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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds) Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International, pp 181-190.

Jake OO (2002).Pharmaceutical Interactions between Striga hermonthica (Del.) Benth. and fluorescent rhizosphere bacteria Of Zea mays, L. and Sorghum bicolor L. Moench for Striga suicidal germination In Vigna unguiculata . PhD dissertation, Tehran University, Iran.

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Full Length Research Paper

Biochemical and hematological evaluation of *Costus speciosus* as a dietary supplement to Egyptian buffaloes

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Sixty Egyptian buffalo heifers ageing about one year old, with an average weight of about 250 to 260 kg were allotted randomly according to their live body weight and age into three equal groups; Group I which received a basal ration; Group II which received a basal ration with fine ground *Costus speciosus* roots in a concentration of 2.5 kg/ton ration and Group III which received a basal ration with fine ground *C. speciosus* roots in a concentration of 5 kg/ton ration. Blood samples were collected from each group and divided into two blood samples, one for serum separation and the other for hematological study. Separated serum samples were subjected to the biochemical analysis of glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, uric acid and electrophoretic pattern. The obtained data revealed a decrease in serum glucose and cholesterol levels which may be utilized in anabolic pathways during this period. While total protein, albumin, α_1-globulin, β-globulin, hemoglobin, packed cell volume (PCV) and lymphocytes were significantly increased especially in Group III. In addition, the erythrocytes antioxidant status was significantly improved by *C. speciosus* supplementation. We could conclude that supplementation of *C. speciosus* powder to the Egyptian buffalo heifers improves the health status, total antioxidant capacity and hematology. So, we advise owners to add *C. speciosus* ground powder to the ration of heifers.

Key words: Heifers, *Costus speciosus*, total antioxidant capacity, hematology.

INTRODUCTION

Natural product is a source of bioactive compounds and has potential for developing some novel therapeutic agent. Over the last decade, there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment (Jawla et al., 2009). Herbal drugs are derived either from the whole plant or from their different parts like leaves, bark, roots, flowers, seeds, etc., and also from plant excretory products like gums, resins and latex (Rajashree et al., 2012). Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries. However, the blind dependence on synthetics is over and people are returning to nature, with hope of safety and security (Singab, 2012).

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Zingiberaceae is a family of about fifty two genera and more than 1,300 species distributed throughout tropical Africa, Asia, and the Americas. Many species are very important for example, shell ginger (*Alpinia*), summer tulip (*Curcuma alismatifolia*), ginger lily (*Hedychium*), torch-ginger (*Etlingera elatior*), ginger (*Zingiber*), turmeric (*Curcuma*) and cardamom (*Amomum Elettaria*) (Jiang et al., 2000). *Costus speciosus* is commonly called Crepe ginger. In Sanskrit, it is known as Keyu and in Hindi as Kust (Khare, 2007). *C. speciosus* is a *Zingiberaceae* erect plant, up to 2.7 m high; root stock tuberous stem, sub-woody at the base, occurring in the moist and wet evergreen areas of the Indo-Malayan region and Sri Lanka. Within India, it occurs from Central and Eastern Himalayas to Southern India (Dutta and Dutta, 1998).

C. speciosus contain diosgenin, 5α-stigmast-9(11)-en-3β-ol, sitosterol-β-D-glucoside, dioscin, prosapogenins A and B of dioscin, gracillin and quinones. In addition, it contains α-tocopherol (Husain et al., 1992). Traditionally, *C. speciosus* is used in the treatment of fevers, cough, worm infestations, skin diseases and snake bites. The effects of *C. speciosus* with regard to the following compounds diosgenin, prosapogenin B of dioscin, diosgenone, cycloartanol, 25-en-cycloartenol and octacosanoic acid which extracted from it (Qiao et al., 2002). *C. speciosus* has an anti-inflammatory, anthelmintic, astringent, bitter, depurative, purgative, and stimulant effect while its roots were used as a remedy in fevers, coughs, anti-diabetic, hepatoprotective (Biman and Kamaruz, 2008), the antihyperglycemic, antihyperlipidemic and antioxidant properties of *C. speciosus* has been reported by Bavarva and Narasimhacharya (2008). In addition, its rhizome juice is applied on the head for relief of headache (Gupta, 2010).

An alkaloid extracted from *C. speciosus* rhizomes is a smooth muscle relaxant and enhances antispasmodic activities (Srivastava et al., 2011). Extract of *C. speciosus* rhizomes stimulate the uterine contraction due to non-estrogenic effects (Wanwisa et al., 2011). Plant-derived antioxidants such as tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins and proanthocyanins could delay or provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species and the concomitant lipid peroxidation, protein damage, and DNA strand breaking (Jha et al., 2010). *C. speciosus* has an antioxidant activity measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, total antioxidant capacity, nitric oxide scavenging activity, ion chelating activity, hydroxyl radical scavenging activity and its correlation with total phenolic content (Nehete et al., 2010).

Heifers in this stage of life need special care, so our study aimed to investigate the effect of *C. speciosus* supplementation in Egyptian buffalo on some serum parameters, erythrocytic antioxidant status and its hematological picture.

MATERIALS AND METHODS

Experimental design

The field experiment was carried out at the farm of Faculty of Veterinary Medicine, Damanhour University, Al-Bostan district to study the effect of different concentrations of *C. speciosus* to the ration of Egyptian buffalo for the duration of one month. Sixty Egyptian buffalo heifers ageing about one year old, with an average weight of about 250 to 260 kg were allotted randomly according to their live body weight and age into three equal groups (twenty Egyptian buffalo heifers in each) and housed in a separate part of a shaded pen. Group I (received a basal ration); Group II (received basal ration with fine ground *C. speciosus* roots in a concentration of 2.5 kg/ton ration) and Group III (received a basal ration with fine ground *C. speciosus* roots in a concentration of 5 kg/ton ration). Concentrate mixtures were given twice daily at 10 a.m. and 2 p.m. while wheat straw was offered *ad lib*. Drinking water was available for animals during the day. The animals in treated groups were noticed for any clinical signs along the experimental period.

Medicinal plant

C. speciosus roots were obtained and identified in the Faculty of Agriculture, Damanhour University. Specimens of *C. speciosus* rhizomes were preserved at -20°C as a standard stock. The rhizomes were washed, cut, grind, and refined. The ground powder was added to the ration at the concentration of (2.5 and 5.0 kg/ton ration).

Blood samples

The blood samples were collected from the jugular vein by using a sterile sharp needle with wide pore. Two samples were collected from each animal; the samples used for hematological analysis and separation of washed red blood cells (RBCs) were collected in clean and dry test tube containing di-sodium ethylenediaminetetraacetic acid (EDTA) as an anticoagulant while serum samples were collected in dry clean tubes and separated by centrifugation at 3,000 RPM for 10 min. Then, clear serum supernatant were aspirated carefully and subjected to biochemical analysis.

Biochemical analysis

Serum samples were subjected to laboratory analysis of blood glucose (Trinder, 1969), cholesterol (Zak et al., 1954) and alanine transaminase (ALT; EC 2.6.1.2) and aspartate transaminase (AST; EC 2.6.1.1) (Reitman and Frankel, 1957), creatinine (Bartles et al., 1972), urea (Kaplan, 1984), and uric acid (Fossati et al., 1980). Electrophoretic patterns of serum proteins were done by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) performed according to the method recorded by (Laemmli, 1970) while the erythrocytes were washed by using physiological saline, and erythrocyte hemolysates were prepared using digitonin as described by (Kornburg and Korecker, 1955). Hemolysates were used for determination of malondialdehyde (MDA) (Ohkawa et al., 1979), total antioxidant capacity (TAC) (Koracevic et al., 2001) and hemolysates protein (Lowry et al., 1951).

Hematological analysis

Hematological measurements were done by fully automated blood cell count, Exigo, Boule Medical AB, Sweden.

Statistical analysis

The raw data were analyzed according to Statistical Analysis System (SAS) (1996), with one-way analysis of variance (ANOVA), with a value of $P < 0.05$ indicating significance.

RESULTS

All treated animals showed no abnormal clinical signs. Biochemical data due to supplementation of *C. speciosus* were summarized in Tables 1, 2 and 3. After 30 days of treatment with *C. speciosus* by 2.5 kg/ton ration, the serum levels of glucose and cholesterol were significantly decreased. Moreover, there are no obvious changes in the serum ALT, AST, creatinine, uric acid, and urea were stated in comparison to control (Table 1). Furthermore, the concentrations of erythrocytic MDA were significantly decreased. On the contrary, glutathione (GSH) contents in erythrocytes were significantly increased (Table 2).

At the end of the experiment, serum levels of glucose and cholesterol were significantly decreased in the group treated with 5.0 kg of *C. speciosus*/ton ration. Furthermore, the levels of ALT, AST, creatinine, uric acid and urea had no changes in relation to group one. The MDA concentrations in RBCs were statistically highly significantly decreased. On the other hand, GSH was highly significantly increased (Table 2). The electrophoretic patterns of this group were statistically significantly increased in serum total protein, albumin, α_1-globulin and γ-globulin while serum α_2-globulin levels were highly significantly increased. Moreover, no significant changes were observed in the level of β-globulin (Table 3).

