ameliorate cytogenetic damage in *Allium cepa*.

Keywords: Medicinal plant, recipe, mitotic index, chromosomal aberration, *Allium cepa*.

INTRODUCTION

The increasing use of medicinal herbs is a clear evidence of public interest in having alternatives to conventional medicine. However, despite the profound therapeutic advantages possessed by medicinal plants, some of their constituents have been found to be potentially toxic, mutagenic, carcinogenic and teratogenic (Ping et al., 2012). Long-term use of herbs to treat or manage diseases can induce cellular damages (Oyedare et al., 2009) and thus increase the side effects and potential toxicity of the medicinal plants, hence, the need to assess their potential toxicity. *Luffa cylindrica, Nymphaea lotus* and *Spondias mombin* are medicinal plants commonly used in Nigeria in the traditional management of cancer. They have been reported to have great medicinal value,
with treatment of intestinal disorders, antioxidant, anti-inflammatory, antiviral, antiparasitic, antibacterial, antimicrobial, antipatotoxic, and antihelminthic activities (Ajao and Shonukan, 1985; Corthout et al., 1994; Ademola et al., 2005; Muthumani et al., 2010; Igwe et al., 2011; Velmurugan et al., 2011). Herbal preparations in Nigeria are mostly made as a recipe. It is generally believed that herbs, when used in combination are more active than when used individually. In a previous study (Oyeyemi and Bakare, 2013), we evaluated the genotoxic and antigenotoxic effects of aqueous extracts of \textit{L. cylindrica}, \textit{N. lotus} and \textit{S. mombin} in \textit{Allium cepa}. Herein, we investigated the cytogenotoxicity of aqueous extracts of four recipes containing two or three of these plants using the \textit{Allium cepa} assay. In addition, we also evaluated the anti-genotoxic effects of the recipe having a combination of the three plants.

**MATERIALS AND METHODS**

**Collection and Identification of plants**

The leaves of \textit{S. mombin}, whole plants of \textit{N. lotus} and fruits of \textit{L. cylindrica} were collected within the premises of the University of Ibadan, Nigeria and then taken to the University of Ibadan herbarium for authentication where voucher specimens (\textit{S. mombin} UIH-22350, \textit{N. lotus} UIH-22349, \textit{L. cylindrica} UIH-22348) were deposited. The leaves and whole plants were washed with tap water, shade dried, ground and stored in the dark while the fruits were washed with tap water and used fresh.

**Preparation of extracts**

Four different recipes were prepared from these plants using the following combinations:

2. \textit{N. lotus} and \textit{S. mombin} (NS).
4. \textit{N. lotus} and \textit{L. cylindrica} (NL).

For LNS, 15 g each of ground \textit{S. mombin} and \textit{N. lotus} and 20 g of \textit{L. cylindrica} were boiled in 1 L of tap water. For NS, NL and SL, 25 g each of the combined plant materials were boiled in 1 L of tap water. The resultant mixture from each combination was filtered using Whatman® no.1 (11 μm) filter paper and the filtrate of each preparation taken as the stock solution was kept at 4°C until use.

**Allium cepa assay**

Onions (\textit{Allium cepa}, L., 2n = 16, Family \textit{Amaryllidaceae}) obtained commercially in Ibadan, Nigeria, were sun-dried for 2 weeks and used in the modified \textit{A. cepa} assay (Fiskesjo, 1997; Bakare et al., 2009) to evaluate the potential genotoxic effects of the recipes and anti-genotoxic effects of LNS only. Twelve onion bulbs were used per concentration of each of the test samples. Five concentrations (v/v): 1, 2.5, 5, 10 and 20% of each recipe were used. Tap water and lead nitrate (10 ppm) solution were utilized as the negative and positive control, respectively.

For the genotoxicity study, a series of 12 bulbs were placed on top of 100 ml beakers filled with the different concentrations of each of the recipe (85 to 100 ml of each of the recipe depending on the size and placement of the onion on the beaker) and incubated in the dark at room temperature for 72 h with the test samples being changed at 24 h interval. The same number of bulbs and treatment were used for the controls. At 48 h, the meristematic region of the roots from 2 bulbs was cut and processed for slide preparation. In the antigenotoxicity study, a series of 12 bulbs were placed on top of beakers filled with lead nitrate (10 ppm) solution for 24 h. After the lead nitrate treatment, the bulbs were treated with five different concentrations of LNS for 48 h and incubated in the dark at room temperature. At 24 h of treatment with LNS, the meristematic region of the roots from 2 bulbs was cut and processed for slide preparation.

