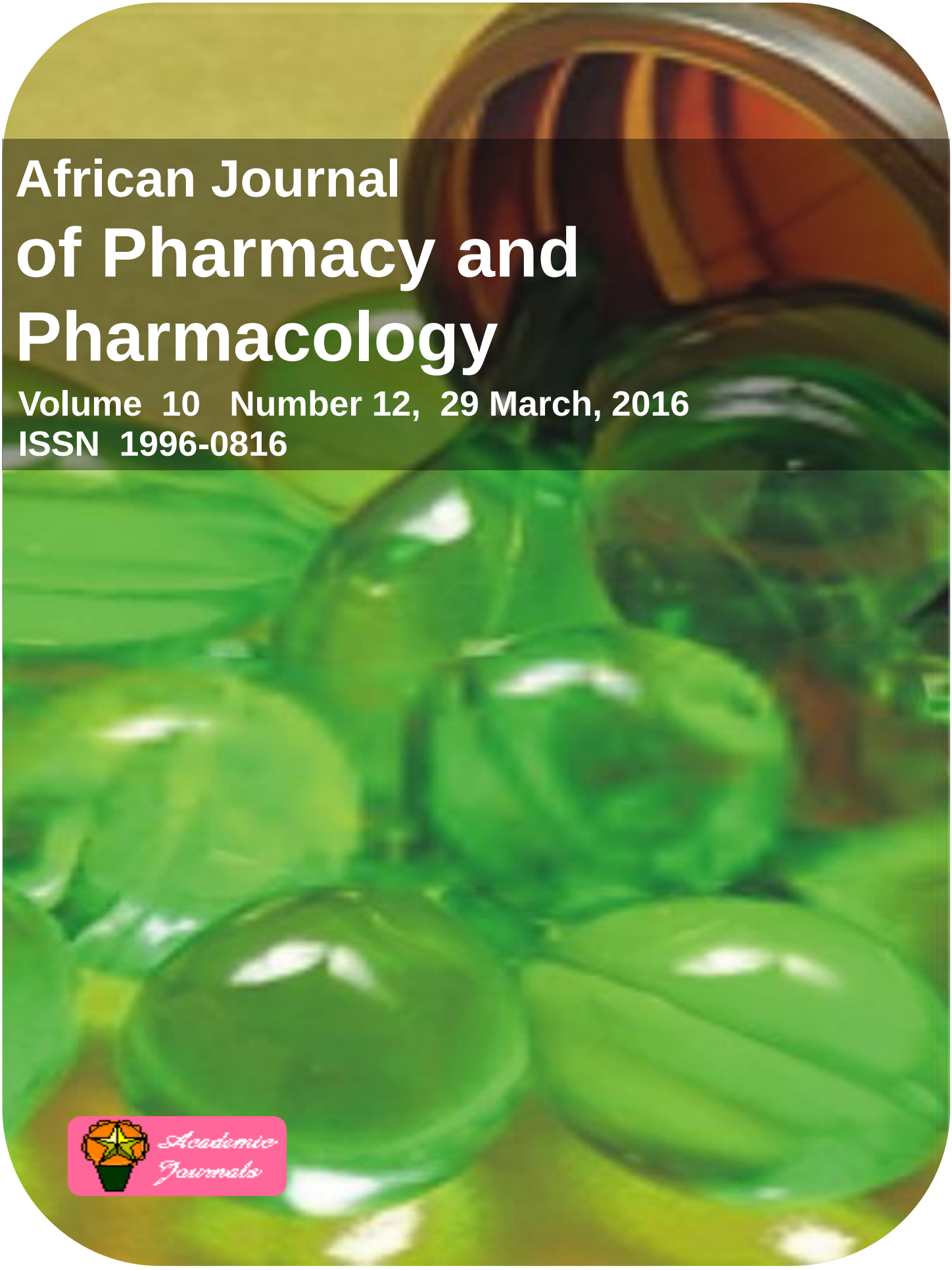

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Full Length Research Paper

Anticonvulsant and anxiolytic activity of the leaf aqueous and ethanolic extracts of *Melanthera scandens* in a rat model

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Modern drug therapy of epilepsy is complicated by the inability of drugs to control seizures in some patients and side effects that range in severity from minimal impairment of the central nervous system to death from aplastic anemia or hepatic failure. Medicinal plants used in traditional medicine for the treatment of epilepsy have been scientifically shown to possess promising anticonvulsant activities in animal models for screening for anticonvulsant activity and can be a source of newer anticonvulsants. The aim of this study was to investigate the preliminary phytochemical properties, anticonvulsant and anxiolytic activities of *Melanthera scandens* aqueous and ethanolic extracts.. Phytochemicals from the aqueous and ethanolic extracts were screened by standard methods. Anticonvulsant activity was evaluated against pentylenetetrazol (PTZ)-induced seizure model in rats. The effect of the extract at oral dose levels of 250, 500 and 1000 mg/kg was evaluated in an experimental rat model, using diazepam (5 mg/kg) as positive control. Anxiolytic activity was performed using elevated plus maze method. Phytochemical screening revealed that *M. scandens* extracts contain carbohydrates, flavonoids, saponins, glycosides, tannins, terpenoids, phenols and phytosterols. The aqueous extract at a dose of 500 mg/kg significantly increased seizure latency (P=0.0023), while the ethanolic extract did not have a significant effect on seizure latency. Both extracts significantly reduced the seizure severity (P= 0.0155), and provided up to 100% protection against PTZ induced death at 1000 mg/kg. Both extracts had no significant effect on the duration of PTZ induced seizures. Both extracts were found to increase the number of entries and the time spent in the open arms of the maze at a dose of 250 mg/kg, indicating anxiolytic activity, which was not seen at higher doses (500 and 1000 mg/kg). The total numbers of entries into the closed arm were significantly reduced at 500 and 1000 mg/kg oral doses of both extracts, indicating a reduction in locomotor activity of the rats. The results obtained in this study suggest that both the aqueous and ethanolic extracts of *M. scandens* possess anticonvulsant and anxiolytic activities in a rat model.

Key words: *Melanthera scandens*, Pentylenetetrazole (PTZ), anticonvulsant, anxiolytic.

INTRODUCTION

Epilepsy is one of the most common and widespread neurological disorders in the human population (Surajit et al., 2012).In modern medicine, epilepsy is considered to be a chronic brain syndrome of various etiology characterized by recurrent seizures and usually associated with loss or disturbance of consciousness.

There may be a characteristic body contraction (convulsion). The seizure is due to excessive electrical discharge in the brain and the seizure pattern depends not only on the cause but the origin, extent, intensity and type of epileptic discharge in the brain (Muazu and Kaita, 2008). Epilepsy is usually controlled, but not cured, with medication (Sridharamurthy et al., 2013). Currently available anticonvulsant drugs are able to efficiently control epileptic seizures in about 50% of the patients; another 25% may show improvement whereas the remainder does not benefit significantly. Furthermore, undesirable side effects of the drugs used clinically often render treatment difficult so that a demand for new types of anticonvulsants exists (Kamalraj, 2011). Anxiety affects most of the population nearly one- eighth of the total population worldwide. About 500 million people suffer from neurotic, stress related and somatoform problems and 200 million suffer from mood disorders.

Benzodiazepines, being a major class of compounds used for treatment of anxiety present a narrow margin of safety between the anxiolytic effect and unwanted side effects (Nitesh et al., 2012). *Melanthera scandens,* belonging to the *Asteraceae* family, is a scandent annual or perennial herb up to 4 m high that is widely distributed across tropical Africa. The infusion of leaves is used as an emetic, cough and febrile headache medicine. In Côte d'Ivoire, the leaves are used as a purgative and an antidote against poisoning (Affia et al., 2011).In Bushenyi, western Uganda, infusion of the leaves is used among others in the management of seizures in addition to being used to clean teeth. The crude leaf extract of *M. scandens* has been reported to contain anti-inflammatory and analgesic activities (Jude et al., 2012). Despite the fact that *M. scandens* is well known to possess interesting properties in traditional medicine it has not been studied for its anxiolytic and anticonvulsant activities. This study was aimed at providing experimental support for the traditional medicinal use of the leaf water and ethanolic extracts of *M. scandens* in the management of epilepsy as well as anxiety. We hypothesized that the leaf aqueous and ethanolic extracts of *M. scandens* are effective in prevention of PTZ induced convulsions and have anxiolytic activity in rats.

This current study was conducted to determine the anticonvulsant and anxiolytic activity of the leaf water and ethanolic extracts of *M. scandens.*

MATERIALS AND METHODS

Plant materials and extraction

The whole plant was collected in April 2014 from a bush in Nyakabirizi, Bushenyi district, Western Uganda. The, plant *M. scandens,* was authenticated by Dr. Eunice Olet, a Botanist from the Department of Biology, Mbarara University of Science and Technology (MUST), Uganda and a Voucher number (Silvano Twinomujuni 001) was kept in the herbarium for reference. Extraction was done according to Sutapa et al. (2012) with modifications in which the fresh plant leaves were washed and shade dried for two weeks and then ground. The powder (300 g) was dissolved in 2 L of distilled water or 2 L of 95% ethanol at room temperature, with intermittent shaking for 24 h, filtered using a filter paper to obtain water and ethanol extracts respectively. The liquid aqueous filtrate was evaporated to dryness in an oven at 60°C over 4 days while the ethanolic extract was dried at 40°C over 3 days. The dried crude extracts were stored in a refrigerator at 4°C until use for the proposed experiment. The yield of ethanol extract and water extract were11.9% (39.6 g/300 g) and 17.8% (53.5 g/300 g) of dry weight respectively.

Chemicals and drugs

PTZ was obtained from Sigma (USA); ethanol was procured from Scharlab S.L (Spain); diazepam injection was procured from Gland Pharma Ltd, India. Prior to use, all drugs/chemicals were freshly prepared in distilled water to the desired concentration.

Animals

Male Wistar rats (120 to 200 g) used in this study were obtained from the animal house facility of the Department of Pharmacology, MUST. They were maintained on a 12 h on and 12 h off light/dark schedule with free access to food and water, except during experimental procedures when they were fasted for 12 h before the experiment. All procedures involving animals were conducted in accordance with the Guidelines for the Humane Care and Use of Laboratory Animals published by National Institutes of Health, United States (Zandieh et al., 2010; OECD, 2000). The Institutional Review Board of Mbarara University approved the research. All laboratory experiments were conducted between 9:00 and 17:00 h. Animals were grouped in five groups of 8 animals each for each type of test conducted.

Phytochemical screening

Extracts of the dry powdered leaves of *M. scandens* were analyzed for the presence of various phytochemicals like alkaloids, terpenoids, flavonoids, phytosterols, proteins, reducing sugars, glycosides, phenols, tannins, saponins, free aminoacids and arginine using standard phytochemical analysis procedures specified by Trease and Evans (2012).

Elevated plus maze test

The elevated plus maze that was used in this study is the one described by Alicia and Cheryl (2007). The plus maze consists of two opposite open arms, 50 cm long and 10 cm wide, crossed with two closed arms of the same dimensions with 30 cm-high walls.

The arms are connected with a central square, 7.5 cm × 7.5 cm,

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Table 1. Phytochemical screening of the water and ethanolic leaf extracts of *M. scandens*.

Phytochemical constituents	Phytochemical test	Aqueous extract	Ethanolic extract
Alkaloids	Dragendorff's test	-	-
Terpenoids	Liebermann Burchard's Test	-	-
Flavonoids	Shinoda's Test	+	-
Phytosterols	Chloroform	+	+
Proteins	Millon's test	+	+
Reducing sugars	Fehling's test	+	+
Glycosides	Molisch's test	-	-
Phenols	Ferric chloride	+	+
Resins	-	+	+
Tannins	Ferric chloride	+	+
Saponins	Frothing test	+	+
Free aminoacids	Ninhydrin test	+	+
Arginine	Sakaguchi test	+	+

(+) Indicates present, (−) indicates absent.

to give the apparatus the shape of a plus sign. The whole apparatus was placed on an even floor to avoid unnecessary movements. Study animals were put into eight groups each containing 8 animals (n=8) and the following treatments were administered per group: Group I was treated with 250 mg/kg water extract (WE); group II 500 mg WE; group III 1000 mg WE; group IV 250 mg ethanolic extract (EE); group V 500mg EE; group VI 1000 mg EE; group VII 3 mg/kg Diazepam while the group VIII was treated with distilled water. 60 min after oral treatment with the extracts, each animal was placed individually in the center of the maze, facing the closed arm, after which the number of entries and time spent in the enclosed and open arms was recorded during the next five minutes using a video tracking system. Animals, which are anxious, are expected to spend more time in the closed arms while animals, which are not anxious, make more entries into and spend more time in the open arms. An arm entry was defined as the presence of all four feet in that particular arm. The maze was cleaned after each trial to remove any residue or odor of the animals. After the elevated plus maze test, the animals were returned to the cages to be used for anticonvulsant activity test.