The data illustrated in (Table 4 and 5) stated the hematological effects of the treated groups at 30^{th} day of experiment and revealed that the total count of erythrocytes (TEC) was significantly ($P < 0.05$) increased in all buffaloes heifers supplemented when compared with control. Consistent with this, the total leucocytic counts (TLC) were ($P < 0.05$) significantly increase, this increase was more pronounced in heifers in Group III. The present finding also revealed that the erythrocytic contents of hemoglobin (Hb) were statistically significantly increased in in Group III in comparison to control one. The same results were observed in packed cell volume (PCV) which was significantly ($P < 0.05$) increased (Table 4). The data summarized in Table 5 indicated that on the 30^{th} day of the experiment, the percentages of lymphocytes were significantly ($P < 0.05$) increased in all buffaloes heifers supplemented with *C. speciosus* when compared with control which more pronounced in Group III. On the contrary, the percentages of monocytes were significantly decreased ($P < 0.05$) in all buffaloes heifers supplemented with *C. speciosus* after 30^{th} day treatment when compared with one control. Moreover, the percentages of basophils, eosinophils and neutrophils ($P < 0.05$) animals supple-mented with *C. speciosus* had non-significant changes.

DISCUSSION

Our study revealed a significant decrease of serum glucose level after supplementation of *C. speciosus* in the buffaloes ration, this finding might be attributed to either the increase in insulin units released by the beta cells of islet of Langerhans and the increase in sensitivity of cell receptors to insulin consequently increased glucose utilization or both. The hypoglycemic action of eremanthin, a component of *C. speciosus* was caused by potentiation of insulin release from the existing beta cells of islets of Langerhans and increased the sensitivity of insulin to uptake glucose (Li et al., 2004). Its hypoglycemic action was accompanied by an increased hepatic hexokinase activity. Hexokinases provided glucose-6-phosphate, the substrate of glycogen synthase which also activated and increased the hepatic glycogen (Bouche et al., 2004).

Generally, blood glucose levels were decreased by *C. speciosus* supplementation due to the increase in glycogenesis and glycolysis and the reduction in gluconeogenesis (Bavarva and Narasimhacharya, 2008). Costunolide isolated from *C. speciosus* was found to possess normo-glycemic and hypolipidemic effect in streptozotocin-induced diabetic rats. In the study of oral administration of costunolide (20 mg/kg bwt) was significantly decreased the plasma glucose level ($P < 0.05$), glycosylated hemoglobin and at the same time markedly increased plasma (Eliza et al., 2009a). In India, diabetics eat one leaf of *C. speciosus* daily to keep their blood glucose low (Benny, 2004). *C. speciosus* leaf water and methanol extracts effectively reduced the insulin resistance in rats by significantly lowering serum glucose at baseline after one month of the onset of experimental medication (Subasinghe et al., 2012).

C. speciosus affects the lipid metabolism by a significant decrease in serum total cholesterol. This finding came in accordance with that stated and the hexane extract of the rhizome possesses a hypolipidemic activity (Daisy et al., 2008). Moreover, costunolide isolated from the plant significantly decreases serum total cholesterol, and triacylglycerol (Eliza et al., 2009a). In addition, the ethanolic extract of *C. speciosus* of administration reduced plasma and hepatic total cholesterol and triacylglycerol concentrations in diabetic rats (Bavarva and Narasimhacharya, 2008).

These results were in agreement with that of ElRokh et al. (2010) who proved that, the hypercholesterolaemic rats treated with aqueous ginger infusion in the three

Table 1. The mean values of serum glucose (g/dl), cholesterol (mg/dl), ALT (U/L), AST (U/L), creatinine (mg/dl), uric acid (mg/dl) and urea (mg/dl) in Group I, Group II and Group III.

Group		Glucose	Cholesterol	ALT	AST	Creatinine	Uric	Urea
Group I	0 day	45.67±0.13^{a}	145.32±0.01^{a}	25.34±0.01^{a}	35.23±0.01^{a}	0.92±0.01^{b}	1.60±0.01^{a}	14.08±0.04^{a}
	30th day	45.33±0.49^{a}	143.41±0.49^{b}	25.47±0.39^{a}	37.33±0.35^{a}	1.01±0.01^{a}	1.50±0.12^{a}	14.10±0.04^{a}
Group II	0 day	45.47±0.02^{a}	145.23±0.01^{a}	26.23±0.01^{a}	36.80±0.01^{a}	0.91±0.01^{b}	1.60±0.01^{a}	14.12±0.04^{a}
	30th day	37.30±0.45^{b}	124.80±0.41^{c}	26.07±0.43^{a}	35.50±0.24^{a}	0.90±0.01^{b}	1.40±0.08^{ab}	13.70±0.16^{a}
Group III	0 day	45.24±0.01^{a}	145.55±0.01^{a}	25.24±0.01^{a}	36.65±0.01^{a}	0.90±0.01^{b}	1.60±0.01^{a}	14.03±0.03^{a}
	30th day	35.47±0.33^{c}	106.32±0.37^{d}	20.97±2.64^{a}	35.4±0.37^{a}	0.89±0.01^{b}	1.51±0.04^{a}	13.93±0.21^{a}

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means ± SE.

Table 2. The mean values of erythrocytic MDA (nmol/mg protein) and TAC (mmol/mg protein) levels in Group I, Group II and Group III.

Group		MDA	TAC
Group I	0 day	0.25±0.01^{a}	0.27±0.01^{d}
	30th day	0.27±0.01^{a}	0.31±0.01^{c}
Group II	0 day	0.25±0.01^{a}	0.27±0.01^{d}
	30th day	0.13±0.01^{b}	0.64±0.01^{b}
Group III	0 day	0.25±0.01^{a}	0.27±0.01^{d}
	30th day	0.10±0.02^{b}	0.84±0.01^{a}

Means within the same column carrying different letters are significantly different at ($P<0.05$). Values are expressed as means ± SE.

different induced significant decreases in all lipid profile parameters. It has been reported that ginger improves dietary (cholesterol, fructose, or high-fat diet) or streptozocin-induced lipid derangements in rodents (Beattie et al., 2011).

C. speciosus supplementation has a healthy effect on liver and kidney functions. Bavarva and Narasimhacharya (2008) reported that the diabetic control group exhibited significantly higher amounts of urea and creatinine while the ethanolic extract of *C. speciosus* administered diabetic rats registered significantly lowered urea and creatinine serum level. Other herbs from *Zingiberaceae* family as *Zingiber officinale* is a useful agent for the prevention of renal ischemia reperfusion-induced injuries (Maghsoudi et al., 2011) and carbon tetrachloride renal induced injuries (Hamed et al., 2012). The ginger extract rendered significant protection against induced nephrotoxicity, which was evident from the lowered serum urea and creatinine levels in the mice (Ajith et al., 2007). It may regard to the fact that the ginger exhibit antioxidant activity and anti-free radicals abilities that stimulate the liver performance and urea synthesis (Polasa and Nirmala, 2003).

Free radicals play an important role in oxidative stress related to the pathogenesis of various important diseases. Many properties of plant products are associated with the presence of phenolic compounds which are essential for plant development and play an important role in their defense mechanisms. The inclusion of these compounds in the regular diet might be beneficial to health by lowering the incidence of diseases (Halliwell, 1997). Oxidative stress of erythrocytes of the 30th day was investigated by determination of the MDA level (as lipid peroxidation product) and total antioxidant capacity. The antioxidant activity of *C. speciosus* extracts might be due to redox properties of the phenolic contents which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Nehete et al., 2010; Baskar et al., 2012). Administration of either costunolide (20 mg/kg daily) or eremanthin (20 mg/kg day), a constituent of *C. speciosus*, for 60 days, caused a significant reduction in thiobarbituric acid reactive substances (TBARS) level and a significant increase in GSH content in the treated rats when compared to untreated diabetic rats (Eliza et al., 2010).

The antioxidant activity of *C. speciosus* rhizome might be due to the presence of phytoconstituents such as flavonoids and phenolic compounds (Jha et al., 2010). In regard to serum protein, the electrophoretic pattern had shown a significant increase in total protein in Group III in comparison to control. This result is in accordance with that of Eliza et al. (2009b). Concentration of total protein in serum of ginger-supplemented broilers tended to be higher at 21 days and was higher at 42nd day of age compared with that of control broilers (Zhang et al., 2009). The blood plasma chemistry analysis revealed that protein, albumin and globulins levels of experimental fish supplemented by ginger at the rate of 5 g/kg of diet were significantly higher than that of control fish (Immanuel et al., 2009).

In the same context, hematological investigation, at 30th day, in all treated animals supplemented with *C. speciosus*

Table 3. The mean vales of electrophoretic pattern at 30th day in Group I, Group II and Group III.

Group	Total protein (g/dl)	Albumin (g/dl)	α_1-globulin (g/dl)	α_2-globulin (g/dl)	β-globulin (g/dl)	γ-globulin (g/dl)
Group I	6.16±0.066^{b}	2.89±0.003^{b}	0.15±0.01^{b}	0.97±0.012^{c}	0.86±0.003^{a}	1.29±0.045^{b}
Group II	6.34±0.026^{b}	2.80±0.003^{b}	0.16±0.01^{b}	1.13±0.007^{b}	0.80±0.009^{b}	1.45±0.006^{b}
Group III	7.09±0.058^{a}	3.12±0.009^{a}	0.19±0.01^{a}	1.17±0.006^{a}	0.85±0.009^{a}	1.76±0.03^{a}

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means ± SE.

Table 4. The mean values of TEC (10/mm), TLC (10/mm), Hb (g/dl) and PCV (%) in Group I, Group II and Group III.