**Root growth and cytogenetic analysis**

In the genotoxicity and anti-genotoxicity studies, the length of the roots of the remaining 10 onion bulbs at each concentration were measured (in cm) at 72 h and used as an index of general toxicity (root growth inhibition). From the weighted averages for each concentration, the percentage root growth inhibition in relation to the negative control and the EC$_{50}$ for each extract was determined (Fiskesjo, 1985). The American Society for Testing and Materials (ASTM, 1994) minimal statistical guidelines for conducting early seedling growth tests were used in the analysis of measured root length.

For the slide preparation, the cut root tips were fixed in ethanol: glacial acetic acid (3:1, v/v). After which the roots were hydrolyzed in 1 N HCl at 60°C for 5 min and then washed thrice in distilled water. Root tips (2 to 3) were squashed on each slide and stained with aceticarmine for 10 min. Six slides were prepared for each concentration out of which four were used for microscopic observation at 1000× magnification (4000 cells were observed per concentration). Chromosomal aberrations were characterized and classified. The mitotic index was calculated as the number of dividing cells per total cells scored at each concentration. The frequency of aberrant cells (%) was calculated based on the number of aberrant cells per total cells scored at each concentration of the extract (Bakare et al., 2009).

**Statistical analysis**

The SPSS 17.0® software package was utilized. Data on root length, mitotic index and chromosomal aberrations were compared using analysis of variance (ANOVA) followed by Dunnett test (p<0.05). The EC$_{50}$ was determined from the root length data using Probit regression analysis. Correlation between root length and mitotic index was determined using Pearson correlation coefficient.

**RESULTS**

The result of the root length parameters is presented in Table 1. There was a marked inhibition of root growth at all tested concentrations for all the recipes. The inhibition of root growth was concentration dependent for all the recipes except for NL. The EC$_{50}$ values obtained are 12.4, 12.2, 24.8 21.8 and 13.9% for LNS, NS, SL and LNS+ lead nitrate respectively. The results of microscopic effects are summarized in Tables 2 to 6. A decrease in the mitotic index (MI) value was observed at the tested concentrations of each of the recipe. The MI was positively correlated (LNS, r = 0.43; NS, r = 0.15; SL, r =
Table 1. Effect of different combinations of aqueous extracts of Spondias mombin, Nymphaea lotus and Luffa cylindrica with or without Lead nitrate on root growth of Allium cepa.

<table>
<thead>
<tr>
<th>Conc. (%)</th>
<th>LNS</th>
<th>NS</th>
<th>SL</th>
<th>NL</th>
<th>LNS+Pb(NO3)2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Growth in % of control</td>
<td>Mean±SD</td>
<td>Growth in % of control</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>NC</td>
<td>4.32±0.44</td>
<td>100.0</td>
<td>4.32±0.44</td>
<td>100.0</td>
<td>4.32±0.44</td>
</tr>
<tr>
<td>1.0</td>
<td>4.03±0.81</td>
<td>93.3</td>
<td>3.65±0.47*</td>
<td>84.5</td>
<td>4.69±0.36</td>
</tr>
<tr>
<td>2.5</td>
<td>4.11±0.65</td>
<td>95.1</td>
<td>3.24±0.53*</td>
<td>75.0</td>
<td>4.25±0.60</td>
</tr>
<tr>
<td>5.0</td>
<td>2.75±0.28*</td>
<td>63.7</td>
<td>2.53±0.48*</td>
<td>58.6</td>
<td>4.23±0.76</td>
</tr>
<tr>
<td>10.0</td>
<td>1.67±0.24*</td>
<td>32.7</td>
<td>1.86±0.41*</td>
<td>43.1</td>
<td>3.02±0.40*</td>
</tr>
<tr>
<td>20.0</td>
<td>1.42±0.13*</td>
<td>10.2</td>
<td>1.57±0.19*</td>
<td>36.3</td>
<td>3.17±0.58*</td>
</tr>
<tr>
<td>PC</td>
<td>3.50±0.70</td>
<td>107.6</td>
<td>4.65±0.70</td>
<td>107.6</td>
<td>4.65±0.70</td>
</tr>
</tbody>
</table>

EC50 (%) 12.4 12.2 24.8 21.8 13.9

*Values are significant compared to the negative control (tap water) at p<0.05. LNS = combination of extracts of L. cylindrica, N. lotus and S. mombin. NS = combination of extracts of N. lotus and S. mombin. SL = combination of extracts of S. mombin and L. cylindrica. NL = combination of extracts of N. lotus and L. cylindrica. NC = Negative control (tap water). PC = Positive control (10 ppm Lead nitrate).