Pentylenetetrazol (PTZ) induced seizures

Two days after elevated plus maze test, the same animals were orally treated with the extracts as follows: Group I was treated with 250 mg/kg water extract (WE); group II 500 mg/kg WE; group III 1000 mg /kg WE; group IV 250 mg/kg ethanol extract (EE); group V 500 mg/kg EE; group VI 1000 mg/kg EE; group VII 5 mg/kg diazepam (positive control) while the group VIII was treated with distilled water. Doses above were chosen basing on the results of acute toxicity test and the need to compare the activity for similar dose levels for the two extracts. The LD50 for the ethanol extract was estimated at 6.708 g/kg however the water extract was found safe up to 9.0 g/kg.

After 60 min, PTZ (60 mg/kg, i.p) was administered to all the groups. Each animal was individually placed in glass cage and observed for convulsive behavior for 30 min. The time of seizure onset, seizure duration and seizure behavior score (seizure severity) were recorded. Grading of seizure severity was done using a scoring system according to Alele and Rujumba (2011) as follows: Grade **0**: no signs of motor seizure activity during the 30 min observation period; Grade 1: staring, mouth or facial movements; Grade 2: head nodding or isolated twitches; Grade 3: unilateral/bilateral forelimb clonus; Grade 4: rearing; Grade 5: loss of posture, jumping; Grade 6: clonic/tonic seizures; Grade 7: full tonic; Grade 8: death.

Statistical analysis

Values for seizure latency, duration, severity time spent and entries into the open and closed arms of the plus maze were statistically analyzed by two-way analysis of variance (ANOVA) followed by multiple comparison tests. Comparison between controls and test groups was performed by Bonferroni, Tukey's and Sidak's post hoc tests using GraphPad Prism version 6.0. Data shown are mean ±Standard error of mean (SEM) for 8 rats per group. P values less than 0.05 ($P<0.05$) were taken to be the c criterion for statistical significance.

RESULTS

Phytochemical screening

Phytochemical screening revealed the presence, in both extracts, of phytosterols, proteins, reducing sugars, glycosides, carbohydrate, tannins, phenols, saponins, free amino acids and Arginine except flavonoids which were present only in the water extract (Table 1).

PTZ induced seizures

There was a significant increase in the time taken to see the first signs of PTZ induced convulsions with MS WE at 500 mg/kg compared to distilled water while there was no significant effect on the time taken for seizure onset in the MS EE treated rats compared to distilled water (Table 2). Both MS WE and MS EE did not have any significant

Table 2. Effect of *M. scandens* ethanolic and water extracts on seizure latency.

Treatment	Doses (mg/kg)	Seizure onset (s)	Seizure protection (%)	Level of significance
Diazepam	5	Nil	100	**P<0.001****
Distilled water	-	34 ± 9.0	37.5	
MS WE	250	50 ± 2.0	75	P=0.9137
MS WE	500	106 ± 37	75	**P=0.0023***
MS WE	1000	78 ± 7.6	100	P=0.1453
MS EE	250	54 ± 9.7	50	P=0.8281
MS EE	500	46 ± 3.6	87.5	P=0.9671
MS EE	1000	63 ± 7.7	100	P=0.5229

Distilled water, Diazepam (5 mg/kg), *M. scandens* water extract (MS WE), *M. scandens* ethanolic extract (MS EE) were administered orally 60 min before the injection of PTZ (60 mg/kg, i.p); values are the mean ± S.E.M. for 8 rats. *P < 0.05 significantly different compared to vehicle treated group, Two-way ANOVA followed by Tukey's test. ** P < 0.05 significantly different compared to the positive control treated group, Two- way ANOVA followed by post Tukey's test.

Table 3. Effect of *M. scandens* Ethanolic and aqueous extracts on seizure duration.

Treatment	Doses (mg/kg)	Seizure duration (min)	Seizure protection (%)	Level of significance
Distilled water		9.5 ± 2.5	37.5	
MS WE	250	5.8 ± 2.0	75	P = 0.9999
MS WE	500	6.9± 2.5	75	P = 0.9999
MS WE	1000	2.4 ± 0.53	100	P = 0.2151
MS EE	250	3.3 ± 0.27	50	P = 0.5009
MS EE	500	5.4 ± 2.4	87.5	P = 0.9999
MS EE	1000	7.1 ± 1.5	100	P = 0.9999

Distilled water, Diazepam, *M. scandens* water extract (MS WE) and *M. scandens* ethanolic extract (MS EE) were administered orally 60 min before the injection of PTZ (60 mg/kg, i.p); values are the mean time ±S.E.M. for 8 rats. All the p-values **were not** significantly different compared to vehicle treated group, Two-way ANOVA followed by Bonferroni's test Diazepam completely protected the animals from seizures.

Table 4. Effect of M. *scandens* ethanolic and water extracts on seizure severity.

Treatment	Doses (mg/kg)	Seizure score	Seizure protection (%)	Level of significance
Distilled water	-	7.4 ± 0.32	37.5	-
MS WE	250	6.3 ± 0.45	75	P = 0.8785
MS WE	500	5.8 ± 0.77	75	P = 0.2583
MS WE	1000	6.0 ± 0.19	100	**P= 0.0155***
MS EE	250	6.8 ± 0.49	50	P = 0.9999
MS EE	500	6.3 ± 0.37	87.5	P = 0.2583
MS EE	1000	5.1 ± 0.30	100	**P= 0.0155***

Vehicle, Diazepam, *M. scandens* water extract **(**MS WE) **and** *M. scandens* ethanolic extract **(**MS EE) were administered orally 60 minutes before the injection of PTZ (60 mg/kg, i.p); values are the mean ± S.E.M. for 8 rats per group. * P < 0.05 significantly different compared to vehicle treated group, Two-way ANOVA followed by Sidak test Diazepam completely protected the animals from seizures.

effect on seizure duration (Table 3).

The seizure severity score was decreased significantly for both MS WE and MS EE at a dose of 1000 mg/kg (Table 4). Animals that were treated with Diazepam (5 mg/kg), the positive control, did not show any signs of seizure activity.

Table 5. Effect of *M. scandens* Ethanolic and aqueous extracts on the anxiogenic effects.

Treatment	Dose (mg/kg)	Time spent in (m)		Entries into	
		Closed arm	Open arm	Closed arm	Open arm
Distilled water	-	2.04± 0.25	0.70± 0.18	3.4 ± 0.62	2.0 ± 0.50
MS WE	250	2.53 ±0.47	**2.95 ± 0.17***	2.8 ± 0.55	3.1 ± 0.58
MS WE	500	**4.07 ± 0.21***	2.17 ± 0.46	1.8 ± 0.54	2.8 ± 0.58
MS WE	1000	3.12 ± 0.53	1.87 ± 0.53	2.3 ± 0.49	3.3 ± 0.81
MS EE	250	3.26 ± 0.18	1.73 ± 0.18	4.7 ± 0.77	**6.0 ± 0.89*[a]**
MSEE	500	3.04 ± 0.40	1.71 ± 0.34	3.8± 0.47	4.0 ± 0.63
MSEE	1000	**4.30 ± 0.18***	0.93 ± 0.34	3.3 ± 0.36	3.0 ± 0.26
Diazepam	3	1.86 ± 0.38	**3.20 ± 0.36***	2.3 ± 0.62	2.6 ± 0.49

Values are expressed as mean ± SEM (n = 8). Two-way ANOVA followed by Bonferroni multiple comparisons test. $^{*}P < 0.05$ when compared with control group. $^{*a}P < 0.05$ compares aqueous and ethanol extracts at 250 mg/kg.

Effects of MS WE and MS EE on elevated plus maze test

In this test, the number of entries and time spent in the open arms were considered for the analysis of anxiolytic activity and the total number of entries in both the arms (enclosed and open arms) was considered for the evaluation of locomotor activity of animals (Varsha and Patel, 2010). MS WE and MSEE at the lowest dose of 250 mg/kg significantly increased the time spent in the open arm and increased entries into the open arm respectively compared to distilled water. At higher doses (500 and 1000 mg/kg), both extracts did not significantly increase the number of entries or time spent in the open arms. The total time spent in the closed arms was significantly increased at a dose of 1000 mg/kg (MS EE) and 500 mg/kg (MS WE) compared to distilled water. Animals treated with MS EE spent more time in the open arms compared to those treated with MS WE at 250 mg/kg, indicating that the MS EE had better anxiolytic activity than MS WE. Diazepam (3 mg/kg, *i.p*.), the positive control, significantly increased the time spent in the open arms (Table 5).

DISCUSSION

Phytochemical screening revealed the presence, in both extracts, of phytosterols, proteins, reducing sugars, glycosides, carbohydrate, tannins, phenols, saponins, free amino acids and arginine except flavonoids which were present only in the water extract. These findings are in agreement with the findings by Omoyeni et al. (2012); Fagbohun et al. (2012); Okokon et al. (2012) and Ndam et al. (2014). However, we did not find alkaloids and terpenoids, which were not found in the studies cited above.

Our findings demonstrated that *M. scandens* leaf extracts have anticonvulsant effects on PTZ model of epilepsy in rats. MS WE at a dose of 500 mg/kg increased seizure latency, while both MS WE and MS EE significantly reduced the severity of seizures. Pretreatment with *M. scandens* extracts protected the animals against PTZ induced death seizures with the percentage of seizure protection highest at 1000 mg/kg (Table 2). The medicinal value of plants lies in some chemical substances (phytochemicals) that have a definite physiological action on the human body (Amin et al., 2013; Datta et al., 2003; Dubois et al., 1986). The observed anticonvulsant activity in this study can be attributed to *M. scandens* extracts.

PTZ exerts its convulsant effects by inhibiting the activity of gamma amino butyric acid (GABA) at GABA-A receptors (DeSarro et al., 1999). GABA is a major inhibitory neurotransmitter, which is implicated in epilepsy. The enhancement of GABA neurotransmission attenuates convulsions while inhibition of the neuro-transmission of GABA enhances convulsions (Hoang and Hai, 2014). Diazepam is a known conventional antiepileptic agent that generally inhibits sodium currents and enhances GABA transmission. As expected, diazepam (5 mg/kg, *i.p*.) pretreated rats did not have any convulsive episode or show any mortality when treated with PTZ.

Since the extracts showed anticonvulsant effect against PTZ induced seizures, it is probable that they may be interfering with GABA transmission to exert their anticonvulsant effect. This is in agreement with findings of studies carried out by Hui et al., 2014 and Paramdeep et al., 2014, who found out that alkaloids, flavonoids, terpenoids, saponins, and coumarins enhance GABA transmission. Therefore, flavonoids and saponins may be responsible for the anticonvulsant activity observed in this study.

The elevated plus maze is considered to be an etiologically valid animal model of anxiety. The number of entries and time spent in the open arms have been found to be increased by anxiolytics and reduced by anxiogenic agents (Pellow et al., 1985). Both MS WE and MS EE showed anxiolytic activity at a lower dose of 250 mg/kg

however, such activity was not observed at higher doses (500 and 1000 mg/kg) compared to the control. Animals spent more time in the closed arms and made fewer entries into the open arms compared to the control, at higher doses, as locomotor activity could have been impaired following after *M. scandens* extract administration. Available literature reports describe the action of benzodiazepines, such as diazepam, as anxiolytics (at low doses) and as anticonvulsants, also producing sedation and myorelaxant effects at higher doses (Wolffgramm et al., 1994). Reduction in the locomotor activity by Both MS WE and MS EE in the elevated plus maze test may be correlated with central nervous depression. Diazepam reduced the animal's natural aversion to the open arms and maze exploration. This was probably because the dose of diazepam used in this study (3 mg/kg) was higher than that of Varsha and Patel (2010) (1 mg/kg) who found out that diazepam increased the animal's natural aversion to the open arms and promoted maze exploration.

In various studies, (Sandeep and Suresh, 2010; Herrera et al., 2008), flavonoids have been shown to have antianxiety activity. The anxiolytic effect of flavonoids has been attributed to its effect on benzodiazepine receptors and central nervous system (Sandeep and Suresh, 2010). Therefore, flavonoids may be responsible for the anti-anxiety activity observed in this study. This study provides experimental support for the traditional medicinal use of this plant for the management of epilepsy and anxiety.

Conflict of interests

The authors have declared no conflict of interests.

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Full Length Research Paper

Effects of *Hibiscus rosa-sinensis* leaf products on haematological indices, lipid profile and hepatic parameters of hyperlipidemic rat

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Previously, we demonstrated that *Hibiscus rosa-sinensis* leaf contains various secondary metabolites including saponins, tannins and oxalates, which are known to possess documented pharmacological activities. In this present study, we investigated the effects of the leaf products on haematological indices, lipid profile and hepatic parameters using Wistar albino rat bioassay. Data suggested that though the haemoglobin counts, packed cell volume and red blood count were not significantly ($P > 0.05$) affected by the leaf products, lipid profile tests results showed that the blood total cholesterol (TC) and low-density lipoprotein (LDL) of rats increased on feeding with high fatty diet (HFD). Administration of the leaf products dose-dependently resulted in significant decreases ($P < 0.05$) in the TC and LDL levels while the high-density lipoprotein level was further increased. Liver function test (LFT) showed no evidence of hepatotoxicity on the administration of the products as assayed liver enzymes and proteins did not vary between HFD administered animals, treated with or without leaf products. Comparatively, dried leaf products had more potent biological activities than the extracted leaf products. These findings suggested that *H. rosa-sinensis* leaves possess pharmacological potentials for treatment of metabolic syndrome related disease conditions.