Group		TEC	TLC	Hb	PCV
Group I	0 day	8.50±0.26^{c}	6.88±0.36^{c}	9.43±0.31^{b}	29.67±0.24^{c}
	30th day	8.43±0.26^{c}	7.03±0.37^{c}	9.72±0.28^{b}	30.33±0.57^{bc}
Group II	0 day	8.58±0.20^{c}	7.45±0.52^{bc}	9.65±0.27^{b}	29.00±0.37^{c}
	30th day	10.50±0.30^{b}	7.45±0.52^{bc}	9.57±0.35^{b}	32.00±0.52^{b}
Group III	0 day	8.68±0.23^{c}	7.50±0.54^{bc}	9.68±0.25^{b}	29.83±0.56^{c}
	30th day	12.5±0.54^{a}	10.23±0.29^{a}	11.42±0.23^{a}	37.00±0.55^{a}

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means ± SE. TEC = total erythrocyte count, TLC = total leukocyte count, Hb = haemoglobin, PCV = packed cell volume.

Table 5. The mean values of differential leucocytes count (%) in Group I, Group II and Group III.

Group		Lymphocyte	Monocyte	Basophil	Eosinophil	Neutrophil
Group I	0 day	58.17±0.97^{b}	4.00±0.41^{ab}	0.33±0.15^{a}	6.17±0.46^{a}	31.00±0.75^{a}
	30th day	59.67±0.91^{ab}	3.83±0.56^{ab}	0.33±0.15^{a}	5.33±0.39^{a}	29.83±0.34^{a}
Group II	0 day	58.17±0.97^{b}	4.00±0.41^{ab}	0.33±0.15^{a}	6.17±0.46^{a}	31.00±0.75^{a}
	30th day	61.67±0.6^{ab}	2.33±0.24^{bc}	0.33±0.15^{a}	5.00±0.41^{a}	30.67±0.35^{a}
Group III	0 day	59.50±0.83^{ab}	4.17±0.34^{a}	0.33±0.15^{a}	5.83±0.42^{a}	29.67±0.65^{a}
	30th day	62.33±0.96^{a}	1.33±0.35^{c}	0.33±0.15^{a}	4.83±0.67^{a}	31.67±0.57^{a}

Means within the same column carrying different letters are significantly different at ($P < 0.05$). Values are expressed as means ± SE.

there showed a significant increase in the total count of erythrocytes, the total count of leukocytes, hemoglobin and lymphocytes. These results are in accordance with that induced by ginger (Nya and Astin, 2009) and turmeric (Harikrishnan and Balasundaram, 2008). Moreover, a dietary supplement protects mice against radiation-induced lethality by protecting the bone marrow from radiation, this will be evidenced by a significant increase in RBCs count and hemoglobin (Jagetia et al., 2004).

Conclusion

From the obtained results, we advise to use *C. speciosus* ground roots as a feed additive supplement in Egyptian buffalo to improve the health status of those heifers by enhancement of immunity and antioxidant status.

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Full Length Research Paper

A simple method for adherence evaluation to highly active antiretroviral therapy by Brazilian patients from healthcare unit: Focus on a adequately therapeutic compliance

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In general, the indirect measures used to evaluate adherence to medication treatment are self-reporting, daily record of the medication use, pharmacy dispensing records, and others. This study used the indirect method analyzing the dispensing records to evaluate adherence of 295 patients treated with antiretrovirals in Health Unit of Ribeirão Preto (SP), from January 2009 to December 2011. The level of adherence, i. e., regularity, low irregularity, high irregularity and dropout presented values of 23.7, 26.1, 46.1 and 26.4%, and over the 3 years, the dropouts showed a significant increase, with a recidivism rate ranging from 1 to 6. The correlation studies showed a negative correlation (P<0.05) between age and adherence, more pronounced in women (P<0.01). On the other hand, the changes in therapeutic prescriptions was positively correlated with the abandon (P<0.05). Based on these results, peculiarities of populations' adherence are useful in the development of actions aiming to improve the assistance.

Key words: Acquired immune deficiency syndrome virus, highly active antiretroviral therapy, medication non-adherence, correlation studies.

INTRODUCTION

After three decades of the human immunodeficiency virus (HIV) emergence, Brazil has occupied a prominent role, ensuring free access of the population to highly active antiretroviral therapy (HAART). This action contributed to the stabilization of the epidemic and increased the rates of longevity and quality of life, even though the growth was still observed in sub-populations in condition of vulnerability and mortality (Barreto et al., 2011; Brasil, 2013; Fonseca and Bastos, 2007). This growth can be attributed to the poor patient adherence to therapy (WHO, 2003; Ceccato et al., 2004; Melchior et al., 2007), a challenge for the Brazilian Unified Health System (Crespo-Fierro, 1997; Figueiredo et al., 2001).

Despite the difficulty of establishing a measure of adherence to treatment effectiveness, studies show that the expected effects of viral sustainable suppression and improvement of the immune system occur when the patient ingest about 95% of prescribed doses (Paterson et al., 2000; Gross et al., 2006). Lower levels of adherence can lead to selection of resistant viruses resulting

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in treatment failure and new treatment schemes, more complex and costly (Martin-Sanchez et al., 2002; Munakata et al., 2006)

In general, patients present adherence of 20 to 50% to prescription of health care professionals and treatment recommendations, including medicine use ranged from 20 to 50% (WHO, 2003; Brasil, 2007; DiMatteo, 2004; Osterberg and Blaschke, 2005). Adherence is a dynamic and multidimensional phenomenon determined by the inter-relation of economic and social factors that requires shared and mutual responsibility between the individual diagnosed positive for HIV, the health equipment and social network (WHO, 2003; Brasil, 2007).

According to Nachega et al. (2006) is important to know the level of adherence to HAART, since some problems such as long distance from home, difficulty with the dosing schedules, and running out of pills can be identified soon and strategies focused on adherence maximized. However, there is no way to establish a "gold standard" for measuring adherence (WHO, 2003). Direct and indirect methods are used to evaluate medication adherence. Among the direct measures, we can include plasma concentration of antiretrovirals (ARVs) and their metabolites, drug assay in urine and direct observation of the patient receiving the medication (Crozzati, 2007). Among the indirect measures, self-report, the daily record of medicine use, manual/electronic counting of pills, electronic monitoring and drug dispensing records of pharmacies can be used (Bonolo et al., 2007; Carvalho et al., 2003; Johnson et al., 2009; Rocha et al., 2011; Polejack and Seidl, 2010).

For the computerized system and identification by bars code of the dispensed medication, the data records began to be highly accurate and reliable, providing precise and exact information. Although it does not ensure that medications dispensed will be used correctly, it is considered that there is a relation between medication dispensed and their correct use (WHO, 2003; Gomes et al., 2009).

In this context, a public unit for medication dispensing (PUMed) has a special role to the access and the coherent use of the medication (prescription, dispensation and patient use). This PUMed is generally linked to a basic healthcare unit (BHCU) which has multidisciplinary professionals in a city or district (Brasil 2010a, b). Consequently, studies directed towards a single PUMed can reveal the impact of a complete healthcare service for a specific group of patients (Brasil, 2007; Minas, 2008).

Therefore, the aims of this study were to evaluate the adherence of patients by using the individual dispensing records of ARVs and correlate adherence to age, gender and scheme's modifications.

METHODOLOGY

The location of data collection was the pharmacy from PUMed Sumarezinho, situated at the West District of Ribeirão Preto city, State of São Paulo, Brazil. The records of dispensing ARVs (and HAART) were collected during 36 months (from January 1st, 2009 to December 31st, 2011).

The PUMed is from a BHCU assisted by the Faculty of Medicine of Ribeirão Preto, University of São Paulo and the Municipal Secretariat of Health. The PUMed is part of the service of pharmaceutical care of the Municipal Health System of Ribeirão Preto (MHS). This PuMed is linked to the PN-DST/AIDS for providing the dispensing medication from strategic component of pharmaceutical care including ARVs beyond the municipal essential medicines (REMUME). Among the list of ARVs available for dispensing in the municipality are: (1) Nucleotide Reverse Transcriptase Inhibitors (NRTIs): abacavir (ABC), didanosine (ddI), stavudine (d4T), lamivudine (3TC), tenofovir (TDF), zidovudine (AZT) and lamivudine + zidovudine (AZT + 3TC, Biovir®); (2) Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs): efavirenz (EFV) and nevirapine (NVP); (3) and protease inhibitors (PI): atazanavir (ATV), darunavir (DRV), fosamprenavir (FPV), indinavir (IDV), ritonavir (RTV), saquinavir (SQV) and lopinavir + ritonavir (LPV/r, Kaletra®); (4) fusion inhibitor (FI): enfuvirtide (T20) and; (5) integrase inhibitor (II): raltegravir (RAL).

Population and sampling

The population of the west district is estimated at 151,218 inhabitants, according to IBGE (2013).

Antiretroviral drugs were dispensed mainly to resident population in the area of the BHCU. The data was collected from 341 patients who had personal registration of dispensation. Criteria for eligibility were: gender (male, female), age (≥18 years) and on HAART. From these, were selected patients who had the registries in the system of management of the municipality's health, named HygiaWeb, and logistics control system of medications (Siclom) since January 1st, 2009. Patients were excluded based on: (1) did not pickup medication for a period longer than 12 months (n=26); (2) transfer registered (n=9); (3) obits (n=10) and; (4) pregnant women (n=1) due to the particularity of treatment (Gomes et al., 2009). Finally, after exclusions, the populations studied totaled 295 individuals (86.51%). All patients in use of HAART had their names coded to ensure anonymity (alphanumeric code).