Table 2. Cytological effects of the combination of aqueous extracts of Spondias mombin, Nymphaea lotus and Luffa cylindrica (LNS) on Allium cepa cells

<table>
<thead>
<tr>
<th>Conc. (%)</th>
<th>No of dividing cells</th>
<th>MI</th>
<th>Disturbed spindle</th>
<th>Sticky chromosome</th>
<th>Distributed metaphase</th>
<th>Chromosome lag</th>
<th>C-mitosis</th>
<th>Bi-metaphase cells</th>
<th>Anaphase bridge</th>
<th>Total aberration</th>
<th>Frequency of aberration (%)/total cells scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>263</td>
<td>66</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td>1.0</td>
<td>234</td>
<td>59</td>
<td>8</td>
<td>8</td>
<td>22</td>
<td>23</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>137</td>
<td>3.45±0.26*</td>
</tr>
<tr>
<td>2.5</td>
<td>145</td>
<td>36*</td>
<td>42</td>
<td>2</td>
<td>20</td>
<td>9</td>
<td>27</td>
<td>4</td>
<td>3</td>
<td>122</td>
<td>3.05±0.51*</td>
</tr>
<tr>
<td>5.0</td>
<td>197</td>
<td>49*</td>
<td>57</td>
<td>20</td>
<td>9</td>
<td>27</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>124</td>
<td>3.10±1.70*</td>
</tr>
<tr>
<td>10.0</td>
<td>191</td>
<td>48*</td>
<td>62</td>
<td>11</td>
<td>16</td>
<td>25</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>124</td>
<td>3.10±1.70*</td>
</tr>
<tr>
<td>20.0</td>
<td>144</td>
<td>36*</td>
<td>34</td>
<td>5</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>-</td>
<td>2</td>
<td>68</td>
<td>1.70±0.51*</td>
</tr>
<tr>
<td>PC</td>
<td>172</td>
<td>43*</td>
<td>31</td>
<td>20</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>56</td>
<td>1.40±0.41*</td>
</tr>
</tbody>
</table>

*Values are significant compared to the negative control (tap water) at p<0.05. MI = mitotic index NC = Negative control (tap water) PC = Positive control (10 ppm Lead nitrate)

Table 3. Cytological effects of the combination of aqueous extracts of Spondias mombin and Nymphaea lotus (NS) on Allium cepa cell.

<table>
<thead>
<tr>
<th>Conc. (%)</th>
<th>No of dividing cells</th>
<th>MI</th>
<th>Disturbed spindle</th>
<th>Sticky chromosome</th>
<th>Distributed metaphase</th>
<th>Chromosome lag</th>
<th>C-mitosis</th>
<th>Bi-metaphase cells</th>
<th>Anaphase bridge</th>
<th>Total aberration</th>
<th>Frequency of aberration (%)/total cells scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>263</td>
<td>66</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td>1.0</td>
<td>283</td>
<td>71</td>
<td>5</td>
<td>7</td>
<td>8</td>
<td>27</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>93</td>
<td>2.33±0.45*</td>
</tr>
<tr>
<td>2.5</td>
<td>269</td>
<td>67</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>106</td>
<td>2.65±0.51*</td>
</tr>
<tr>
<td>5.0</td>
<td>228</td>
<td>57</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>24</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>91</td>
<td>2.28±0.57*</td>
</tr>
<tr>
<td>10.0</td>
<td>140</td>
<td>35*</td>
<td>33</td>
<td>16</td>
<td>15</td>
<td>29</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>94</td>
<td>2.35±0.65*</td>
</tr>
<tr>
<td>20.0</td>
<td>91</td>
<td>23</td>
<td>27</td>
<td>10</td>
<td>4</td>
<td>14</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>96</td>
<td>1.40±0.50*</td>
</tr>
<tr>
<td>PC</td>
<td>172</td>
<td>43*</td>
<td>31</td>
<td>20</td>
<td>3</td>
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<td>1</td>
<td>2</td>
<td>-</td>
<td>56</td>
<td>1.40±0.41*</td>
</tr>
</tbody>
</table>

*Values are significant compared to the negative control (tap water) at p<0.05. MI: Mitotic index NC = Negative control (tap water). PC = Positive control (10 ppm Lead nitrate)
Table 4. Cytological effects of the combination of aqueous extracts of *Spondias mombin* and *Luffa cylindrica* (SL) on *Allium cepa* cells.