Key words: Liver function test, hematology, lipid profile, hepatic parameters, cholesterolemia, metabolic diseases.

INTRODUCTION

Extended hyperlipidemia, including hypercholesterolemia, characterized by high circulating blood triglyceride (TG) and low HDL cholesterol levels, is a permissive factor interlinked with diverse metabolic syndrome, including atherosclerosis (Bhatnagar et al., 2008), hepatic dysfunctions (Semple et al., 2009), and diabetes mellitus

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(Dixit et al., 2014; Abbate and Brunzell, 1990). Between 2009 to 2012, of people aged 18 years or older with diagnosed diabetes in USA, 65% had serum low density lipoprotein cholesterol greater than or equal to 100 mg/dL or have used anti-hyperlipidemia medications (NDS, 2014). Of various causative factors of hyperlipidemia; sedentary life, poor dieting and genetic factors are the most prominent. In humans, continuous in-take of high amounts of fat ostensibly appears to contribute to hyperlipidemia.

In laboratory settings, hyperlipidemia induction is a well-known tool for investigation of the cholesterol metabolism-related disorders on one hand, as well as testing possible treatments for the reduction of circulating cholesterol levels on the other. Different models exist for hyperlipidemia induction in laboratory animals comparable to what obtains in human patients suffering from various metabolic disturbances (Li et al., 2010; Matos et al., 2005). Considering the prevalence of high cholesterol related metabolic disorders diseases world wide, effective and affordable treatments are urgently needed. In rural areas and as in most developing countries; reliance on natural sources for medications (traditional medicine) is common. Of these sources, plants (sometimes specific parts) are critical components. In therapeutic medicine, plants are also veritable sources of therapies as most of the currently available drugs are derived there off. Unlike primary metabolites which are central metabolites with intrinsic functions in plant growth, development and reproduction; secondary metabolites are not directly involved in those processes but play other critical roles in plant physiology and in its medicinal activity.

Saponin, for instance, is a widely known secondary metabolite of plant origin and has been reported to possess lytic actions against red blood cell membranes because of the affinity of saponin-aglycone moiety for membrane sterols, especially cholesterol, leading to formation of insoluble complexes (Glauert et al., 1962; Bang-ham and Horne, 1962); thus making saponins atypical cholesterol absorption inhibitor. Plant polyphenols are also known to prevent low-density lipoprotein oxidation and thrombocytes aggregation (Hayes et al., 2009; Singh et al., 2008), which are critical conditions that preclude atherosclerosis and coronary obstruction (Parthasarathy et al., 2010). Like saponins, fatty acids, vitamins, tannins and oxalates have also been implicated in several other biological mechanisms and therapeutic applications.

Hibiscus rosa-sinesis is an ornamental plant popularly grown in the tropics. Traditionally, it is a medicinal herb, with high anti-oxidant and vitamins contents, and documented potencies in decreasing chances of developing pyrexia, liver and cardiovascular disease (Bruneton, 1999; Chen, 2003; Imafidon and Okunrobo, 2009). The extracts are also known to block adipogenesis by suppression of the expression of adipogenic transcription factors (Kim et al., 2003). Similarly, other research groups have implicated extracts from diverse genera of Hibiscus plant as having potentials suitable for various metabolism-related therapeutic interventions (Hirunpanich et al., 2006; El-Sadaany et al., 1991; Lin et al., 2007; Hernández-Pérez and Herrera-Arellano., 2010; Hainida et al., 2008; Gurrola-Diaz et al., 2010; Alarcon-Aguilar et al., 2007; Mohd-Esa et al., 2010). Previously, we demonstrated that *H. rosa-sinensis* leaf products contain diverse secondary metabolites including saponins, fatty acids, vitamins, tannins and oxalates. Aware of the documented therapeutic potentials of these metabolites against metabolic dysfunctions, this current study undertook an experimental approach firstly to evaluate the effect of High fat diet feeding on lipid metabolism and liver function, and further examine the protective roles of *H. rosa-sinensis* leaf, using various forms of the leaf products, against damages caused by hyperlipidemia induction in rats.

MATERIALS AND METHODS

Animal, treatment and biological assessment

Forty-four Wistar albino rats (both sexes) weighing 150 to 250 g purchased from the Animal house of the Department of Veterinary Pathology, The University of Nigeria, Nsukka were employed for this study. The animals were housed in metabolic cages and acclimatized for 7 days, then divided into eleven groups of four each, marked A_1-D_1, A_2-D_2, N, P and Q while been maintained with standard grower's mash and tap water *ad albitum* prior to experimentation. They were maintained under normal room temperature with approximately normal 12:12 h dark/light cycle. The weight of each animal was taken pre-test samples administration. Within the first two weeks, rats in groups A_1-D_1, were fed with high fat diet HFD (composition of HFD is as described in supplementary material). They were treated two weeks later with 500 mg dose of the processed leaf samples while those in groups A_2-D_2 which were also fed with high fat diet and were treated two weeks later with 300 mg dose of the processed leaf sample. All doses were given to the animal once daily by oral administration. Group N was also fed with high fatty diet and later fed normal rat growers feed (No treatment). This was to investigate the effects of short-term HFD feeding; while group Q (Negative Control) was also fed with high fat diet throughout the period of the experiment. Neither changes in diet nor remedial treatment were administered. Group P (Positive Control) was fed throughout the thirty days with normal growers rat ration (described in supplementary material). We adopted the Li et al. (2010) model to induce hyperlipidemia as our diet composition and other experimental conditions were similar. Effects of the processed leaf samples on haematology, lipid profile and liver function of the experimental animals were determined according to previous protocols of Burtis and Ashwood (1999). Animals were observed throughout the 30 days for clinical signs/behavioral changes and/or mortality symptoms before and after dosing.

Plant materials

Leaves of *H. rosa-sinesis* were procured, based on ethno-pharmacological information, from the Department of Animal Science, and identified in the Department of Plant Science and Biotechnology, The University of Nigeria, Nsukka; by a taxonomist, Cyprian Okafor. A portion of the leaves was deposited in the Departmental herbarium for reference.

Products preparation

Fresh leaves were harvested, washed with distilled water and drain-dried, then divided into nine portions (100 g each). The 1st portion was analyzed as raw leaf (RL) control. The 2nd portion was blended with water, filtered and the filtrate pasteurized at 70°C for 30 min hereafter referred to as raw leaf extract (RLE). The 3rd, 4th and 5th portions were blanched in hot water at 100°C for 2, 4 and 6 min respectively, after which they were blended with water, filtered and the filtrate pasteurized at 70°C for 30 min and respectively called 2 min blanched leaf extract (B_2LE), 4 min blanched leaf extract (B_4LE) and 6 min blanched leaf extract (B_6LE). The 6th portion was dried at 50°C, milled and called raw dried leaf powder (RDLP). The 7th, 8th and 9th portions were blanched in hot water at 100°C for 2, 4 and 6 min respectively, dried at 50°C, milled into powder and called 2 min blanched dried leaf powder (B_2DLP), 4 min blanched dried leaf powder (B_4DLP) and 6 min blanched dried leaf powder (B_6DLP) respectively.

Blood collection for total cholesterol/serum biochemical analysis

At the end of the study, all animals were fasted overnight prior to necropsy, sacrificed and their blood collected by jugular vein puncture. The remaining portion of the blood sample from the euthanized rats was dispensed into plain tubes and allowed to stand for 3 h. Clotted blood samples were centrifuged at 3 x 10^3 rpm for 10 min. Clear sera were aspirated and stored frozen (at -80°C) for serum biochemical analyses.

Determination of haematological Indices, lipid profile and hepatic parameters

Red and white Blood Cell Counts were determined as described (Schalm et al., 1975). Samples were diluted (1:200) with red blood cell diluting fluid, and loaded into a Neubauer counting chamber. Cells were counted using a light microscope at 40 and 10 magnifications. Packed cell volume (PCV) was determined as described by Coles (1986). A micro capillary tube was nearly filled with the blood sample and sealed at one end. It was centrifuged at 10^4 rpm for 5 min using a micro haematocrit centrifuge and read with haematocrit reader. Cyanomethaemoglobin method (Dacie and Lewis, 2001) was used for determination of the serum Haemoglobin concentration. Low- and High-density Lipoprotein were determined using the methods described by Assman et al. (1984) and Albers et al. (1978) respectively. Triglycerides were determined using glycerol-phosphate oxidase method (Jacobs and Van Demark, 1960). The serum glucose and cholesterol levels were determined spectrophotometrically after enzymatic oxidation (Sood, 2006). Burtis and Ashwood (1999) method was used for the determination of aspartase and alanine aminotransferase activities. To determine Alkaline Phosphatase, 8 ml of blood was collected from each animal by cardiac puncture, transferred into a centrifuge tube and allowed for 30 min to clot before centrifuging using Wisperfuge Model 1384 centrifuge (Tamson, Holland) for 5 min and the resulting supernatant used for the assessment of liver integrity. Total and conjugated bilirubins, and the alkaline phosphatase activity were assayed using p-nitrophenylphosphate as substrate in a phosphate buffered saline (pH 9.8) using the colorimetric method according to Ojiako and Nwanjo (2006). Total protein was estimated following the method of Lowry et al. (1951).

Ethic statement

All animal use and experimentation were approved by the institutional research committee of the University of Nigeria, Nsukka. Guiding Principles for the Care and Use of Laboratory Animals were strictly followed (NIH Publication No. 85-23, 1985), and with full adherence to the Helsinki Declaration.

Data and statistical analysis

All displayed data are mean of independent triplicate experimental results obtained and statistically analyzed using the analysis of variance (ANOVA) in a completely randomized design (CRD). Differences among means were determined with the least significant difference (LSD) at $P < 0.05$ (Steele and Torrie, 1980).

RESULTS

Effects of *H. rosa-sinensis* leaf products on haematological indices of rats

No significant ($P > 0.05$) difference was observed in both the packed cell volume count and Red Blood Count of the experimental animals both in the period of inducing hyperlipidemia, before and after feeding them with the products (Table 1), suggesting that neither the HFD nor the treatment altered PCV and RBC of the experimented animal. Similarly, after hypercholesterolemia induction (feeding with high fatty diet–group Q), WBC count was found not to be significantly ($P > 0.05$) different from non-dyslipidemic rats (P) nor was it significantly different from WBC count of those in which cholesterol was induced but later fed normal commercial rat diet (N). However, animals exhibited significant variation in WBC counts post-products administration. Feeding the rats with the two doses of the leaf products (A_1 and A_2, B_1 and B_2, C_1 and C_2, D_1 and D_2) resulted to lower WBC counts when compared to rats fed diets not containing the leaf products (P, Q and N) as shown in Table 1. A possible explanation could be that the leaf products inhibited WBC production or up-regulated its export from the blood. The normal WBC level in the rats where within the normal range 6 to 18 ($\times 10^3$ ul) according to previous studies. Among the leaf products, the dried leaf products resulted to higher WBC counts compared to their corresponding extracted leaf products.

Effect of *H. rosa-sinensis* leaf products on lipid panel of rats

Blood glucose levels (Table 2) of rats fed normal rat diet (P) was, as expected, significantly ($P < 0.05$) lower than the blood glucose levels in the rest of the rats. When rats were fed high fat diet without any other treatment, the blood glucose rose significantly ($P < 0.05$) higher than rats fed normal diet, though no significant ($P > 0.05$) difference was observed compared to the blood glucose levels of other rats. Interestingly, glycemic levels increased further in high fat diet fed rats fed with normal rat diet. These results suggest that high fat feeds induced

Table 1. Effects of high fatty diet and the processed samples of *H. rosa-sinensis* leaf on the haematology of the experimental rats.