In the present work, record on dispensing drugs to patients with HIV/AIDS dispensed the statement of consent. This study was approved by the Ethics in Research, Teaching Health Center, CONEP-CEP on 03/09/2012.

Dispensing records

The ARVs are dispensed in the PUMed from a BHCU, through individualized assistance. Each patient, at the dispensing, receives the amount of pills for one month of treatment and the date of the next return, according to the medical prescription. For each dispensation along the 36 months of the study, the attendance records and individually dispensing drugs (individual records, HygiaWeb and Siclom) were analyzed. From both, individual form and Siclom/HygiaWeb, the following data were collected: ARVs, quantity dispensed, date of dispensing and changes in the scheme of treatment. Finally, the information was inserted into a database (Excel 2003, Microsoft®).

Evaluation of dispensations

For the analysis, the following variables were considered: number of patients, gender (male and female), age (≥18 years), use of medications and schemes (medications used and scheme according the methodology), as well as the modifications in HAART scheme (number of modifications on schemes during the period).

Regarding medications' dispensing were considered the frequency (monthly) during the period. Anticipated dispensations or dispensations for more than one month of treatment, time and counting pills were adjusted for the monthly period (Gomes et al., 2009; Grossberg et al., 2004). Thus, classification of dispensations were divided into three groups: regular, when there was no failure in withdrawing medications, including frequency (monthly) and amount of pills; Irregular, patients who did not pick up the medications in the correct frequency (monthly), remaining a period of 30 to 60 days without medications were considered; and dropouts, when the patient failed the pickup of medication for longer than 60 days (Bomtempo, 2000; Carmody et al., 2003; Seguy et al., 2007) with the possibility of the return to the treatment.

For each patient who abandoned (> 60 days) and returned to treatment, recidivism rates were calculated. Already for irregularities (up to 60 days) were classified as low and high irregularity. Low irregularity were considered, which is the frequency of pickup of ARVs ≥ 95%, or in other words, from one to two irregular pick up of medications over the period. For high irregularity were considered the patients who picked up the medication at a frequency ≤ 95%, in other words, three or more irregular pickups.

Statistical data analysis

Statistical analyzes were performed by SigmaStat software (SigmaStat for Windows, Version 3.5, Systat Software Inc. 2006). One-way analysis of variance (ANOVA) was used for data analysis (regularity, irregularity and dropouts between the years). For the use of ARVs, the data presented as parametric and correlation test of "Pearson" was used. The P-value was set at $P<0.05$.

RESULTS

Table 1 presents the population's characteristics. From the total, 152 were male (51.5%) and 143 females (48.5%). The male population, female and total presented a normal distribution since the medians and percentiles were close, as well as the low standard deviation between ages. Male/female ratio was 1.06 (Table 1).

Regarding age, the main proportion of AIDS cases was observed between 40 and 49 years (42.7%), followed by age group 50 to 59 years (19.7%).

Comparisons between the rates of irregularities during the period (Figure 1) showed that there were no statistical differences between 2009 (17.2 ± 7.3), 2010 (17.1 ± 4.3) and 2011 (17.1 ± 3.5) with $P>0.05$. The averages of dropouts in the first, second and third years were 6.3 ± 2.5 (1 to 12 months), 9.4 ± 1.8 (13 to 24 months) and 12.3 ± 1.8 (25 to 36 months), respectively. Statistical analysis revealed a significant increase in the number of dropouts ($P<0.01$) from the first to the second year and from the second to the third year (Figure 1). The regularity (n = 295), which involves dispensation of HAART within a period of 30 days, was detected for 70 individuals, or 23.7% of total adherence to treatment (Figure 2A and 3).

Considering low irregularity, the percentage achieved 26.1% (77 patients), however patients with high irregularity, i.e., time without pickup the medication is higher than 5%, was 46.1% (Figure 2A).

In this study, patients who dropout totalized 78, the percentage was 26.4% value higher than patients considered regular (Figure 2A). We should also consider the recidivism rate of these individuals (Figure 2B). In this study it was observed that 78.2% of these individuals (n=61) dropout and returned to the treatment once or twice and 20.4% (17 subjects) did it more frequently, from 3 to 6 times during 3 years (Figure 2B).

Regarding the total population (n=295), statistically significant correlations between the dropout rate and (1) age, (2) change in the treatment scheme of the individual and (3) regularity was observed (Table 2). The regularity demonstrated negative significant statistically correlation more substantial with the dropouts (r=-0.558), in other words, the higher regularity, lower dropouts. In relation to age, the higher the age of the population the lower the dropouts (r=-0.196) and more regularity (r=0.224), as shown in Table 2. In contrast, the change in the treatment scheme is positively correlated to dropouts and negatively to the regularity. This implies that the higher the change in the treatment scheme of the individual the higher the dropout rate.

Concerning male (n=152), a statistically significant influence on these correlations was not observed (Table 2). Only a statistically negative with respect to the regularity and dropout rate was observed (r=-0.534). However, females (n=143) had a strong influence on the correlations, particularly to dropouts and change in treatment scheme (Table 2). Finally, no correlation was observed between age and change in the treatment scheme of patients regarding the general population and genders.

Between January 2009 and December 2011, considering the first record of dispensing for each patient, 49 different HAART's schemes were detected. Therefore in the course of the records there was an increase in the number of schemes to 80, or an increase higher than 63% in the number of combinations of HAART for the treatment of patients during the period.

The combinations of HAART more prescribed were triple schemes combining two NRTIs with a NNRTI, being the most common medication AZT+3 TC (62.7%). The most frequent combinations with AZT +3TC was with EFZ (28.1%) and NVP (13.3%). The most prevalent of PI scheme with AZT +3TC medications was LPV/r (9.5%).

DISCUSSION

According to Brazilian government, in 2011, the national population ratio was of 1.7, narrowing over the years (Szwarcwald et al., 2000; Dhalia et al., 2000 Ribeirão Preto, considering cases from 1985 to 2011, ratios were 2.12 and 1.57, respectively. One factor that may explain the decrease in the proportion of HIV cases between men and women is the increased rates of heterosexual transmission to women (84.6%) (Brasil, 2011). In Ribeirão Preto, the higher exposure category of the 5628 cases from

Table 1. Descriptive analyses of the population studied.

Data	Population		
	Total	Male	Female
Localization	Ribeirão Preto (state of São Paulo), Brazil		
N	295	152	143
Age (years)			
Mean	44.6	44.3	44.9
Standard deviation	9.1	8.9	9.2
Minimum	18	18	28
Maximum	75	75	71
Median	44	45	44
Percentile 25	39	39	38
Percentile 75	50	49.5	51

Table 2. Correlation between parameters related to the use of ARVs on the Basic Healthcare Unit.

Population	Total (n=295)			Male (n=152)			Female (n=143)		
	Dropout	Age	Scheme change	Dropout	Age	Scheme change	Dropout	Age	Scheme change
Age	-0.196*	-	-	-0.157	-	-	-0.240**	-	-
Scheme change	0.132*	-0.003	-	0.010	0.059	-	0.245**	-0.072	-
Regularity	-0.558**	0.224**	-0.115*	-0.534**	0.137	0.009	-0.585**	0.331*	-0.237**

*P<0.05; **P<0.01

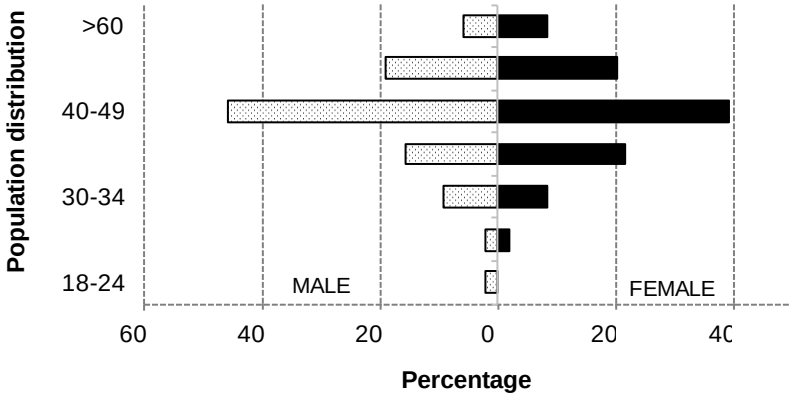

Figure 1. Distribution of the population studied regarding age range (n total=295, n males= 152, n females= 143).

from 1985 to 2011, is heterosexual (43.8%) followed by injecting drug users (IDU) (29.35%) (Ribeirão, 2013).

The studied population had the lowest ratio male/ female, this is an expressive female predomination, and considering heterosexual category as high risk, the PUMed should pay attention to this scenario. Santos et al. (2009) suggested, after analyzing the vulnerability to HIV among Brazilian women, the need to think about prevention strategies focused on women and not just focus on their individual behaviors.