<table>
<thead>
<tr>
<th>Conc.(%)</th>
<th>No of dividing cells</th>
<th>MI</th>
<th>Disturbed spindle</th>
<th>Sticky chromosome</th>
<th>Distributed metaphase</th>
<th>Chromosome lag</th>
<th>C-mitosis</th>
<th>Binucleated cell</th>
<th>Total aberration</th>
<th>Frequency of aberration (%) total cells scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>263</td>
<td>66</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>27</td>
<td>0.68±0.41</td>
</tr>
<tr>
<td>1</td>
<td>132</td>
<td>33'</td>
<td>13</td>
<td>14</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>33</td>
<td>0.83±0.83</td>
</tr>
<tr>
<td>2.5</td>
<td>240</td>
<td>60</td>
<td>30</td>
<td>29</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>65</td>
<td>1.63±0.71*</td>
</tr>
<tr>
<td>5</td>
<td>188</td>
<td>47'</td>
<td>26</td>
<td>20</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>51</td>
<td>1.28±0.43*</td>
</tr>
<tr>
<td>10</td>
<td>162</td>
<td>41'</td>
<td>17</td>
<td>17</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>34</td>
<td>0.85±0.21</td>
</tr>
<tr>
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<td>53'</td>
<td>17</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>32</td>
<td>0.80±0.22</td>
</tr>
<tr>
<td>PC</td>
<td>172</td>
<td>43'</td>
<td>31</td>
<td>20</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>56</td>
<td>1.40±0.41*</td>
</tr>
</tbody>
</table>

*Values are significant compared to the negative control (tap water) at p<0.05. MI: Mitotic index NC = Negative control (tap water). PC = Positive control (10 ppm Lead nitrate).

Table 5. Cytological effects of the combination of aqueous extract of *Nymphaea lotus* and *Luffa cylindrica* (NL) on *Allium cepa* cells.

<table>
<thead>
<tr>
<th>Conc.(%)</th>
<th>No of dividing cells</th>
<th>MI</th>
<th>Disturbed spindle</th>
<th>Sticky chromosome</th>
<th>Distributed metaphase</th>
<th>Chromosome lag</th>
<th>C-mitosis</th>
<th>Bi-metaphase cells</th>
<th>Anaphase bridge</th>
<th>Total aberration</th>
<th>Frequency of aberration (%) total cells scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>263</td>
<td>66</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>27</td>
<td>0.68±0.41</td>
</tr>
<tr>
<td>1</td>
<td>121</td>
<td>30'</td>
<td>6</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>18</td>
<td>0.45±0.13</td>
</tr>
<tr>
<td>2.5</td>
<td>134</td>
<td>34'</td>
<td>15</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>24</td>
<td>0.60±0.32</td>
</tr>
<tr>
<td>5</td>
<td>143</td>
<td>36'</td>
<td>10</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>18</td>
<td>0.45±0.33</td>
</tr>
<tr>
<td>10</td>
<td>111</td>
<td>28'</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>0.25±0.10</td>
</tr>
<tr>
<td>20</td>
<td>85</td>
<td>21'</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>0.50±0.38</td>
</tr>
<tr>
<td>PC</td>
<td>172</td>
<td>43'</td>
<td>31</td>
<td>20</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>56</td>
<td>1.40±0.41*</td>
</tr>
</tbody>
</table>

*Values are significant compared to the negative control (tap water) at p<0.05. MI: Mitotic index NC = Negative control (tap water). PC = Positive control (10 ppm Lead nitrate).

0.90; NL, r = 0.05) to the root lengths. LNS and NS significantly induced chromosomal aberration (Tables 2 and 3), SL induced significant chromosomal aberrations at concentrations 1, 10 and 20% (Table 4), while NL did not induce significant (p<0.05) aberration at any of the tested concentrations (Table 5). In the anti-genotoxicity study, LNS reduced the frequency of chromosomal aberrations induced by lead nitrate to levels not significantly different from the negative control at concentrations 1, 10 and 20% (Table 6). The observed cytological aberrations include disturbed spindle, chromosome lag, sticky chromosome, distributed metaphase, C-mitosis, anaphase bridge, bi-nucleated cells, vagrant chromosome, non-disjunction at anaphase and bi-metaphase cells (Figure 1).