Animal groups + Samples doses	Haemoglobin (g/dl)	PCV (%)	RBC (x10^6/ul)	WBC (x10^3/ul)
Animals fed HFF + 500 mg RDLP (A_1)	13.933^a ± 0.757	39.667^a ± 4.726	6.790^a ± 0.020	10.467^{ab} ± 0.473
Animals fed HFF + 300 mg RDLP (A_2)	15.900^a ± 0.173	39.667^a ± 1.528	7.003^a ± 0.206	10.567^{ab}± 0.982
Animals fed HFF + 500 mg B_2DLP (B_1)	13.533^a± 1.266	38.667^a ± 2.309	5.957^a ± 0.901	8.717^{abc} ± 0.325
Animals fed HFF + 300 mg B_2DLP (B_2)	14.100^a ± 1.253	38.667^a ± 1.000	6.073^a ± 0.287	11.217^{ab} ± 0.256
Animals fed HFF + 500mg RLE (C_1)	13.900^a±2.751	40.000^a ± 3.606	5.863^a ± 1.510	6.137^c ± 4.225
Animals fed HFF + 300mg RLE (C_2)	13.433^a ±1.286	41.000^a ± 2.646	6.183^a ± 0.114	8.483^{abc} ± 2.314
Animals fed HFF + 500mg B_2LE (D_1)	13.800^a±0.819	40.000^a ± 3.606	6.023^a ± 0.016	8.183^{bc} ± 0.711
Animals fed HFF + 500 mg B_2LE (D_2)	13.933^a ± 3.765	39.000^a ± 9.000	6.443^a ± 0.508	8.573^{abc} ± 4.026
Animals fed HFF only, No treatment (Q)	14.367^a ± 0.945	40.333^a ± 3.512	6.110^a ± 0.020	10.99^{ab} ±2.000
Animals fed HFF and later fed normal rat grower ration (N)	13.833^a ± 0.404	35.333^a ± 4.509	6.360^a ± 0.480	12.020^a ± 1.025
Animals fed normal grower ration (P)	14.6667^a ±0.987	40.000^a ± 1.00	5.847^a ± 0.586	12.100^a ± 1.100

Presented data are means ± standard deviation of three determinations. Lower case letter superscripts are P-values. Data on the same column with different superscripts are significantly different ($P < 0.05$), while those in same column with same superscripts are not significantly different ($P > 0.05$). A_1= Animal fed with high fatty diet and treated with 500 mg of raw dried leaf powder (RDLP), A_2 = Animals fed with high fatty diet and treated with 300 mg of raw dried leaf. B_1 = Animal fed with high fatty diet and treated with 500 mg of 2 min blanched dried leaf powder (B_2DLP), B_2 = Animals fed with high fatty diet and treated with 300 mg of 2 min blanched dried leaf powder. C_1 = Animal fed with high fatty diet and treated with 500 mg of raw leaf extract (RLE), C_2 = Animals fed with high fatty diet and treated with 300 mg of raw leaf extract. D_1 = Animal fed with high fatty diet and treated with 500 mg of 2 min blanched leaf extract (B_2LE), D_2 = Animals fed with high fatty diet and treated with 300 mg of 2 min blanched leaf extract. Q = (Negative Control) Animals fed with high fatty diet only, no treatment, N = Animal fed with high fatty diet and later fed with normal (growers) diet/ration. P =Animals fed with normal rat growers diet alone (Positive Control). HFF = High fatty feed.

a rise in both cholesterol and blood glucose concentrations. Inclusion of the dried leaf products to diet resulted to slightly higher blood glucose compared to corresponding extracts. The differences can be attributed to higher carbohydrate content of the dried leaf products compared to the extracted leaf products, which were between 73 to 79% and 13 to 16% of the products respectively. Cholesterol levels in the animals fed normal ration were low but were increased by high fat diet feeding. This shows that the high fat diet was effective in inducing hypercholesterolemia in the rats. Remarkably; the products, especially the dried products, significantly ($P < 0.05$) reduced the cholesterol level in all treatments. However, blanching effects were variable. While in the dried leaf products (A_1, A_2, B_1 and B_2), the blanched leaf products (B_1 and B_2) were slightly less effective in lowering the blood cholesterol compared to the un-blanched products (A_1 and A_2); but in the extracted leaf products (C_1, C_2, D_1 and D_2), the blanched products (D_1 and D_2) were slightly more effective in lowering blood cholesterol compared to un-blanched products (C_1 and C_2). Similarly, data of the triglyceride concentration (Table 2) were variable. Low Density Lipoprotein (LDL) and triglyceride levels increased dramatically on feeding with high fat feed. The leaf products slightly reduced the blood triglycerides and LDL in all treatments. When the rats were fed high fat diet, the high-density lipoprotein decreased slightly. Treatment of the rats with normal rat ration after feeding with high fat diet caused an obvious increase in their high density lipoprotein level which also was found to be significantly ($P < 0.05$) higher than the high density lipoprotein level found in the other animals.

Effect of H. rosa-sinensis leaf products on liver function tests

Generally, blood albumin generally increased ($P < 0.05$) significantly on administration of the leaf products compared to unfed control, suggesting that the leaf products stimulated the synthesis of albumin. With respect to this present study, comparing the total bilirubin value of animals in group P with those fed with leaf products (A_1, A_2, B_1, B_2, C_1, C_2, D_1 and D_2), it can be observed that their total bilirubin values were slightly lower than the range stated above and higher than the normal range for rats (0.2 to 0.5 g/dl). The effects

Table 2. Effects of high fatty diet and the processed samples of *H. rosa-sinensis* leaf on the lipid profile of the experimental rats.

Animal groups + Samples doses	Blood glucose (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Low-density lipoprotein (mg/dl)	High density lipoprotein (mg/dl)
Animals fed HFF +500mg RDLP (A_1)	$69.333^{a} \pm 2.517$	$48.033^{de} \pm 2.954$	$48.733^{a} \pm 16.738$	$12.733^{f} \pm 2.533$	$35.300^{bcd} \pm 0.520$
Animals fed HFF +300mg RDLP (A_2)	$61.333^{a} \pm 6.506$	$51.567^{de} \pm 3.459$	$55.700^{a} \pm 24.719$	$16.633^{f} \pm 1.401$	$34.933^{bcd} \pm 4.400$
Animals fed HFF +500mg B_2DLP (B_1)	$60.333^{a} \pm 10.693$	$52.000^{de} \pm 9.062$	$53.533^{a} \pm 9.235$	$12.867^{f} \pm 1.026$	$39.333^{bc} \pm 9.452$
Animals fed HFF +300mg B_2DLP (B_2)	$55.000^{a} \pm 33.151$	$50.400^{de} \pm 9.854$	$38.200^{a} \pm 11.995$	18.433ef ± 2.714	31.967cd ± 7.557
Animals fed HFF +500mg RLE (C_1)	$54.333^{a} \pm 13.013$	$73.000^{bc} \pm 18.330$	$57.033^{a} \pm 17.470$	$30.900^{bc} \pm 3.005$	$49.333^{abc} \pm 17.897$
Animals fed HFF +300mg RLE (C_2)	$54.000^{a} \pm 14.177$	$81.467^{abc} \pm 12.944$	$48.600^{a} \pm 26.692$	$23.667^{de} \pm 0.577$	$51.767^{ab} \pm 12.564$
Animals fed HFF +500mg B_2LE (D_1)	$61.333^{a} \pm 4.933$	$67.233^{cd} \pm 22.148$	$62.300^{a} \pm 22.364$	$26.167^{cd} \pm 7.654$	$41.067^{bcd} \pm 14.860$
Animals fed HFF +300mg B_2LE (D_2)	$57.333^{a} \pm 22.811$	$73.200^{bc} \pm 19.755$	$42.033^{a} \pm 10.550$	$24.133^{d} \pm 3.459$	$49.067^{abc} \pm 18.243$
Animals fed HFF only, No treatment (Q)	$55.730^{a} \pm 0.030$	$91.870^{ab} \pm 0.020$	$46.730^{a} \pm 0.030$	$65.533^{a} \pm 0.351$	$30.127^{d} \pm 0.025$
Animals fed HFF and later fed normal rat grower ration (N)	$59.333^{a} \pm 8.145$	$99.400^{a} \pm 4.221$	$64.500^{a} \pm 10.776$	$34.733^{b} \pm 3.842$	$64.667^{a} \pm 4.163$
Animals fed normal grower ration (P)	$41.333^{b} \pm 3.512$	$38.867^{e} \pm 6.180$	$33.333^{a} \pm 10.786$	$8.567^{g} \pm 4.332$	$30.300^{d} \pm 4.709$

Presented data are means ± standard deviation of three determinations. Lower case letter superscripts are P-values. Data on the same column with different superscripts are significantly different ($P < 0.05$) while those in same column with same superscripts are not significantly different ($P > 0.05$). A_1= Animal fed with high fatty diet and treated with 500 mg of raw dried leaf powder (RDLP), A_2 = Animals fed with high fatty diet and treated with 300 mg of raw dried leaf. B_1 = Animal fed with high fatty diet and treated with 500 mg of 2 min blanched dried leaf powder (B_2DLP), B_2 = Animals fed with high fatty diet and treated with 300 mg of 2 min blanched dried leaf powder. C_1 = Animal fed with high fatty diet and treated with 500 mg of raw leaf extract (RLE), C_2 = Animals fed with high fatty diet and treated with 300 mg of raw leaf extract. D_1 = Animal fed with high fatty diet and treated with 500 mg of 2 min blanched leaf extract (B_2LE), D_2 = Animals fed with high fatty diet and treated with 300 mg of 2 min blanched leaf extract. Q = (Negative Control) Animals fed with high fatty diet only, no treatment, N = Animal fed with high fatty diet and later fed with normal (growers) diet/ration. P =Animals fed with normal rat growers diet alone (Positive Control). HFF = High fatty feed.

of treatment of the rats with the leaf products showed that the rats treated with dried leaf products generally had lower AST than rats treated with corresponding leaf extracts. This may tend to suggest that the aqueous leaf extracts were more hepatotoxic than the dried leaf products. Blanching resulted to higher AST in the rats compared to the corresponding un-blanched counterparts, suggesting that blanching of the leaf products may be more damaging to the liver. Also, it appears that a threshold exists beyond which damage could be more pronounced in the liver. The leaf products lowered the ALT contents significantly ($P < 0.05$) when compared to rats fed normal rat ration (P) or those induced with cholesterol in a non-dose dependent manner. Again, we find that the dried leaf products gave lower ALP values compared to the un-dried products. This suggests that the dried leaf products may be more effective in preventing liver damage than the leaf extracts (Table 3).

DISCUSSION

High serum LDL and/or Low serum HDL concentration occasioned by increase in oxidative stress (which can be altered by poor nutrition) are known risk factors of atherosclerosis and other metabolic disorders (Chander et al., 2003; Bhatnagar et al,. 2008). Similarly, rise in blood cholesterol concentration is also a precursor for ischemic heart disease and other cardiovascular disorders (Aparna, 2003; Bhatnagar et al., 2008). The pharmacological and biological roles of the secondary metabolites of *H. rosa-sinensis* as hypoglycaemic and hypolipidemic agents are well established. The data presented herein are also consistent with the hypocholesterolemic activity previously reported for other plant products. In this study, we employed leaf products with assayed secondary metabolites comprising of saponin (0.06 to 0.19%), tannin (0.05 to 0.2%) and oxalate (0.14 to 0.92%) dry weight ranges. We demonstrated that oral administration of HDF to Wistar rats resulted to surge in blood total cholesterol and LDL as reported previously (Li et al., 2010; Matos et al., 2005). While total glucose

Table 3. Effects of high fatty feed and the processed samples of *Hibiscus rosa-sinensis* leaf on the liver function of the experimental rats.

Animal groups +Samples doses	Total Protein (g/dL)	Albumin (g/dl)	Total Bilirubin (g/dL)	Conjugated Bilirubin (g/dL)	Aspartate Amino Transaminase (µ/L)	Alanine Amino Transaminase (µ/L)	Alkaline phosphatase (µ/L)
Animals fed HFF +500 mg RDLP (A_1)	$5.933^{a} \pm 0.751$	$3.133^{cd} \pm 0.208$	$0.123^{a} \pm 0.087$	$0.020^{a} \pm 0.010$	$17.667^{de} \pm 7.095$	$4.333^{d} \pm 0.577$	$10.333^{a} \pm 6.658$
Animals fed HFF + 300 mg RDLP (A_2)	$6.100^{a} \pm 0.346$	$3.633^{ab} \pm 0.306$	$0.053^{b} \pm 0.031$	$0.017^{a} \pm 0.012$	$15.000^{e} \pm 14.178$	$7.667^{bcd} \pm 2.309$	$10.667^{a} \pm 1.155$
Animals fed HFF +500 mg B_2DLP (B_1)	$6.067^{a} \pm 0.116$	$3.367^{ab} \pm 0.252$	$0.050^{a} \pm 0.010$	$0.020^{a} \pm 0.006$	$15.000^{e} \pm 3.000$	$6.667^{cd} \pm 1.528$	$14.000^{a} \pm 8.185$
Animals fed HFF +300 mg B_2DLP (B_2)	$5.600^{a} \pm 0.458$	$3.833^{a} \pm 0.252$	$0.070^{a} \pm 0.027$	$0.017^{a} \pm 0.026$	$19.000^{cde} \pm 4.583$	$6.333^{dc} \pm 0.577$	$13.333^{a} \pm 4.163$
Animals fed HFF + 500 mg RLE (C_1)	$6.667^{a} \pm 0.702$	$3.500^{abc} \pm 0.265$	$0.100^{a} \pm 0.102$	$0.047^{a} \pm 0.040$	$28.333^{abcd} \pm 10.017$	$7.333^{cd} \pm 1.528$	$10.333^{a} \pm 3.055$
Animals fed HFF + 300 mg RLE (C_2)	$6.267^{a} \pm 0.551$	$3.733^{ab} \pm 0.153$	$0.087^{a} \pm 0.055$	$0.083^{a} \pm 0.110$	$21.333^{bcde} \pm 9.074$	$6.667^{cd} \pm 3.055$	$14.000^{a} \pm 5.292$
Animals fed HFF + 500 mg B_2LE (D_1)	$5.567^{ab} \pm 1.007$	$3.567^{ab} \pm 0.252$	$0.160^{a} \pm 0.063$	$0.047^{a} \pm 0.025$	$32.333^{ab} \pm 10.970$	$6.333^{cd} \pm 1.155$	$17.000^{a} \pm 9.849$
Animals fed HFF + 300 mg B_2LE (D_2)	$5.500^{ab} \pm 0.866$	$3.400^{bc} \pm 0.361$	$0.147^{a} \pm 0.051$	$0.073^{a} \pm 0.021$	$31.667^{abc} \pm 8.622$	$6.667^{cd} \pm 2.517$	$20.000^{a} \pm 9.000$
Animal fed HFF only, No treatment (Q)	$5.687^{b} \pm 0.153$	$2.753^{de} \pm 0.265$	$0.177^{a} \pm 0.150$	$0.060^{a} \pm 0.020$	$17.500^{de} \pm 2.646$	$11.167^{ab} \pm 0.070$	$15.000^{a} \pm 0.030$
Animals fed HFF and later fed normal grower ration (N)	$4.400^{c} \pm 0.300$	$2.933^{cd} \pm 0.252$	$0.193^{a} \pm 0.021$	$0.063^{a} \pm 0.035$	$37.667^{a} \pm 3.512$	$10.667^{abc} \pm 0.577$	$18.000^{a} \pm 4.000$
Animals fed normal grower ration (P)	$5.900^{a} \pm 0.300$	$2.667^{e} \pm 0.208$	$0.177^{a} \pm 0.142$	$0.063^{a} \pm 0.015$	$17.000^{de} \pm 3.000$	$12.667^{a} \pm 7.371$	$16.333^{a} \pm 4.726$