Regarding the percentage of AIDS cases in this population, there are many differences when comparing to São Paulo state, which prevails in the range between 30 and 39 years (38.4%), followed by the range of 40 to 49 years (20,2%) (São Paulo, 2011). In Ribeirão Preto, the most prevalent age group according to the first diagnosis is 30 to 39 (2120 from 5637 patients, 37.6%) and 20 to 29 years (1855 from 5637 patients, 32.9%)

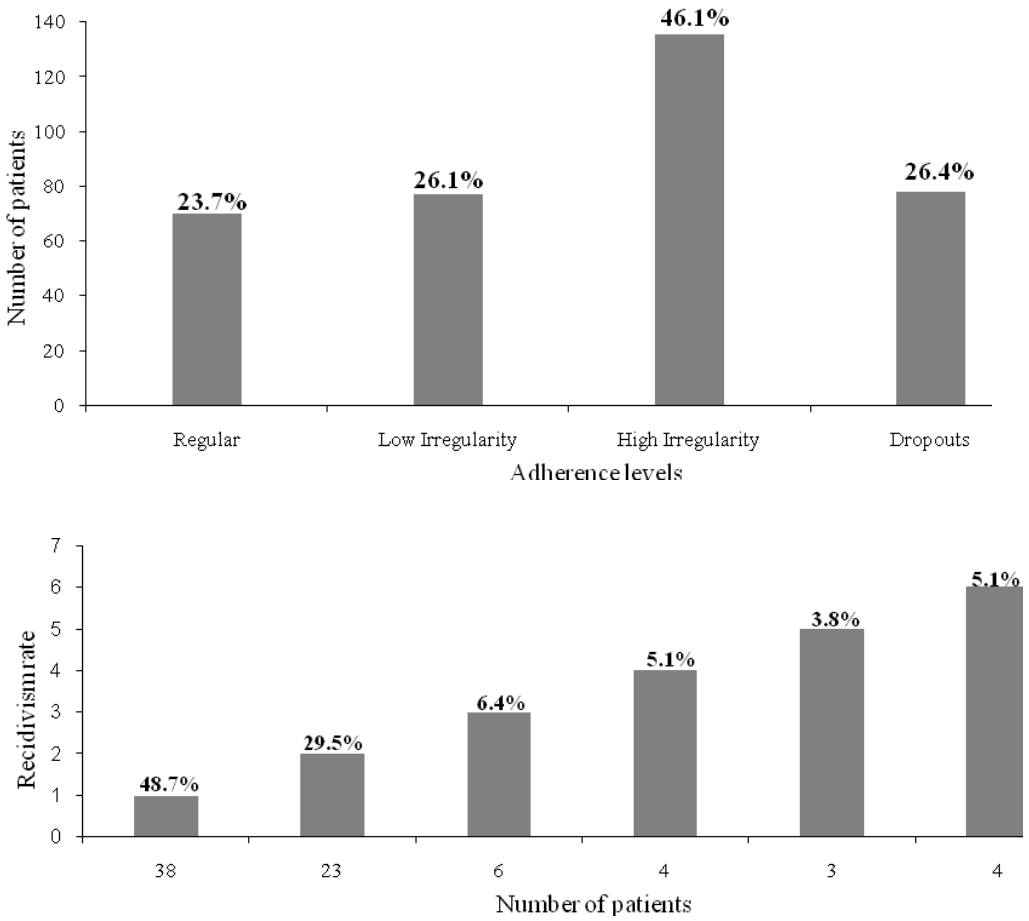

Figure 2. Adherence levels (A) and recidivism rate (B) of related patients in the study (n=295).

years. A projection from the first diagnosis to today for patients in Ribeirão Preto shows that the largest proportion of age groups are 40 to 49 (2360 of 5616, 42.02%) and 50 to 59 (1349 of 5616; 24.02 %), the same age groups presented by the studied population. Therefore, we observe that age in this study is more pronounced than in the state of São Paulo. Due to increasing of life expectancy, it is necessary to develop special actions for control and prevention of HIV infection in patients older than 40 years.

Evaluation of adherence

In their studies performed in Philadelphia (USA), Grossberg et al. (2004) concluded that, despite the self-reports indicate that patients would be 100% adherent to treatment, only 41% were considered adherent by the pharmacy dispensing records. According to Bedell et al. (2000), only one third of patients from an academic center in Boston (USA) used their medications as prescribed and despite they understand the risks of the nonadherence, the level of adequately compliance to medication used was lower than recommended. Here it was noticed that the chronic HAART users had low adherence (Figure 3). According to Jordan et al. (2000), for chronic diseases there are decreases in adherence with the time of treatment because, in general, patients feel asymptomatic, become comfortable and opt out of the treatment.

According to Paterson et al. (2000), to decrease the viral load is necessary ingestion of 95% of the prescribed medications. When analyzing patients at high irregularity period without pickup, the value added up 46.1% (Figure 2A). Hence, actions such as residence visits, educational campaigns and supervised medication use should be encouraged to inhibit this practice of patients. According to Safren et al. (2001), another way to increase HAART adherence could be the self monitoring condition (using daily report, recording the number of pills prescribed and the number of pills taken) and life step condition (utilizing cognitive-behavioral, problem-solving and motivational interviewing techniques).

The percentage of individuals considered regular and irregular in our findings were not similar to previous studies (11.8 and 57.9%; 55.9 and 44.1%; 64.1 and 35.9%,

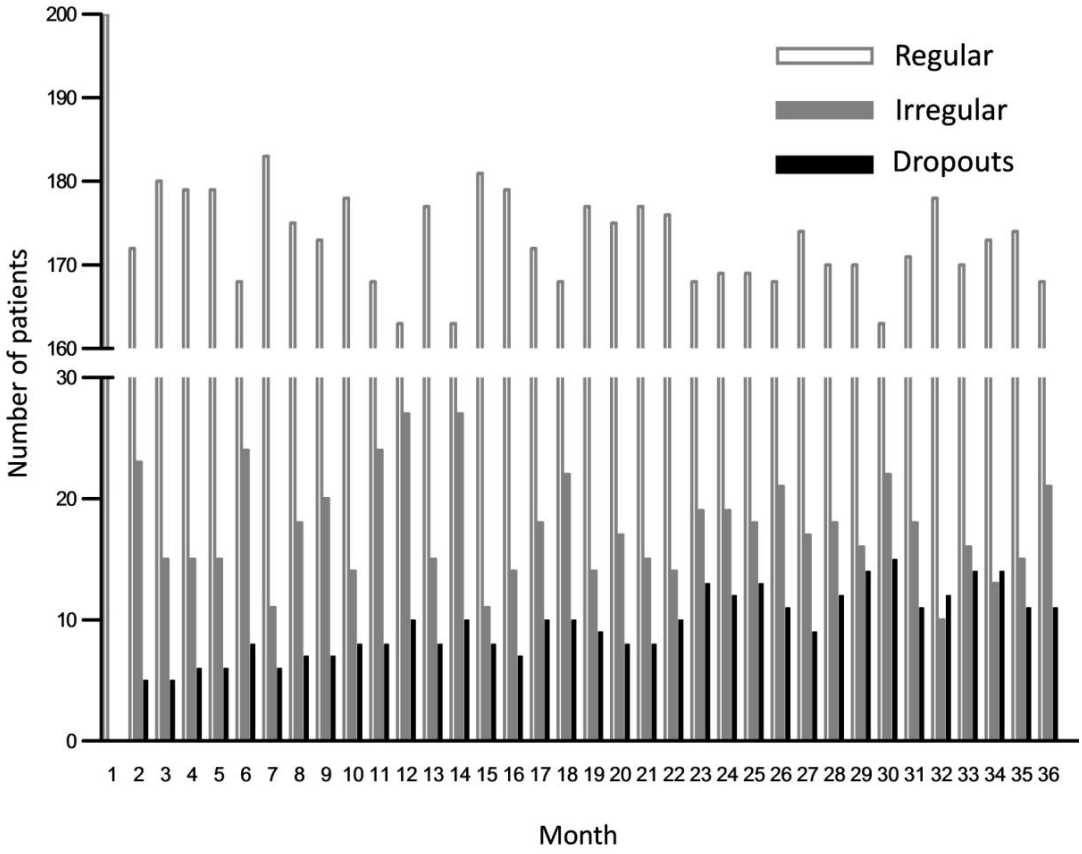

Figure 3. Regularities, irregularities, and dropouts of patients in HAART from BHDU from January 2009 (1st month) to December 2011 (36th month). n=200: patients who pickup ARVs from month 1 to 36.

respectively) (Gomes et al., 2009; Brito et al., 2006; Nogueira et al., 2007). Data from WHO (2003) reported that only about 1/3 of individuals use the medication as prescribed. Furthermore, Rocha et al. (2011) found that 19.4% of patients which were considered adequately compliant by self-report had, according to the pharmacy records, dropedout of the treatment. Hence, a comparison between all studies is very important to establish methodological standardization to estimate the adherence. These differences observed between the several studies may be due to the difficulty of classifying adherence to HAART. The studies usually establish different cut off points. For instance, Bonolo et al. (2007) compared adherence studies found in cut off points ranging from 80 to 100% of adherence for achieving the treatment efficacy. In addition, other variables may influence the evaluation of treatment adherence, such as access and services with high quality, physical structure of the BHCU and inter-relations between the multidisciplinary equip.

Special attention should be directed to patients who dropout the HAART. In this study, these individuals totaled 78 (26.4%). Brito et al. (2006) reported 35.9% of dropouts, while Gomes et al. (2009) 30.3%. Despite these high values, we should also consider the recidivism rate of these individuals. Here we observed that 78.2% of patients dropout and returned to treatment once or twice and 20.4% did it from 3 to 6 times.

The effectiveness of HAART is strictly associated to treatment adherence. Raffa et al. (2006) determined the viral level and the genotype resistance by analyzing plasma of patients in HAART. They reported that adherence between 80 to 90% increased the viral genotypic mutations at a higher rate of those who adhered in a lower or higher range. They concluded that ingestion of HAART in this interval can cause virologic resistance and treatment failure. Therefore, adherence studies are extremely important since we can identify the characteristics of patients, those at low adherence, using strategic tools to improve this parameter. In this context, it is worth mentioning that pharmaceutical care (Foisy and Akai, 2004), pharmacotherapy monitoring and health promotion activities (ongoing education) (Rueda et al., 2006) are very important for patients diagnosed as HIV positive. These actions can improve the adherence to treatment and, for more vulnerable groups such as children and elderly people, the contamination and side effects by HIV.