**DISCUSSION**

Medicinal plants have been widely used by both ancient and modern man of all cultures for treating different ailments. A single plant processed in different formulations can be used to cure a wide range of diseases (Adegbite and Sanyaolu, 2009). However, the historic role of medicinal herbs in the treatment and prevention of diseases and in the development of pharmacology do not assume their safety for uncontrolled use by an uninformed public (Mathews et al., 1999). Studies of genotoxicity and anti-genotoxicity can help evaluate the safety
Table 6. Cytological effects of the combination of aqueous extract of *Spondias mombin, Nymphaea lotus* and *Luffa cylindrica* (NLS) on *Allium cepa* cells pretreated with lead nitrate.

<table>
<thead>
<tr>
<th>Conc. (%)</th>
<th>No of dividing cells</th>
<th>MI</th>
<th>Disturbed spindle</th>
<th>Sticky chromosome</th>
<th>Distributed metaphase</th>
<th>Chromosome lag</th>
<th>C-mitosis</th>
<th>Bi-metaphase cells</th>
<th>Anaphase bridge</th>
<th>Total aberration</th>
<th>Frequency of aberration (%)/total cells scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>263</td>
<td>66</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>2</td>
</tr>
<tr>
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<td>4</td>
<td>7</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>57</td>
</tr>
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<td>54</td>
<td>-</td>
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<td>-</td>
<td>1</td>
<td>74</td>
</tr>
<tr>
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<td>242</td>
<td>61</td>
<td>63</td>
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<td>9</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>107</td>
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<td>-</td>
<td>-</td>
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<td>87</td>
<td>2.18±0.16*</td>
</tr>
<tr>
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<td>43</td>
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<td>1</td>
<td>1</td>
<td>-</td>
<td>56</td>
<td>1.40±0.41*</td>
</tr>
</tbody>
</table>

*Values are significant compared to the negative control (tap water) at p<0.05. MI: Mitotic index NC = Negative control (tap water). PC = Positive control (10 ppm Lead nitrate)

**Figure 1.** Chromosomal aberrations (arrowed) induced in *Allium cepa* root tips by the different combinations of aqueous extracts of *Spondias mombin, Nymphaea lotus* and *Luffa cylindrica*. (a, b) sticky chromosomes at metaphase (c) multipolar division (d and e) spindle disturbance at anaphase (f) anaphase bridge (g) vagrant chromosome (h) c-mitosis Magnification: 1000×

and effectiveness of herbal health products (Bast et al., 2002). All the recipes used in this study reduced root growth and were thus mitodepressive. The EC_{50}, in the order NS ≤ LNS < LNS+PbNO3 < NL < SL, showed the cytotoxic nature of each of the recipes. This indicates that one or more, or combination of the phytochemicals present in these recipes is/are toxic to *A. cepa* cells.
Preliminary phytochemical analysis showed the presence of tannins, saponins, sterol, glycosides and resins in *S. monbin*; tannins, saponins and sterols in *N. Lotus*; alkaloids, saponins, sterol and resins in *L. cylindrica* (Oyeyemi and Bakare, 2013). Reduction in root growth could be as a result of alteration in the duration of the mitotic cycle, resulting from direct interaction of the meristematic cells of *A. cepa* root tips with the phytochemicals present in plant materials.

MI is used as an indicator of cell proliferation biomarkers which measures the proportion of cells in the M-phase of the cell cycle (Ping et al., 2012). It is an acceptable measure of cytotoxicity for all living organism (Smaka-Kinel et al., 1996). (MI significantly lower than the negative control indicates alteration in the growth and development of *A. cepa* (Hoshina, 2002); while MI higher than the negative control is as a results of increase in cell division leading to disordered cell proliferation and formation of tumor tissue (Leme and Marin-Morales, 2009). Decrease in MI observed herein is probably due to either chromatin dysfunction or disturbance in the cell cycle induced by the interaction of the phytochemicals with the DNA. This may be due to increase in the period of G2 (Van't Hoff, 1968), complete arrest of mitotic cycle at the G2 as observed by Bruner (1971) or complete halt of metabolic activities preventing the cell to enter mitosis (Metin and Burun, 2010). Mitodepressive effect signifies that these recipes have the ability to block the synthesis of DNA and nucleoproteins (Schulze and Krischer, 1996). This action occurring in the interphase nucleus suggests that the recipes may not even allow the initiation of the biosynthesis of nucleoproteins and DNA (Akinboro and Bakare, 2007). The inhibitory and mitodepressive effects of the recipes may probably be part of the mechanism/mode of actions utilized in the management of cancer traditionally. Evidence in support of this has been documented on some plant extracts with anticancer therapy (Sheng et al., 2000; Kura’s et al., 2007).