Presented data are means ± standard deviation of three determinations. Lower case letter superscripts are P-values. Data on the same column with different superscripts are significantly different ($p < 0.05$) while those in same column with same superscripts are not significantly different ($P > 0.05$). A_1= Animal fed with high fatty diet and treated with 500 mg of raw dried leaf powder (RDLP), A_2 = Animals fed with high fatty diet and treated with 300 mg of raw dried leaf. B_1 = Animal fed with high fatty diet and treated with 500 mg of 2 minutes blanched dried leaf powder (B_2DLP), B_2 = Animals fed with high fatty diet and treated with 300 mg of 2 minutes blanched dried leaf powder. C_1 = Animal fed with high fatty diet and treated with 500 mg of raw leaf extract (RLE), C_2 = Animals fed with high fatty diet and treated with 300 mg of raw leaf extract. D_1 = Animal fed with high fatty diet and treated with 500 mg of 2 minutes blanched leaf extract (B_2LE), D_2 = Animals fed with high fatty diet and treated with 300 mg of 2 minutes blanched leaf extract. Q = (Negative Control). Animals fed with high fatty diet only, no treatment, N = Animal fed with high fatty diet and later fed with normal (growers) diet/ration. P =Animals fed with normal rat growers diet alone (Positive Control). HFF = High fatty feed.

(TC) also increased rather slightly, assayed hematological parameters were largely un-affected. Leaf products treatment resulted to increasing concentrations of HDL suggesting that the leaf products possess therapeutic potentials against low HDL engendering disease conditions. Both high fatty diet and the leaf products given to animals at both high and low doses did not reduce its albumin level as obtained data fell within the range of 3 g/dl as reported previously (Thapa and Walia, 2007) and also within the normal albumin level of rats (3.8 to 4.8 g/dl).

Albumin level below 3 g/dl (usually found in hepatitis condition) is a prognostic factor in chronic liver disease caused by decreased albumin synthesis. Neither the high fat diet nor products treatment affected bilirubin concentration. Improper functioning of liver coupled with serum bilirubin levels more than 17 µmol/L are underlying markers of liver disease, while normal total bilirubin level is usually between 0.2 to 0.9 g/dl (2 to 15 µmol/L) in rats.

Furthermore, slight reduction in bilirubin concentration is suggestive that the leaf products have potentials to impart processes leading to haemoglobin breakdown in the experimental rats. Bilirubin is a yellow-orange pigmented molecule and primarily by-product of heme (a component of haemoglobin) degradation. Reduced oxidative stress (due to LDL protection from oxidation) is a promising strategy towards therapeutic interventions against atherosclerosis related cardiovascular problems of coronary heart disease, stroke, peripheral arterial disease and aortic disease (Hayes et al., 2009; Singh et al., 2008; Aviram et al, 2000; Retsky et al, 1993).

The products also seem to significantly lower normal plasma alanine aminotransferase, a key catalyst of the alanine cycle compared to both untreated hyperlipidemic and normal control rats. In conclusion, our findings underscored the fact that sub-chronic exposure of rats to high fat diet, using lard as a source of fat, could induce metabolism conditions, typical of hyperlipidemia, and that the *H. rosa-sinensis* leaf products

administration in the hyperlipidemic rats have promising potentials to improve these conditions.

Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of the compliance of women with breast cancer to treatment in a reference hospital in a city of North-east, Brazil

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For the translation, transcultural adaptation and a pilot test of the instrument were done. Also, the compliance of the breast cancer patients to the treatment was measured. The questionnaire "Cancer Patient Self-Report Questionnaire Non-Adherence" was translated and adapted; and a pilot test with 30 patients was performed. For this study, the questionnaire, Morisky-Green test, was also used. A hundred and thirteen patients diagnosed with breast cancer and who used oral chemotherapy were evaluated. The evaluated patients' average age was 57 years. 82.5% of them had invasive lobular carcinoma cancer, and almost 50% had a family history of breast cancer. The evaluation of the compliance shows that only 31.85% of the patients completed the medical treatment. Patients who presented adverse reactions were more susceptible to stopping the treatment. The low compliance on the medicated treatment is worrying and shows the need to improve it and further research to identify which factors contribute to the non-compliance to such treatment.

Key words: Breast cancer, compliance, oncology.

INTRODUCTION

Breast cancer is the second most present type of cancer in Brazil and in the world. In the last years, the incidence of breast cancer increased. Due to the rise of early diagnosis of the cancer, the mortality has fallen, highly increasing the prevalence of breast cancer (Ferlay et al., 2015). The breast neoplasia has a genetic origin, be it through mutation or hereditary matters (Bilimoria and Morrow, 1995). The therapeutics focuses on surgery, with or without the use of radiotherapy and chemotherapy as well as oral chemotherapy which prevents the recurrence of the disease (WHO, 2003).

The use of chemotherapy for a long time turns compliance into a challenge. As in developed countries, the compliance of long-term treatment is approximately 50% and in developing countries, this number is lower due to lack of financial resources (Vermeire et al., 2001;

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Souza et al., 2013).

Several reasons and factors may influence compliance. Non-compliance is higher among women under 35 years of age, with more advanced stages of the disease (stages III and IV), drinkers, those undergoing chemotherapy and the increase on the number of months between diagnosis and starting of treatment (Brito et al., 2014). For these and other reasons, it is necessary to assess compliance on chemotherapy so that health staff can take appropriate decisions.

Therefore, we aimed to carry out the translation, cultural adaptation and a pilot test of the instrument and measure compliance to the treatment by patients with breast cancer.

MATERIALS AND METHODS

This is an observational, descriptive and exploratory study with a cross-sectional design. Data collection was performed at the Oncology Center of Emergency Hospital (HUSE), located in the municipality of Aracaju, from October to December 2012. Female patients with breast cancer were treated at HUSE Oncology Center. The sample consists of the target population of patients who met the following inclusion criteria: Age less than 18; breast cancer diagnosis using oral adjuvant therapy; agreed to participate by signing a free and informed consent form (ICF).

The sample size was determined by estimating a ratio of registered patients at the Oncology Center, considering a non-compliance rate of 76%, with absolute estimate precision of 8% and a confidence level of 95%. The calculated value was 109 patients and 113 patients were collected.

Socio-demographic data were collected on the diagnosis: type, histological grade, staging and date of diagnosis, hormonal stage as premenopausal or postmenopausal of the woman on the day of the diagnosis, the age of the woman at menarche, menopause if the woman has already been affected by it, parity, age of children if the woman has any, abortions, family history regarding breast cancer occurrence. It was verified whether treatment was already started or not, and if started, what form of treatment such as surgery, radiation therapy or chemotherapy and which is the current treatment. When was it started and how long has the woman been undergoing treatment? It will be checked for other diseases and medications used in addition to oral chemotherapy.

Compliance and non-compliance to treatment as a dependent variable

Three instruments for data collection were used: one for socio-demographic and clinical data, and two questionnaires for assessing compliance in women with breast cancer and using oral adjuvant therapy.

For assessing treatment compliance, Morisky and Green test was applied; it was translated into Portuguese language (Strelec et al., 2003; Abreu and Koifman, 2002). A questionnaire of self-reporting of non-compliance in cancer patients (Cancer Patient Self-Report Non-Adherence Questionnaire) was used (Kondryn et al., 2009).

The process of translation and cultural adaptation of the Cancer Patient Self-Report Non-Adherence Questionnaire was conducted based on methodologies described in the literature. The entire process was executed in five stages: (1) translation into Portuguese by two translators; (2) consensus of the two translations; (3) back-translation into English, (4) evaluation of semantic equivalence and (5) pilot test with 30 patients (Guillemin et al., 1993; Wild et al., 2005).

Step 1: Translation of the instruments from mother tongue, English to Portuguese by two people, independently; the translators were fluent in English language, had no idea of the issued addressed in the questionnaire and without knowledge of health supply.

Step 2: Consensus of the two translations was done by a meeting between the translators and a mediator. The mediator reconciled the differences in translation and did the final translation in consensus with everyone.

Step 3: Back-translation of the Portuguese version was made in step 2 by two translators independently; they have different life experiences, are native English speakers and are fluent in the target language. The translators were blinded during the process from the original version of the questionnaire and production of consensus.

Step 4: Semantic equivalence evaluation by two experts, ALF and WB, along with the back-translators; the translated versions were equivalent to the questionnaire in its original version. They came up with a consensus on the final version of the questionnaire in Portuguese.

Step 5: To assess the applicability of the instrument, a pilot test with cross section was performed in 30 women diagnosed with breast cancer; they were undergoing treatment at the Oswaldo Leite oncology unit, located in the Emergency Hospital of Sergipe in September 2012. Data collection was performed by personal contact of researchers with patients at the time they were in the hospital waiting for an outpatient visit. There were no refusals by the participants or sample loss at any stage of the study.

Data analysis was done using the *EPI-info software v.3.5*. The vision statistical analysis was used to investigate the distribution and relationship between the variables. Means or medians and standard deviations were used to summarize the data when needed. Two-way significance tests, including chi-square or Fisher's exact test were performed to verify the association between categorical variables. Student tests or Mann-Whitney tests were used to compare means between continuous variables. A 95% confidence interval ($\alpha \leq 0.05$) was used.

In accordance with Resolution CNS 196/96 of the National Board of Health, the project was registered in SISNEP and submitted to the Committee of Ethics and Research of the Federal University of Sergipe where it was given favorable opinion with CAAE: 00841712.4.0000.0058. To the research subjects, the right to confidentiality was assured, as well as non-maleficence, autonomy and information on the purpose, procedures, possible discomforts and benefits of the research; their agreement to participate in the study was attested by signing a free and informed consent form (ICF).

RESULTS

The items were translated independently by two professionals in step 1. Item 5 is expressed in the sentence "How Often do you forget to take a dose of your prescribed oral (taken by mouth) medicines?" in the original instrument. The translation process generated two results “Com que frequência você se esquece de tomar uma dose de seu medicamento oral prescrito? (Ingerido pela boca)” and “Com que frequência você esquece de tomar a dosagem de seus medicamentos

orais prescritos?". The difference between "dose" and "dosage" was found in translations to Portuguese in step 2. We opted for the "dose" term, which means the amount needed to elicit a desired therapeutic response in the patient, since "dosagem" refers to how often the dose is taken.

Verbs were translated mostly into the past tense of the indicative case of a recall questionnaire; for example, the Item 7: "Have you ever taken the wrong dose?" was translated into: "Você já tomou a dose errada?"

Item 16 "Have you ever forgotten to care for your central line?" generated two results in step 1: "Você alguma vez esqueceu de cuidar da sua linha central?" and "Você já se esqueceu de cuidar do seu eixo central?" Translations into Portuguese were literal and the term "central line" was not consistent in any translation with cancer treatment; as Brazil refers to central venous access done in some patients (Table 1).