Correlations between the parameters related to the use of antiretrovirals

We observed that the higher regularity, lower dropouts in the studied population. In relation to age, its negative value correlated with dropouts. In contrast, change in the treatment scheme is positively correlated with dropouts and negatively associated with regularity. This implies to say more changes in treatment scheme of the individual is associated with a higher dropout rate. Concerning only males, there were no statistically significant influence on these correlations, except regarding regularity and dropout rate, as expected. Females strongly influenced the correlations in general population, particularly for dropouts and changes in treatment scheme. Finally, we did not observe any correlation between age and changes in treatment scheme for general population or genders.

This study demonstrates that there are relevant tendencies to dropouts in relation to age and gender. Multidisciplinary should be direct efforts in order to improve the regularity of these patients. Thus, knowing the particularities in adherence of a population through simple statistical studies, actions can be promoted such as coherent use of drugs specifically targeted to groups as a way to increase adherence at levels higher than 95% with subsequent increase of treatment efficacy.

Characterization of changes in treatment schemes

The combinations of HAART medical prescriptions were schemes associating two NRTIs, with a NNRTI being the most common medication AZT + 3TC (62.7%). The most frequent combinations with AZT + 3TC was with EFZ (28.1%) and NVP (13.3%). The most prevalent of PI scheme with AZT + 3TC medication was LPV/r (9.5%). In studies that also evaluated the therapy used, the more prescribed schemes were also schemes combining two NRTIs and one NNRTI, with values similar to the scheme AZT + 3TC + EFV (30.7, 26.9 and 34.1%) (Gomes et al., 2009; Blatt et al., 2009; Fonseca et al., 2012).

Conclusion

Therefore, a well-studied data associated to precise statistical analysis can contribute significantly within particular characteristics of population and thus be able to use this information to develop actions to improve the treatment. For this reason, it is a necessary knowledge of the multidisciplinary staff to use this information properly in an interdisciplinary way, acting to serve the population in health promotion and continuous education in a humanistic way, thus increasing adherence.

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Full Length Research Paper

Physicochemical characterisation of *Irvingia wombolu* gum in tramadol encapsulated granules

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The objectives of the work were to evaluate the binder properties of gum from *Irvingia wombolu* seed cotyledons and to compare with sodium carboxymethylcellulose (SCMC) in tramadol encapsulated granules. Tramadol granules was formulated by wet granulation using gum derived from the seed cotyledons of *I. wombolu* as binder at concentrations of 2.5, 5.0, 7.5, 10.0 and 15.0% w/w. The binder properties of the gum were compared with that of SCMC. The flow properties of the granules were studied by direct and indirect methods. The tramadol capsules were evaluated using necessary official tests. The phytochemical and physicochemical properties of the gum were also studied. The results showed that tramadol granules exhibited good flow for the production of quality capsules. Tramadol capsules formulated with *Irvingia wombolu* gum and SCMC, respectively complied with BP specification for capsules weight uniformity with percentage deviations below 10%. Capsule disintegration time ranged from 4.80 ± 0.43 min to 5.90 ± 0.45 min for tramadol capsules formulated with *I. wombolu* gum and were not significantly affected by concentration of gum in the formulation ($p < 0.05$). However, tramadol capsules formulated with *I. wombolu* gum exhibited faster disintegration time than SCMC ($p < 0.05$) whose disintegration time occurred at 14.20 ± 0.87 min. The results of phytochemical analysis of *I. wombolu gum* showed that the gum contains alkaloids, flavonoids, saponin, tannins and glycosides. Therefore, natural gum from *I. wombolu* has good potential to be used in formulating normal release tramadol capsules.

Key words: *Irvingia wombolu* gum, physicochemical characterization, micromeritic studies, capsule production.

INTRODUCTION

Tramadol is a synthetic 4-phenyl-piperidine analogue of codeine that is a weak μ-opioid receptor agonist (Howard and Huda). It is indicated for the management of moderate to moderately severe pain including chronic pain and pain associated with molar extraction in adults (Wantana et al., 2011). Tramadol is an effective and well-tolerated agent to reduce pain resulting from trauma, renal or biliary colic and labour, and for the management of chronic pain of malignant or nonmalignant origin, particularly neuropathic pain (Wantana et al., 2011).

Irvingia wombolu commonly called bush/wild mango, or dika nut, is an edible Africa indigenous fruit tree that produces edible fruits and seeds (Atangana et al., 2002; Harris, 1996). *Irvingia* belongs to the family Irvingiaceae; the fruit of *I. wombolu* is sour and is consumed locally and the edible kernels are used for culinary purposes (Fajimi et al., 2007). In Nigeria, the kernels are used as a condiment and are highly valued for their food thickening properties (Ndjouenekeu et al., 1996; Fajimi et al., 2007) in preparing “ogbono” or draw soup. Gums from plants are mainly long chain, straight or branched chain polysaccharides that contain hydroxyl groups which bond to water molecules (Emeje et al., 2008). These gums are generally non-toxic and widely available, hence the continued interest (Emeje et al., 2008). A number of plant gums have been investigated as binding, suspending or

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emulsifying agents in both solid and liquid dosage formulations (Chukwu et al., 1994; Nasipuri et al., 1999; Odeku and Itiola, 1998; Emeje et al., 2008). Binders confer structural strength required by granules during processing, handling, packaging and transportation. The widening availability of natural gums with specific characteristics offers flexibility of application with respect to improving the bioavailability of drugs and manipulating their release profile (Momoh et al., 2011). Also, the use of synthetic polymer matrix materials often goes along with detrimental effects on incorporated drug during manufacturing of formulations or during the erosion of the polymers after application (Reithmeir et al., 2001). The aim of the work is to formulate tramadol encapsulated granules using a natural gum from the seed cotyledons of *I. wombolu* and to evaluate the *in vitro* properties of the capsules.

MATERIALS AND METHODS

Chemicals and reagents

Lactose (Merck, Germany), sodium carboxymethylcellulose, acetone (BDH, England), magnesium stearate, tramadol (May and Baker, England), distilled water (Lion water, Nsukka, Nigeria) were used for this study. *I. wombolu* seed gum was obtained from a batch processed in our laboratory. All other reagents and solvents were of analytical grade and were used as supplied.

Extraction of *I. wombolu* gum

I. wombolu seed were purchased from the market of Nsukka, Enugu State, Nigeria in the month of June, 2010. The plant material was authenticated by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka. The voucher specimen of the plant studied was kept in the herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. *I. wombolu* seeds were milled using an equipment of hammer mill type (500# grinder/Fuyu Metal, Linyi Fuyu Metal Products Co., Ltd, China) and soaked in water containing 1% sodium metabisulphite for about 12 h, it was filtered and the gum was precipitated using acetone. The precipitated gum was dried for 2 h in a tray dryer (Manesty Ltd, Liverpool, England) at 40°C. The dried gum was milled in an end runner mill (Pascal Engineering Co Ltd, England) and finally passed through 55 mm sieve (Turgens & Co., Germany).

Phytochemical screening

Phytochemical tests were carried out on the powdered gum for the presence of alkaloids, tannins, saponins, flavonoids, resins, oils, steroids, glycosides, terpenoids, acid compounds, carbohydrates, reducing sugars and proteins. The tests were carried out using standard procedures of analysis (Harborne, 1993; Sofowora, 1993; Trease and Evans, 2002).

Rheological properties of *I. wombolu* gum

A 3 %w/v of *I. wombolu* gum was prepared and the viscosities were determined at temperatures of 25, 40, 80, 60 and 100°C, respectively (Onyechi, 2008).

Solubility

The solubility of the *I. wombolu* gum was tested in water (cold and hot), n-hexane, petroleum ether, chloroform ethyl ether, acetone, ethanol and methanol.

Preparation of granules

Granules were prepared by wet granulation method using *I. wombolu* gum as binders at concentrations 2.5, 5.0, 7.5, 10.0 and 15% w/w. Details of granulation are given in Table 1. Lactose used as filler and tramadol were mixed for 10 min in a tumbler mixer. The powder mixtures were moistened with the appropriate amount of binder solution. The homogeneous wet mass was then screened through a 1.7 mm sieve and the wet granules dried in a hot air oven at 55°C for 1 h. Thereafter, the dried granules were screened through a 1.0 mm sieve (Lachman et al., 1990; Shendge et al., 2010).

Characterisation of granules

Bulk and tapped densities

A 25 g quantity of each batch of tramadol granules was placed in a 100 ml measuring cylinder. The volume occupied by the sample was noted as the bulk volume. The bulk density was calculated as shown in Equation 1:

$$\text{Bulk density } (\ell_B) = \frac{\text{Mass of powder (M)}}{\text{Bulk volume of powder } (V_B)} \quad (1)$$

The cylinder was tapped on a wooden platform by dropping the cylinder from a height of one inch at 2 s interval until there was no change in volume reduction. The volume occupied by the sample was then recorded as the tapped volume. The tapped density was calculated using the formula:

$$\text{Tapped density } (\ell_T) = \frac{\text{Mass of powder (M)}}{\text{Tapped volume of powder } (V_T)} \quad (2)$$

Flow rate and angle of repose

A funnel was properly clamped on to a retort stand. The funnel orifice diameter, base diameter and efflux tube length were appropriately measured. A 25 g quantity of the granule was placed into the funnel with the funnel orifice closed with a shutter. The time taken for the entire sample in the funnel to flow through the orifice was noted. The flow rate was gotten by dividing the mass of the sample by the time of flow in seconds. The dynamic angle of repose was determined by measuring the height of heap of powder formed using a cathetometer; the radius was obtained by dividing the diameter by two. Angle of repose (ɵ) for each granule sample was calculated using Equation 3 (Aulton, 2007; Ngwuluka et al., 2010):

$$\Theta = \tan^{-1} \frac{\text{height of powder heap}}{\text{radius of powder}} \quad (3)$$

Table 1. Composition of tramadol capsules.