Chromosomal aberrations are changes in the structure of chromosomes resulting from breaks or exchange of chromosomal materials (Sultan and Celik, 2009). All the recipes utilized except NL induced chromosomal aberrations (p < 0.05) at one or more concentrations. These did not induce significant aberration at the 20% which was the highest concentration tested. A possible explanation for this is that there was a higher level of cytotoxicity at this concentration thus probably leading to fewer cells dividing, with most of them at prophase stage of cell division. Individually, the aqueous extract of *S. monbin* and *N. lotus* was reported to be non-genotoxic while that of *L. cylindrica* was genotoxic in *A. cepa* (Oyeyemi and Bakare, 2013). Herein, the aqueous extract of LNS (resulting from the combination of the three plants) was observed to be genotoxic; likewise the extract of NS. The actions of herbal products have been known to be due to the combined actions of many types of chemical compounds in the complex mixture (Wang et al., 2009). This suggests that the genotoxicity of LNS and NS in *A. cepa* was as a result of the interaction; synergistic, additive or antagonistic, of the phytochemicals present in the combined plants. Interestingly, the extract of NL was not genotoxic; which may mean that some phytochemicals present in *N. lotus* antagonized the phytochemical(s) responsible for the genotoxicity of *L. cylindrica* or perhaps blocks its binding site.

Most of the observed aberrations were due to spindle failure (such as disturbed spindle and distributed metaphase) which indicates the interaction of the phytochemical constituents of the recipes with the spindle apparatus. Alkaloids have been reported to inhibit mitosis and also bind to tubulin, preventing the formation of the mitotic spindle (Khakdan and Piri, 2012). The presence of alkaloids in the recipes might have contributed to the success acclaimed with these plants in the traditional management of cancer in Nigeria; however they may be genotoxic to normal cells if they are not selective in their mode of action.

Lead nitrate is a potent mutagen which has been reported to be mutagenic in *A. cepa* (Liu et al., 1994), wheat (Truta et al., 2011), mice (Madhavi et al., 2007) and human cultured cells (Yedjou and Tchounwou, 2007). In this study, it induced mostly disturbed spindle and chromosome lag. The extract of LNS showed anti-genotoxic effect at some of the tested concentrations by reducing the frequency of lead nitrate induced cytological aberrations to levels not significantly different from the negative control. It acted as a bio-antimutagen by suppressing the process of lead nitrate induced mutation in *A. cepa* and inhibited further DNA damage probably by inhibiting the binding of free radicals to DNA. The extract also probably induced cell death in cells with lead nitrate induced DNA damage. This was expressed as significant inhibition of cell division in lead nitrate pretreated cells which implies cytotoxic and anti-proliferative effect of the extract. Induction of apoptosis in *A. cepa* cells by plant extract have been previously reported (Celik and Aslanturk, 2010). Human exposure to genotoxic substances present in food and the environment is inevitable. Various genotoxic physical and chemical agents are known to act as mutagenic, co-carcinogenic and/or carcinogenic agents (Mitscher et al., 1986). Consumption of natural antigenotoxic and antimutagenic substances can protect from the effect of these genotoxic substances thereby protecting from life threatening diseases (such as cancer) caused by mutagens. The findings herein suggest that the tested recipes showed potential genotoxic and anti genotoxic activity not exhibited by the individual extract in *A. cepa*. We are currently assessing potential oxidative damage, mutagenic and anti-mutagenic effect of the extracts *in vivo* and *in vitro*. This is expected to provide further information on the mechanism of action of these plants and their recipes in biologic system.
Conclusion

The recipes tested in this study induced chromosomal aberrations which are due to spindle failure. This is a common feature of some orthodox medicine used in chemotherapy such as vincristine and vinblastine. Hence, this may be the mechanism underlying the success acclaimed by traditional healers in using these plants to treat cancer. However, caution should be taken in using these recipes since they may not be selectively mutagenic to cancer cells.

ACKNOWLEDGEMENTS

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Conflict of interest

Authors have none to declare.

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African Journal of Pharmacy and Pharmacology

Related Journals Published by Academic Journals

- Journal of Medicinal Plant Research
- African Journal of Pharmacy and Pharmacology
- Journal of Dentistry and Oral Hygiene
- International Journal of Nursing and Midwifery
- Journal of Parasitology and Vector Biology
- Journal of Pharmacognosy and Phytotherapy
- Journal of Toxicology and Environmental Health Sciences