For the pilot study 30 patients were interviewed with an average age of 57 years (± 12.6). All were diagnosed positive for breast cancer during the period of 17/09/2012 to 28/09/2012, in Oswaldo Leite Oncology Unit, located in the Emergency Hospital of Sergipe. The studied population was 113 patients with an average age of 57 ± 13 years. The evaluated patients were all women. The most present breast cancer subtype with 82.5% is the invasive lobular cancer followed by lobular carcinoma *in situ* with 10%. Hormonal status of 72% women were postmenopausal; only 20% were premenopausal, 5.5% had undergone hysterectomy and 1.8% stopped menstruating as a result of chemotherapy. As for menarche 12 4% happened to be less than 12 years. Other risk factors, such as hormone replacement were present in only 6.2% of the studied population. As nulliparity was observed in 12.3% of patients, looking at the family history of breast cancer we obtained a percentage of 49.1%. The assessment of compliance with the adjuvant pharmacotherapy made by Morisky and Green questionnaire shows a fulfillment of only 31.85% (36) of patients, and 68.14% (77) was considered non-adherent.

The second tool used to determine compliance with not only pharmacotherapy, but treatment as a whole, is a self-report questionnaire that consists of two parts. The first is composed by two questions in which in positive cases, the patient has a high risk of non-compliance to the treatment. The questions are dichotomous and only 9.7% already thought about stopping all treatments. The second part, also called low risk of non- compliance made in the form of Likert scale, obtained a score of 2.6 (± 2.4).

Comparing the average scores of patients who considered quitting with those who did not consider quitting, we achieved a statistically significant difference (Mann-Whitney = 19.97, p = 0.04). The average scores of the patients who had diarrhea were statistically higher than those patients who did not have (Mann-Whitney = 10.48, p = 0.001). But when we evaluated the scores of people who had bleeding or fever with the scores of people who had no bleeding or fever, there were no significant differences. Ingestion of 5 or more medicaments is characterized as polypharmacy. These values were found in over 10% of patients in this study and studies of literature.

DISCUSSION

The translation was well accepted by patients, since there was little or no difficulty in understanding the issues. It was also effective in identifying people with the most prone behavior of not completing their treatment, making it necessary for further monitoring by health professionals.

The sample population of more than 80% has lobular cancer attacker, which, according to literature, is also the most frequent between the breast cancers; also lobular carcinoma *in Situ* was observed, with 10%, which is also the value found in literature (Adami et al., 2008). And contrary to the study of Santa Catarina, over 70% of invasive ductal carcinoma was found (Moreno et al., 2012).

The hormonal status of women is a risk factor for the onset of breast cancer. Literature shows that the greater the exposure time to the female hormones, for example, the difference between menarche and menopause the higher the probability of breast cancer (Brazil, 2009). And for every year that menarche delays, the risk of having breast cancers diminishes by 5%(Key et al., 2001). Hormone replacement is an important risk factor that increases the vulnerability of women towards breast cancer and was found in 6.2% (Kirk and Hudis, 2008). It is already known that the use of hormone replacement therapy for more than five years may increase the risk of breast cancer by 34% with isolated estrogens and by 53% when combined with progesterone (Key et al., 2001). Another important risk factor is genetic inheritance; all cancers are genetic. This risk factor is very important and appears in almost 50% of patients (Brazil, 2009).

Non-compliance in this study was found in around 70% of patients. This demonstrates a low compliance rate and increases the likelihood of recurrence. Closer values found in literature were 43.6% of non- compliance (Kirk and Hudis, 2008). The self-reported questionnaire for evaluating any treatment consists of two parts: the first part is called high risk of non- compliance to treatment. It has two questions with dichotomous answers and only 9.7% already think about stopping treatment. This demonstrates that patients like the treatment. The second part, also called low risk of non- compliance made in the form of Likert scale, obtained a score of 2.6 (± 2.4); the literature article scores a 4.3 (± 4.2), which shows the good compliance to the treatment (Kondryn et al., 2009).

The average scores of patients that thought about stopping the treatment compared to the average scores

Table 1. Transcultural translation and semantic equivalence of the original instrument in English (O), consensus of versions for the Portuguese (TC), consensus of back-translation to English (RC) and final version in Portuguese (F).

O	TC	RC	F
1. Have you ever considered stopping ALL your treatment?	1.Você já pensou em parar TODO o seu tratamento?	1. Have you ever thought about stopping ALL of your treatment?	1. Você já pensou em parar TODO o seu tratamento?
2. Have you ever considered stopping PARTS of your treatment? IF YES, please say which part(s) of treatment you have considered stopping?	2. Você já pensou em parar PARTES do seu tratamento? SE SIM, por favor, diga que parte(s) do tratamento você pensou em parar?	2. Have you ever thought about stopping PARTS of your treatment? IF YES, please say which part (s) of the treatment you have thought about stopping?	2. Você já pensou em parar PARTES do seu tratamento? SE SIM, por favor, diga que parte(s) do tratamento você pensou em parar?
3. Have you ever tried to change with the medical team the timing of any aspect of your treatment?	3. Você já tentou mudar com a equipe médica qualquer aspecto do seu tratamento?	3. Have you ever consulted with your medical professionals about changing any aspects of your treatment?	3. Você já tentou mudar com a equipe médica qualquer aspecto do seu tratamento?
4. Have you ever tried to change the type of treatment you are receiving/will be receiving?	4. Você já tentou mudar o tipo de tratamento que você está recebendo ou vai receber?	4. Have you ever tried to change the type of treatment that you are receiving or will receive?	4. Você já tentou mudar o tipo de tratamento que você está recebendo ou vai receber?
5. How often do you forget to take a dose of your prescribed oral (taken by mouth) medicines? If you have forgotten to take a dose(s), can you give a reason(s) for this	5. Com que frequência você se esquece de tomar uma dose de seu medicamento oral prescrito? (ingerido pela boca) Se você se esqueceu de tomar uma dose (s) do seu medicamento pode dizer o por quê?	5. How often do you forget to take your prescribed oral medication? (taken by mouth) If you have missed a dose (s) of your medication, why?	5. Com que frequência você se esquece de tomar uma dose de seu medicamento oral prescrito? (ingerido pela boca) Se você se esqueceu de tomar uma dose (s) do seu medicamento pode dizer o por quê?
6. Do you ever take your oral drugs at the wrong time?	6. Alguma vez você já tomou seus medicamentos orais na hora errada?	6. Have you ever taken your prescribed oral medications at the wrong time? If yes. How often?	6. Alguma vez você já tomou seus medicamentos orais na hora errada?
7. Have you ever taken the wrong dose?	7. Você já tomou a dosagem errada?	7. Have you ever taken the wrong dosage? If yes. How often?	7. Você já tomou a dosagem errada?
8. When you feel well, do you ever stop taking your oral prescribed drugs?	8. Quando se sente bem, alguma vez já parou de tomar seu medicamento oral prescrito?	8. When you feel good, have you ever stopped taking your prescribed oral medication?	8. Quando se sente bem, alguma vez já parou de tomar seu medicamento oral prescrito?
9. When you feel worse, do you ever stop taking your medicines?	9. Quando se sente pior, já parou de tomar seus medicamentos?	9. When you feel worse, have you ever stopped taking your prescribed oral medication?	9. Quando se sente pior, já parou de tomar seus medicamentos?
10. Are you the one responsible for making sure you take your prescribed tablets/medicines? If NO, who is responsible?	10. Você é o único responsável por certificar-se de tomar os comprimidos/medicamentos prescritos? Se não, quem é o responsável?	10. Are you solely responsible for making sure you take your medication? (prescribed and injection) If not, who is responsible?	10. Você é o único responsável por certificar-se de tomar os comprimidos/medicamentos prescritos? Se não, quem é o responsável?
11. Have you ever missed any out-patient appointments?	11. Você já perdeu uma consulta ambulatorial?	11. Have you ever missed any outpatient appointment (treatment, exams and consultations)? If yes, how often?	11. Você já perdeu uma consulta ambulatorial?
12. Have you ever missed any in-patient admissions?	12. Você já perdeu alguma admissão hospitalar?	12. Have you ever missed any inpatient appointments (treatment, examinatiobs and consultations)? If yes, how often?	12. Você já perdeu alguma admissão hospitalar?

Table 1. Contd.

13. Have you ever refused help at home from any of the following people - District Nurse, GP, Liaison Nurse? If you have ever refused help at home from these people, can you give a reason for this	13. Alguma vez você já recusou a ajuda em domicilio de algum desses profissionais – Enfermeiro ou Medico? Se você já se recusou a receber a ajuda de alguns desses profissionais, você poderia dar uma razão para isso?	13. Have you ever refused medical care at your home from any of these medical professionals - Nurse or Doctor? If you have refused to receive help from these professionals, could you please give a reason(s) why?	13. Alguma vez você já recusou a ajuda em domicilio de algum desses profissionais – Enfermeiro ou Medico? Se você já se recusou a receber a ajuda de alguns desses profissionais, você poderia dar uma razão para isso?
14. Since diagnosis have you ever failed to seek medical help for: a temperature, diarrhea, bleeding, If you have answered YES to any part of Question 14, how long is the longest period of time before you have got medical help?	14. Desde o diagnóstico você já teve dificuldades ao procurar ajuda médica quando você teve febre, diarreia, sangramento. Se você respondeu sim para qualquer parte da pergunta 14, quanto tempo demorou para você consegui ajuda medica?	14. Since your medical diagnosis, have you ever had difficulties in finding medical attention when you had: If you answered yes to any part of this question, how long did it take you to get medical attention?	14. Desde o diagnóstico você já teve dificuldades ao procurar ajuda médica quando você teve febre, diarreia, sangramento. Se você respondeu sim para qualquer parte da pergunta 14, quanto tempo demorou para você consegui ajuda medica?
15. Have you ever forgotten to follow your mouth care routine?	15. Você já se esqueceu de seguir sua rotina de cuidados com a boca?	15. Have you ever forgotten to follow your routine for oral health care?	15. Você já se esqueceu de seguir sua rotina de cuidados com a boca?
16. Have you ever forgotten to care for your central line?	16. Você já se esqueceu de cuidar de seu acesso central?	16. Have you ever forgotten to take care of your central line? If yes, how often?	16. Você já se esqueceu de cuidar de seu acesso central?
17. Since diagnosis have you ever refused a medical examination?	17. Desde o diagnóstico você já se recusou a fazer um exame médico?	17. Since your diagnosis, have you ever refused to do any medical examination?	17. Desde o diagnóstico você já se recusou a fazer um exame médico?
18. Do you feel you have been adequately supported in dealing with your treatment? If yes, by whom? mother, father, siblings, friend, partner, doctors, nurses, psychologists, other (please state)	18. Você sente que foi apoiado de forma adequada ao lidar com seu tratamento? Se sim, por quem? Mãe, Pai, Irmãos, Amigo, Parceiro, Médicos, Enfermeiros, Psicólogos ou Outros (por favor, indique)	18. Do you feel that you have received adequate support to deal with your treatment? If yes, from whom? Mother, Father, Brother, Friend, Partner, Doctors, Nurses, Psychologists and/or others (please specify)	18. Você sente que foi apoiado de forma adequada ao lidar com seu tratamento? Se sim, por quem? Mãe, Pai, Irmãos, Amigo, Parceiro, Médicos, Enfermeiros, Psicólogos ou Outros (por favor, indique)
19. Please feel free to comment upon any other aspects of the cancer treatment you have found particularly demanding.	19. Por favor sinta-se a vontade para comentar sobre qualquer outro aspecto do tratamento de câncer que você considerou particularmente exigente?	19. Please feel free to comment on any aspect of your cancer treatment that you have found particularly challenging:	19. Por favor sinta-se a vontade para comentar sobre qualquer outro aspecto do tratamento de câncer que você considerou particularmente exigente?

*Questions 1, 2, 10, 14 and 18 are dichotomous, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17 are structured through the Likert scale and the question 19 is an essay question.

of patients who did not think of stopping had a statistically significant difference (Mann-Whitney = 19.97, p = 0.04). This demonstrates that patients who thought about quitting the treatment are more likely not to comply with it.

The average scores of the patients who had diarrhea were statistically higher than those patients who did not have (Mann-Whitney = 10.48, p = 0.001). This was also observed by other authors (Kondryn et al., 2009). But when we evaluated the scores of people who had bleeding or fever with the scores of people who did not have bleeding or fever, there was no significant difference. This data show that adverse effects are an obstacle to the continuity of the treatment, as also found by daCosta et al., (2014). Therefore, knowledge of the patients about possible adverse events improves compliance as already reported in the literature (Flores and Mengue, 2005).

Ingestion of 5 or more medicaments is characterized as polypharmacy. Polypharmacy was defined in the literature as one of the factors that negatively influence performance; although not found in a similar study, because they have different populations (Lessa, 1998; Flowers and Mengue, 2005; Kondryn et al., 2009).

Conclusion

It is concluded that the translation and cultural adaptation

of the "Cancer Patient Self-Report Non-Adherence Questionnaire" was well accepted and understood by the targeted audience. There is a low level of compliance with drug therapy and a good level of compliance with treatment. Therefore, it is necessary to further research on identifying the factors that lead to non-compliance with drug therapy.