Ingredient	Quantity/capsule (mg)				
	F1	F2	F3	F4	F5/G
	2.5% binder	5.0% binder	7.5% binder	10.0% binder	15.0% binder
Tramadol	50.0	50.0	50.0	50.0	50.0
Binder*	2.5	5.0	7.5	10.0	15.0
Magnesium stearate	1.0	1.0	1.0	1.0	1.0
Lactose qs	100.0	100.0	100.0	100.0	100.0

**Irvingia wombolu* gum, sodium carboxymethylcellulose (SCMC).

Table 2. Results of phytochemical constituents of *I. wombolu* seed gum.

Phytochemical constituent	Remark†
Alkaloids	+
Saponins	+
Reducing sugars	-
Tannins	+
Glycosides	+
Flavonoids	+

†- Absent, + present.

Compressibility index and Hausner's quotient

Carr's compressibility indices (%) of the granules were obtained using the formula (Aulton, 2007; Ngwuluka et al., 2010).

$$\text{Carr's index}\ (\%) = \frac{\ell_T - \ell_B}{\ell_T} \times 100 \quad (4)$$

While Hausner's ratio was obtained using the formula:

$$\text{Hausner's ratio} = \frac{\ell_T}{\ell_B} \quad (5)$$

Where ℓ_T and ℓ_B are tapped and bulk density, respectively.

Preparation of capsules

Initially, granules were treated with magnesium stearate (lubricant) and the capsules were filled manually using 100 mg of tramadol granules per capsule (Ofoefule, 2002).

Evaluation of capsules

Disintegration time test

Disintegration time test was conducted using an Erweka ZT 120 basket and rack assembly and 0.1 N HCl maintained at 37.0 ± 1.0°C as the disintegration medium. Ten capsules from each batch were used for the test and the procedure being as stipulated in the British Pharmacopoeia (BP) (2009).

Uniformity of mass

Twenty capsules were randomly selected from each batch. The content of each capsules were weighed individually using an electronic balance (Ohaus Adventurer, China) and the individual weights recorded. The mean weight, standard deviation and percentage deviation were calculated (BP, 2009).

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 14.0 (SPSS Inc. Chicago, IL.USA). All values are expressed as mean ± standard deviation (SD). Data were analysed by one-way Analysis of Variance (ANOVA). Differences between means were assessed by a two-tailed student's T-test. $P < 0.05$ was considered statistically significant.

RESULTS

Phytochemical constituents of *I. wombolu* gum

The results of the phytochemical analysis of the gum are shown in Table 2. The results revealed that the gum contains alkaloids, saponins, tannins, flavonoids and glycosides in substantial quantities. Reducing sugars was however not found in the gum.

Solubility

I. wombolu gum was soluble in hot and cold water (0.1% w/v). However, the gum was insoluble in n-hexane, petroleum ether, chloroform ethyl ether, acetone, ethanol and methanol.

Rheological properties of gum

The effect of temperature on the viscosity of *I. wombolu* gum is shown in Figure 1. From the results, increase in temperature increased the viscosity of the gum. Therefore, this gum could be used as binders in wet granulation without affecting the properties of the gum.

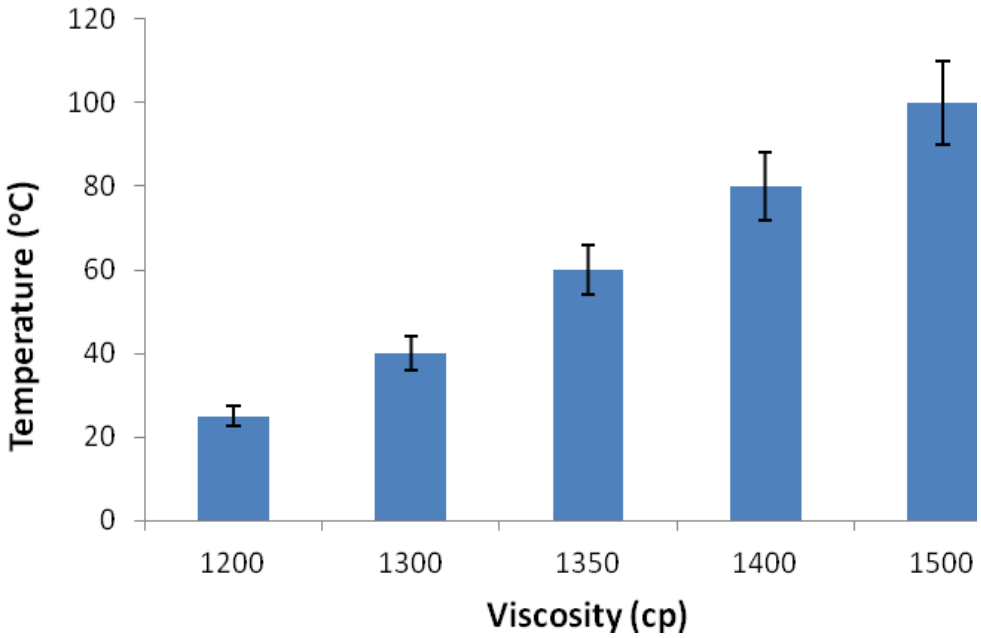

Figure 1. Effect of Temperature on 3% dispersion of the gum.

Table 3. Micromeritic properties of tramadol granules formulated with *I. wombulu* gum.

Batch (%)	ℓ_B (g/ml)*	ℓ_T (g/ml)*	A.R (°)*	H.R	C.I (%)	Flow rate (g/s)
F1 (2.5)	0.43±0.05	0.81±0.09	13.10±0.05	1.88	46.90	0.10
F2 (5)	0. 42±0.03	0.80±0.07	14.00±0.03	1.90	47.50	0.10
F3 (7.5)	0.42±0.11	0.67±0.05	20.10±0.03	1.60	36.30	0.10
F4 (10)	0.39±0.05	0.50±0.07	22.50±0.09	1.25	22.00	0.09
F5 (15)	0.49±0.11	0.78±0.03	19.02±0.17	1.60	37.20	0.09
G (15 SCMC)	0.42±0.17	0.81±0.12	19.65±0.01	1.90	48.10	0.08

Values shown are mean ± SD (*n = 3); F1 to F5: tramadol granules prepared with different concentrations of *I. wombolu* gum, G: tramadol granules prepared with 15% SCMC; ℓ_B and ℓ_T = bulk and tapped densities, AR = angle of repose, HR = Hausner's ratio, CI = Carr's compressibility index, SCMC: sodium carboxymethylcellulose.

Table 4. Weight uniformity of tramadol capsules.

Batch/tablet code (%)	Weight (mg ± CV)*
F1 (2.5)	102.00±1.09
F2 (5)	100.00±0.50
F3 (7.5)	102.00±2.53
F4 (10)	102.00±3.70
F5 (15)	100.00±2.04
G (15 SCMC)	100.00±1.73

*Mean for 20 capsules, F1 to F5: tramadol granules prepared with different concentrations of *I. wombolu* gum, G: tramadol granules prepared with 15% SCMC, $p < 0.05$ was considered significant.

Flow properties of tramadol granules

The results obtained from micromeritic studies presented in Table 3 showed that *I. wombolu* granules exhibited good flowability.

Properties of capsules

Capsule weight uniformity

The results of capsule weight uniformity presented in Table 4 showed that capsule weight ranged from 100.00 ± 0.50 to 102.00 ± 1.09 mg. The results indicate that

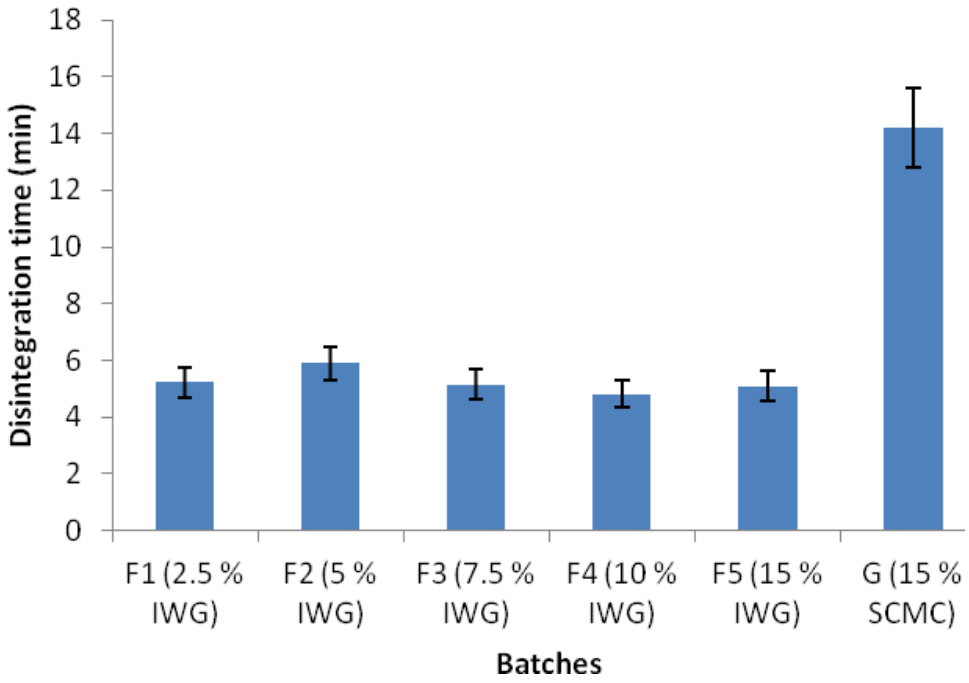

Figure 2. Disintegration time of tramadol capsules; batches F1 to F5: tramadol granules prepared with different concentrations of *I. wombolu* gum, G: tramadol granules prepared with 15% SCMC, $p < 0.05$ was considered significant.

tramadol capsules formulated with *I. wombolu* gum and SCMC, respectively complied with BP specification for capsules weight uniformity.