Conflict of interest

The authors have not declared any conflict of interest

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Full Length Research Paper

Evaluation of clinical parameters in people living with HIV undergoing pharmacotherapeutic monitoring: Viral load, CD4+ T lymphocytes and adherence to antiretrovirals

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The aim of this study was to evaluate the clinical indicators (viral load- VL, CD4 lymphocytes and adherence) of HIV+ patients, at the beginning of treatment with antiretrovirals (ARV), during pharmacotherapeutic monitoring (PTM) in a specialized center in Fortaleza, Ceará. The longitudinal study, according to the Dáder method, was used for patients with HIV (n = 100) from 2008 to 2012, beginning at the time of dispensation of the antiretroviral therapy. The data were analyzed using SPSS®. To evaluate the VL and CD4 levels, the Wilcoxon's test was carried out and the patients were used as temporal controls for themselves regarding the outcomes assessed at the beginning and end of the PTM. Adherence was determined by self-report and pharmacy dispensing records (PDR) of antiretrovirals. There was a mean reduction of 89.45% (SD = 0.28986) in total VL ($p < 0.001$). For CD4+ lymphocytes, a mean increase of 124.14% (SD = 1.31756) was detected ($p <0.001$) during the PTM. Most treated patients showed high rates of adherence by self-report (95.0%, n = 100) and (76.0%) PDR methods. The findings of the present work demonstrated the potential benefits of PTM on treatment adherence, which may have been decisive for the successful improvement of the assessed clinical indicators. The inclusion of PTM for people living with HIV/AIDS (PLHIV/AIDS) in clinical services should be encouraged at the level of secondary health care.

Key words: HIV, AIDS, viral load, adherence, pharmaceutical care.

INTRODUCTION

The highly active antiretroviral therapy (HAART) has resulted in increased patient survival rates. Thus, continuing clinical assessment by objective indicators is important. CD4+ T-cell and viral load (VL) measurements are fundamental parameters for deciding when to the start and evaluate effectiveness of the antiretroviral therapy (Brito, 2012).

Adherence to antiretroviral drugs is a fundamental and

decisive factor for successful virological suppression and immune function preservation in people living with HIV/AIDS (PLHIV/AIDS). To achieve an optimal therapeutic result in the long term, more than 95% of antiretroviral doses must actually be taken (Chen et al., 2007) and that represents one of the major challenges for patients and professionals dealing with HIV (Felix and Ceolim, 2012). This is one of the reasons why the pharmacist must effectively participate in the specialized care team treating HIV patients around the country, since these professionals are one of the most important links in the chain of logistics regarding drug use. Strengthening the patient-pharmacist relationship can lead to the best therapeutic results and quality of life (Vieira, 2007).

The Brazilian government offers support to HIV patients, so they can have access to antiretroviral drugs (Gomes et al., 2009). In general, Brazilian HIV+ patients are first seen by a physician (when they receive the diagnosis) and are later treated by other members of the multidisciplinary team, especially in specialized care centers (Brasil, 2010). However, studies on adherence show that the process of understanding health and disease, and especially the importance of correct administration of medication are still incipient in this model of care and require the implementation of new strategies for improving care in the complex field, which is, the treatment of PLHIV.

The monitoring of PLHIV involves a broad dimension of closely-associated knowledge, skills and interfaces and the detailed understanding is crucial for the decision-making process of the best strategies for a successful therapy (Silveira et al., 2010). Thus, the analysis of several indicators, such as the clinical (virological and immunological count) and the therapeutic ones (adherence rate), combined with the socioeconomic profile of each patient, becomes an important tool for the monitoring of these patients and the pharmacist can strategically collaborate with the process (Okoye et al., 2014).

Therefore, pharmaceutical care through pharmaco-therapeutic monitoring (PTM) can have a positive role, aiming at achieving rational pharmacotherapy, as well as defined and measurable clinical outcomes (Opas, 2002). During PTM, the provided pharmaceutical care helps HIV-positive patients address the factors that lead to poor adherence; improves their knowledge on the disease and the treatment plan, and especially, helps them to understand and accept the need for high therapy compliance (Dader et al., 2008).

Based on this context, the aim of this study was to demonstrate the evolution pattern of the clinical indicators, viral load and CD4+ T- lymphocytes, in a sample of HIV-positive patients monitored in a pharmaceutical care program since the start of antiretroviral treatment, and also to disclose their sociodemographic and adherence profile.

MATERIALS AND METHODS

This was a longitudinal, follow-up study, carried out between November, 2008 and January, 2012 in a secondary care reference unit with a specialized service for PLHIV, the José de Alencar Center of Medical Specialties (CEMJA). Patients were selected according to the following inclusion criteria: adult outpatient patients aged ≥ 18 years, using antiretroviral therapy (treatment-naive), who had not participated in any pharmaceutical intervention study and agreed to participate by signing a term of consent. Each patient served as his or her own control. The pharmacotherapeutic monitoring (PTM) was the main intervention and lasted for nine (9) months, being developed according to the Dáder et al. (2008) method, which involves the following steps: 1. Service provision; 2. initial interview, 3. situation status; 4. study phase 5. global assessment, 6. pharmaceutical intervention and 7. evaluation of the outcomes. Periodic evaluations were made to assess the effectiveness of the performed pharmaceutical interventions, which were continually documented in a PTM form designed by a group of experts. The form included data on the sociodemographic profile, habits and lifestyle of the PLHIV; pharmacotherapeutic and pharmaceutical care data, adherence and other information related to medication use. Table 1 shows the established parameters, tools and frequency of measurements according to the follow-up period. The study was designed according to the guidelines and norms for research involving human subjects and was approved by the Ethics Committee in Research of the Federal University of Ceará (Protocol 191/08). To ensure confidentiality of the obtained information, the data were analyzed in aggregate form.

Regarding adherence evaluation, the literature (Polejack and Seidl, 2010) recommends using at least two assessments to increase result accuracy. In this study, the self-report (Delgado and Lima, 2001) and the Pharmacy Dispensing Records (Brasil, 2010) methods were chosen. The assessment by self-report was carried out through direct interviews using a semi-structured questionnaire consisting of seven questions and answers graded according to Likert scale. After obtaining the results, the answers to each question were summed and divided by the total number of questions and the value obtained was converted into a dichotomous scale used to define 'adherent' and 'non-adherent' to treatment. All the monthly records of antiretroviral (ARV) dispensation were analyzed through the pharmacy dispensing record method in the Pharmaceutical Care Unit (PCU) of CEMJA until the 9th month of PTM. Thus, ARV dispensations were expressed by their prevalence during a nine-month period, regardless of the time of occurrence of the same, and categorized into three groups according to the recommended protocol of Pharmaceutical Services, Ministry of Health (Brasil, 2010): a) Regular (adherent): When there was no irregularity, either in time or in the quantity dispensed until the 9th month of follow-up; b) Irregular (non-adherent): when the time between the dispensations was at least one day longer than the average time, or when the number of dispensed tablets was less than 95% of the total number of tablets expected for each ARV scheme prescribed until the 9th

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Table 1. Parameters, tools and frequency of measurements according to the PTM period of HIV-positive patients.

Clinical parameters	Tools, data sources, laboratory tests, location of analysis of indicators	Frequency of measurement
Measurement of CD4+ T lymphocytes	Tool: Recording in an individualized form of pharmacotherapy monitoring of PLHIV/AIDS Data source: Results of laboratory tests in Medical Record Form Laboratory test: Flow Cytometry Method Place of analysis of the test: Central Laboratory of Ceará State	Start (1st month) and End (9th month) of PTM
Measurement of Viral Load	Tool:Recordingin an individualized form of pharmacotherapy monitoring of PLHIV/AIDS Data source: Results of laboratory tests in Medical Record Form Laboratory test: bDna Method Place of analysis of the test: Central Laboratory Of Ceará State	Start (1st month) and End (9th month) of PTM
Measures of Adherence	Tool:Recordingin an individualized form of pharmacotherapy monitoring of PLHIV/AIDS Data source: Drug Control Logistics System (SICLOM – Ministry of Health) Method of Adherence Assessment 1: dispensing pharmacy records of antiretroviral drugs Method of Adherence Measuring 2: Self-report Place of analysis of the indicator: outpatient pharmacy services (SAE/CEMJA) - Unit of Pharmaceutical Care for HIV Patients	- Monthly (during 09 months of PTM – To assess the adherence profile).

month of PTM. The number of times that an irregular dispensation occurred for each patient was computed, regardless of when it occurred; c) Treatment dropout: when the patient remained for more than 90 days without taking ARVs after the coverage period for the last dispensation and did not return until the 9th month of PTM to restart treatment. For a complete evaluation of each case, it was necessary to associate this situation with other monitoring factors, for instance, "no shows" to scheduled medical appointments and no return in six months, in addition to those previously mentioned. Viral load was determined through DNA method and the CD4+ T cells through flow cytometry.

Statistical analysis

Data were entered into a structured database using the Statistical Package for Social Sciences (SPSS) software, version 11.0 and analyzed with the support of statisticians of the Federal University of Ceará. For the information regarding the socioeconomic profile (age, weight, sex, marital status, ethnicity, educational level, occupation and income) and pharmacotherapeutic indicators (adherence), the simple frequencies and percentages were presented for each category, considering only the patients who had available information. Adherence was expressed as their simple frequencies and percentages. Clinical indicators (viral load/CD4+ T lymphocytes) were analyzed by comparing the initial and final profiles using the paired nonparametric Wilcoxon´s test.

RESULTS

Initially, a total of 105 patients were selected; however, four patients were excluded (patients with cognitive difficulties and prisoners) and 01 patient died. Of those remaining (n=100), only <50 patients were used for the evaluation of the clinical indicators (CV and CD4) analyzed in this study, because the others either missed adherence monitoring or did not undergo laboratory tests, invalidating the before/after comparative analysis.

Table 2 shows the sociodemographic parameters of the study sample (n = 100). Regarding the age of the patients, 90.0% (n=100) were aged between 19 and 40 years (mean=35.42, min = 19, max = 66, SD = 10.61). The majority of patients were single (62.0%, n=100) and lived in Fortaleza, state of Ceará (96.0%). There was a predominance of males (69%; n=100) and mixed-race patients (65.0%, n=100). The analysis of schooling showed that a significant number of respondents had finished elementary school (42.0%, n= 42).

The results of the viral load and CD4 + T lymphocytes variables of patients receiving PTM are shown in Tables 3 to 6. Table 3 lists all patients submitted to this assessment at some moments; it was observed that the mean viral load at baseline (mean = 63838.71 copies/mL, SD = 80403.55 copies/mL) was well above the mean value at the end time (mean = 54 copies/mL, SD = 20.044 copies/mL), with the standard deviation at the initial time being also quite high, that is, the viral load measurements at the end time (VL = 37.12%) were more homogeneous and close to the respective mean than at baseline (VL = 125.95%). According to the observed data, a mean reduction of 89.45% (SD = 0.28986) in the viral load of all patients were found in PTM.

Of the 100 PLHIV/AIDS, only 27 had viral load tests at

Table 2. Distribution of PLHIV/AIDS according to their sociodemographic profile, CEMJA, Fortaleza- Ceará (Dec/2008 – Dec/2012).

Variables		N
Age	<30 years	33
	30 to 39 years	35
	40 to 49 years	22
	>50 years	10
	Total	100
City	Fortaleza	96
	Another city	04
Gender	Female	31
	Male	69
	Total	100
Marital Status	Married	36
	Single	62
	Widowed	02
	Total	100
Ethnicity	Mixed-Race	25
	White	65
	Black	10
	Total	100
Education	Illiterate	04
	Incomplete elementary school	27
	Complete elementary school	11
	Incomplete high school	8
	Complete high school	32
	Incomplete College/University	6
	Complete College/University	12
	Total	100

Source: Direct Research, José de Alencar Center of Medical Specialties (CEMJA), November 2008/January 2012.

the beginning and at the end of the PTM. This may have been caused by different reasons, both related to the health system, as well as patient-related factors. Of those who had available test results, 90% (n = 27) of them were below 50 copies/mL, which is the target result for viral load when using antiretroviral therapy.

Considering only the patients in whom viral load measurements were performed at the two different points in time, that is, start and end (n=27), Wilcoxon´s test was used to compare the viral load measurements at these two points in time, leading to the inference that there is a significant difference between the values of initial and final viral loads in this study of pharmacotherapeutic monitoring of PLHIV/AIDS (value of the statistic W = -4.372, p-value <0.001).

The analysis result for the association between the adherence profile and the method of dispensing and the recorded values of viral load found is shown in Table 4. The mean baseline viral loads were well above the mean of the final viral load and the variability around the mean is also substantially higher. The mean reduction in viral load was 86.19% for the non-adherent patients and 90% for the group of adherent patients.

As for the Wilcoxon test as compared to the initial and final viral loads for adherent patients, a significant difference between measurements was found (value of statistic W = --3.724, p-value <0.001). For non-adherent patients, the value of the statistic was W = -2.366, with p-value = 0.018, indicating that there is a difference at the 5% significance level.

Another important indicator for clinical and laboratory monitoring for PLHIV/AIDS is the CD 4 + T lymphocyte count, as it indicates the body's positive immune response and acts decisively to minimize the morbidity and mortality of this disease when levels are found in standardization. According to the observed data (Table 5), the initial CD4 lymphocyte count was higher than 200 in 48.8% (n = 43) of the patients for whom the measurement was available in some of the assessments (initial or final), whereas the final CD4 was higher than 200 in 81.3% (n = 32) of patients. Thus, a considerable increase in the number of leukocytes was noticed in this class for PLHIV assessed during the course of PTM, with an average increase of 124.14% (SD= 1.31756).

Descriptive statistics in Table 5 show a lower T CD4 + lymphocyte count at baseline (minimum of 13 cells/mm^3, mean = 204.88, SD = 111.91 cells/mm^3) than at the end of PTM (maximum = 805 cells/mm^3, mean = 384 and SD = 186.61 cells/mm^3) and this difference was statistically significant when Wilcoxon test was applied (statistic W = -4.433, p-value <0.001), considering only those patients in whom CD4 measurements were obtained at two different points in time, that is, start and end (n=30). The variability around the means had similar values.

Similar to the viral load, the association between adherence and the mean values of CD4+ T lymphocytes was also studied. Based on the intersection of these data, the CD4+ T count was higher at the end time for both groups of patients, adherents and non-adherents, with the greatest difference being observed in the first group (Table 6). Thus, it was found that non-adherent patients had an average increase of 55.96% in the rate of CD4, whereas this increase was 148.94% for adherent patients, emphasizing the importance of adherence to the antiretroviral therapy.

To assess whether this difference had statistical significance, Wilcoxon test was performed to compare the values, which was only possible in 30 monitored individuals, as not all of them had both the test and

Table 3. Statistical profile for the clinical indicator viral load (VL) of PLHIV/AIDS in PTM.

Analysis time	N	Minimum (copies/ml)	Maximum (copies/ml)	Mean (copies/ml)	Standard Deviation (copies/ml)	Coefficient of variation (%)	Median (copies/ml)	Interquartile Range
Baseline viral load	38	< 50	299268.00	63838.71	80403.55	125.95	30138.50	93909.75
Final viral load	30	< 50	149.00	54.00	20.044	37.12	49.00	0.00

Source: Direct Research, José de Alencar Center of Medical Specialties (CEMJA), November 2008/January 2012.

Table 4. Statistical analysis between the viral loads at the baseline/end and adherence of PLHIV/AIDS in PTM.

Adherence classification	Analysis time	N	Minimum (copies /ml)	Maximum (copies /ml)	Mean (copies/ ml)	Standard deviation (copies/ml)	Coefficient of variation (%)	Median	Interquartile range
Non-adherent	Baseline Viral Load	11	49.00	296442.00	51847.27	87146.62	168.08	13303.00	74585.00
	End Viral Load	9	49.00	50.00	49.11	0.33	0.67	49.00	0.00
Adherent	Baseline Viral Load	27	49,00	299268.00	68724.11	78696.99	114.51	57281.00	100871.00
	End Viral Load	21	49.00	149.00	56.09	23.82	42.47	49.00	0.00

Source: Direct Research, José de Alencar Center of Medical Specialties (CEMJA), November 2008/January 2012.

Table 5. Statistical profile for the clinical indicator CD4+ T lymphocytes of PLHIV/AIDS in PTM.

Analysis time	N	Minimum (copies/ml)	Maximum (copies/ml)	Mean (copies/ml)	Standard Deviation (copies/ml)	Coefficient of variation (%)	Median	Interquartile range
Baseline CD4	43	13.00	593.00	204.88	111.91	54.62	200.00	117.00
End CD4	32	71.00	805.00	384.03	186.61	48.59	348.50	255.00

Source: Direct Research, José de Alencar Center of Medical Specialties (CEMJA), November 2008/January 2012.

adherence results. Thus, it can be concluded that the difference between the CD4 counts is significant for adherent patients (W = -4.107 p-value <0.001). We once again emphasize that the number of non-adherent patients is small, which may have compromised the test results (W = -1.680, p-value = 0.093).

In relation to adherence, the self-report method showed an overall adherence rate of 95.0% (n = 100) among HIV patients undergoing pharmacotherapeutic follow-up. On the other hand, the Pharmacy Dispensing Records method showed median, mean and standard deviation values for the time between the dispensations of respectively, 30.00, 33.07 and 15.55 days. Also, the minimum time between dispensations was equal to one day and the maximum was 220 days. The 95% confidence interval for the mean time between dispensations was equal to 30.03 and 36.12. Table 7 shows a good compliance to antiretroviral pharmaco-therapy with a regular adherence rate of 76% for the group undergoing monitoring and 10% (n = 100) for patients that withdrew from the study (> 90 days without returning).

DISCUSSION

AIDS has a strong negative impact on the current context of health. Considering the importance of the pandemic and the need for studies that associate adherence with clinical indicators through new care strategies for PLHIV (Santos et al., 2010), the present study was designed in the context of pharmaceutical care and its interfaces with these patients. In particular, the sociodemographic characteristics of the monitored patients coincide with those of other national and international studies (Echevarría et al., 2004).

Regarding the monitoring of therapeutic success of HIV+ patients, the main clinical indicators are the viral load and CD4 lymphocyte count, because these parameters of immunological evaluation are important in determining factors related to drug therapy (Brazil, 2008; Hirsch et al., 2009). Some data show that a low count of CD4+ T cells may be a risk factor related to the disease that affects the patient's adherence to treatment (Schilkowsky et al., 2011). Studies try to explain this situation using two theories: the physical and cognitive

Table 6. Statistical analysis between the CD4 count at the baseline/end and adherence of PLHIV/AIDS in PTM.

Adherence Classification	Analysis Time	N	Minimum (copies/ml)	Maximum (copies/ml)	Mean (copies/ml)	Standard Deviation (copies/ml)	Coefficient of Variation (%)	Median	Interquartile Range
Non-adherent	Baseline CD4	12	112.00	428.00	219.17	90.26	41.18	221.00	133.25
	End CD4	9	71.00	675.00	374.55	184.19	49.17	355.00	275.50
Adherent	Baseline CD4	31	13.00	593.00	199.35	120.14	60.26	194.00	120.00
	End CD4	23	133.00	805.00	387.74	191.53	49.39	342.00	262.00

Source: Direct Research, José de Alencar Center of Medical Specialties (CEMJA), November 2008/January 2012.

Table 7. Distribution of PLHIV receiving PTM in relation to the adherence profile (dispensing pharmacy records method).

Classification: Pharmacy Dispensing Records			Total
Regular adherence	Irregular adherence	Treatment dropout	
76	14	10	100

Source: Direct Research, José de Alencar Center of Medical Specialties (CEMJA), November 2008/January 2012.

patient receives as the disease progresses (Melchior et al., 2007; Gir et al., 2005; Garrido and Castro, 2005).

In pharmacotherapeutic monitoring studies (Eidam et al., 2006; Martinez, 2012; Rathbun et al., 2005; Ma et al., 2010; Henderson et al., 2011; Moriel et al., 2001; Silveira, 2009) conducted with people living with HIV, pharmaceutical interventions also positively influenced the clinical outcomes through improved adherence to ART and, hence, suppression of viral load and increase in CD4+ count. In this study, both clinical indicators were statistically significant ($p<0.001$) before/after PTM. One possible explanation is the fact that the group that is being studied underwent a closer monitoring, which probably minimizes the concerns about the health-disease process and, consequently, facilitates the adherence to treatment. The attainment of expected levels of CD4+ T cells and viral load is intrinsically related to patient adherence to ART, with the possibility of near-normal values in immunological evaluation indices (Rocha et al., 2001).

Adherence refers to the degree to which the patient's behavior related to the therapeutic regimen fits what was established by the physician and the multiprofessional team. Adherence includes the willingness to undergo treatment and the ability to take the medications as prescribed (Gusmão and Mion, 2006). It is a multifactorial and dynamic process that encompasses physical, psychological, social, cultural and behavioral factors; it requires decisions that are shared and communicated between the PLHIV, the multidisciplinary health care team and their social networks (Saldanha et al., 2009).

Inadequate adherence to treatment of chronic diseases is an important worldwide problem. In developed countries, mean adherence to the continuous use of drugs is 50% and in developing countries, this percentage is even lower (Oigman, 2006). The studies regarding adherence to antiretroviral agents, in particular, have shown rates ranging from 37 to 83% (Sabaté, 2003). A meta-analysis of North American studies reported rates between 28.3 and 69.8% (Kim et al., 2014). This rate depends on the studied drug, method and demographic characteristics. In Brazil, a review (Bonolo et al., 2007) identified that the level of non-adherence to antiretroviral drugs ranged from 5 to 67%. In this case, observational research with these patients, previously done in same place and using the same two methods, albeit without PTM, found a compliance rate of 45.7%.

In this study, most patients receiving PTM showed good adherence with the used methods (95% and 76%) as compared to other services that use traditional dispensing models and do not follow patients through PTM. It should also be noted, that non-dispensation of drugs to those patients with a lower adherence was not due to lack of medication supply, but rather because of non-attendance of the patient or the caregiver at the Pharmaceutical Care Unit to receive the drugs on the day scheduled by the pharmacist.

Another major challenge for those working with HIV+ patients is to choose the most effective method of measuring adherence to drug treatment (Ventura, 2006). The literature does not mention an established method for assessing adherence as the “gold standard” (Chesney,

2006) and all of the methods have advantages and limitations to be overcome. No single method provides a precise result and two or more concomitant methods should be used to improve accuracy (Mcmahon et al., 2011). The self-report method has the advantages of easy application and low cost, but generally leads to overestimated results, which may have occurred in this study that found an adherence rate of 95%.

The method of pharmacy dispensing records was chosen for this study because the Brazilian government already provides an electronic control system for ART, which facilitates the operationalization of measuring adherence through this mechanism. This method has been increasingly the object of interest in studies with PLHIV (Ross-Degnan et al., 2010). Researchers recently conducted a review on this subject and identified 36 studies (24 in developed countries and 12 in developing countries) that evaluated the association between the pharmacy-dispensing data and measurements of adherence and laboratory or clinical results. The data showed that the measurement methods that included the number of days during which a patient received the antiretroviral drugs seemed to be more effective. Four of these studies clearly favored the use of pharmacy data, while only one favored self-reporting (Keith, 2011).

Literature shows that the practice of pharmaceutical care through PTM improves adherence to antiretroviral (Rodrigues et al., 2010; Hernanz et al., 2004) and significantly contributes to the care of PLHIV. Other authors demonstrated that patients who were followed by clinical pharmacists had significant improvement at the initial moment in their CD4+ lymphocyte count, in viral loads and in the management of adverse reactions (March et al., 2007). Souza et al. (2010) considered that pharmacotherapeutic guidance during PTM was effective in promoting continued adherence to antiretroviral treatment, because all patients who adhered to treatment in the intervention group maintained an undetectable viral load.

Some limitations were detected in this study. Initially, the very specific care required for PLHIV/AIDS already constitute a challenge due to social, cultural, economic and psychological dilemmas faced by these individuals, which have an impact in terms of meeting the schedule and adherence to recommendations established between health professionals and patients. Additionally, for technical and operational reasons for facilitate the study, and because this was a convenience sample, through funding by temporal demand, it may have passed on a sample size with a number not as robust and sufficient to perform statistical tests to subsidize a more accurate analysis of the intended outcomes.

Other limitations of the small size of PLHIV/AIDS, was conducting benchmarking follow-up viral load CD4+ lymphocyte measures at the laboratory in the start and end times so that the differential analysis could be performed. This may reflect the difficulties of access and structure of services or even organization between the service unit and the carrying out of laboratory assessments. However, it was often caused by the fact that patients missed the appointment for blood collection. This scenario is quite typical in studies using real-world data. Also, with respect to limitations, for ethical reasons, all patients had to receive pharmaceutical care and it was not possible to use a control group, in which the subjects followed themselves longitudinally, while pharmaceutical care was controlled by the patients themselves during the nine months. Interventions were measured at the beginning and end of the AFT, which limited the interpretation of the findings to be associated with the potential impact of the intervention and pharmaceutical care.

Conclusion

Despite therapeutic and political advances regarding PLHIV in the last decade, a high level of adherence to antiretroviral treatment is still an obstacle to be overcome. The treatment involves a complexity of social, psychological (stigma), pharmacological (adverse reactions and drug interactions) and other factors that impact the implementation of strategies that strengthen holistic care, as well as the monitoring of clinical key indicators such as CD4+ T cells and viral load in order to establish adherence over time.

In this sense, at the time of dispensing, the pharmacist has an excellent opportunity to interact with patients and the interdisciplinary team through an efficient and continuous pharmaceutical care program. The findings of this study showed that most patients undergoing pharmacotherapeutic monitoring improved their assessed clinical indicators (CD4 and viral load), and this may be a reflection of a higher rate of adherence to pharmaco-therapy instituted among patients monitored at the secondary level of health. This highlights the importance of a humane approach in the chain: respecting the psychosocial values during the follow-up of clinical indicators, guidance and monitoring of PLHIV.

The pharmacist can act favorably to achieve the proposed therapeutic goals and improve the quality of life of these patients.

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Conflict of interests

The authors declare that there is no conflict of interest.

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