Disintegration time

The results of capsule disintegration time also presented in Figure 2 showed that tramadol capsules exhibited good disintegration time and complied with BP specifications.

DISCUSSIONS

Phytochemical constituents of *I. wombolu* gum

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals substances to protect themselves and they are also believed to protect humans against certain diseases (Edeoga et al., 2005). The medicinal plants that are moderately rich in alkaloids and tannins have potential health promoting effects (Olajide et al., 2000; Jigam et al., 2010). The results revealed that the gum contains important phytochemicals as shown in Table 2.

Flow properties of tramadol granules

The granules from various batches exhibited good micromeritic properties. Angle of repose and flow rate were within the standard acceptable values required for formulation of quality capsules. Values for angles of repose ≤ 30° generally indicate a free flowing material and angles ≥ 40° suggest a poorly flowing material (Yüksel et al., 2007; Momoh et al., 2012). Carr's index (CI) indicates the flowability and consolidation properties of the powder mixtures. When the CI and Hausner's ratio are adequate, the powder flows at minimum bulk density (Yüksel et al., 2007; Momoh et al., 2012). The results of Carr's compressibility index and Hausner's ratio indicated values that were above the limits for good powder fluidity. This may be due to such factor as the nature of granulation and other factors that could lead to false negative results. The flow of powder during manufacturing dictates the quality of the product in terms of weight and content uniformity of the capsules (Yüksel et al., 2007). The measurement of the flow properties of granules is essential in capsule production because variation in particle flow will automatically cause variation in capsule filled weight and active ingredient variation. The flow property of bulk material results from the cohesive forces acting on individual particles such as van der Waals, electrostatic, surface tension, interlocking and friction (Yuksel et al., 2007).

friction (Yüksel et al., 2007).

Properties of capsules

Capsule weight uniformity

Tramadol capsules formulated with *I. wombolu* gum in functionality as a capsule excipient compared favourably with SCMC and complied with BP specification for capsules weight uniformity as their percentage deviations were significantly below 10% (BP, 2009).

Disintegration time

Encapsulated tramadol granules had disintegration time range from 4.80 ± 0.43 min to 5.90 ± 0.45 min for capsules formulated with *I. wombolu* gum and were not significantly affected by concentration of gum in the formulation. However, the disintegration time of tramadol capsules formulated with *I. wombolu* gum were significantly lower than that of SCMC ($p < 0.05$) whose disintegration time occurred at 14.20 ± 0.87 min.

Conclusion

Tramadol capsules were successfully formulated using different concentrations of *I. wombolu* seed gum. The granules exhibited good flow properties that were within limits for good granule flow and hence, for quality capsule production. The weight uniformity and disintegration time of tramadol capsules formulated with *I. wombolu* gum complied with BP specifications as did those of tramadol capsules formulated with SCMC. Therefore, natural gum from *I. wombolu* could be used in formulating normal release tramadol capsules.

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Full Length Research Paper

Antioxidant effects of *Ixora coccinea* Linn. in a rat model of ovalbumin-induced asthma

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Oxidative stress, specifically lipid peroxidation, contributes to the pathogenesis of asthma. A natural antioxidant could be a potential therapeutic intervention. Hydro-alcoholic extract of *Ixora coccinea* (ICE) exhibit the anti-asthmatic activity in an ovalbumin (OVA) induced asthmatic rat model. These facts led us to examine their antioxidant activities. The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity and the intracellularly antioxidant activity of ICE were determined. The protective effect of ICE against 2,2′ azobis (2-amidinopropane) hydrochloride (AAPH)-induced red blood cell lysis was also evaluated. It was found that ICE could scavenge DPPH with an IC_{50} of 283.3 µg/ml and protected red blood cell against AAPH-induced hemolysis with an IC_{50} of 72.92 versus 52.08 µg/ml for ascorbic acid. Erythrocytes obtained from the ICE-administrated rats showed an enhanced resistance to hemolysis. In OVA-induced asthma, rats were sensitized and challenged with ovalbumin. The effect of ICE at 1500 mg/kg per os on malondialdehyde (MDA) production and lung catalase activity were determined. ICE significantly reduced the lipid peroxidation and enhanced catalase activity in lung ($p < 0.05$). In conclusion, the hydro-alcoholic extract of *I. coccinea* possesses an antioxidant activity and protective effect against free-radical-induced hemolysis. This may explain the traditional use of this plant as a remedy against asthma and other diseases.

Key words: Asthma, oxidative stress, antioxidants, *Ixora coccinea.*

INTRODUCTION

Many decades of research have produced a significant amount of data showing increased oxidative stress in asthma and indicating a potential role for oxidants in the pathogenesis of the disease (Caramori and Papi, 2004). A number of studies have clearly demonstrated that oxidative stress is an important consequence of the inflammatory response in asthma (Nadeem et al., 2005; Wood et al., 2003). Detrimental effects of oxidative stress on airway function include: airway smooth muscle contraction, airway hyper-responsiveness and epithelial shedding, each of which contribute to the airway obstruction that is characteristic of asthma (Wood et al., 2003). Indeed the lung is continuously exposed to oxidants, either generated endogenously by metabolic reactions (for example, from mitochondrial electron transport during respiration or released from phagocytes) and exogenously from air pollutants or cigarette smoke (Kirkham and Rahman, 2006). These agents may cause direct tissue oxidation, release of endogenous oxidants and inactivation of antioxidant defense mechanisms (Rai

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and Phadke, 2006). Host defense against the potentially damaging effects of reactive oxygen species (ROS) is provided by a range of antioxidants. These may be endogenous, such as the antioxidant enzymes (superoxide dismutase, glutathione peroxidase, catalase), thiols (glutathione) and metal-binding proteins (lactoferrin, transferrin, ceruloplasmin) or exogenous, including a variety of antioxidants obtained from the diet such as tocopherols, carotenoids, flavonoids and ascorbate. Thus, it is likely that the use of antioxidants to restore the oxidant-antioxidant balance may be effective in the treatment of asthma (Kirkham and Rahman, 2006).

Medicinal herbs have are a very valuable source for natural antioxidant products. The antioxidant activity of many extracts and constituents from medicinal herbs has been widely documented *in vivo* and *in vitro*. *Ixora coccinea* Linn (Rubiaceae) is a common flowering shrub native to Asia which can be found growing in the tropical and subtropical climates of the world (Baliga and Kurian, 2012). Leaves are given in diarrhea; flowers are used in the treatment of dysentery, leucorrhoea, dysmenorrhoea, hemoptysis and catarrhal bronchitis (Ghani, 2003). Recently, potent anti-ulcerogenic (Arunachalam et al., 2009), antidiabetic (Yasmeen and Prabhu, 2011) and anti-diarrhoeal (Prabhu et al., 2010) properties of *I. coccinea* have been reported. The extracts of *I. coccinea* were found to be chemoprotective, antiviral, antimitotic, modulatory on cyclophosphamide-induced toxicity in mice and to act as anti-inflammatory agent (Ratnasooriya et al., 2005; Latha and Panikkar, 1999, 2000). In our previous paper (Missebukpo et al*.,* 2011), we have reported that hydro-alcoholic extract of *I. coccinea* leaves exhibit the anti-asthmatic activity in an ovalbumin (OVA)-induced asthmatic rat model. These facts led us to examine their antioxidant activities.

Recent studies reveal *in vitro* antioxidant effect of methanolic extract of the flower, leaf and stem of *I. coccinea* (Bose et al., 2008; Banerjee et al., 2011). *In vivo* antioxidant activities applicable for various diseases are experimented. For example, Bose et al. (2010) showed that *I. coccinea* extract decreased significantly lipid level in plasma and prevented hyperlipidemia, and this effect provided evidence for their antioxidant properties. Other report showed that *I. coccinea* and *I. perviflora* extract have hepatoprotective properties on CCl_4 induced liver damage in rat and this hepatoprotective effect is due to their *in vivo* antioxidant activities (Bose et al., 2011). However, the study that deals with *in vivo* antioxidant effect of *I. coccinea* extract is scarce and their antioxidant activity in animal model of ovalbumin induced asthma is not documented. In search of the mechanism of action of the extract in asthma, the aim of this study was to investigate the antioxidant activity of *I. coccinea* extract (ICE) in cell and cell-free systems, and in addition, the ability of ICE to inhibit ROS generation in model of OVA-induced asthma.

MATERIALS AND METHODS

Plant

The leaves of *I. coccinea* were collected on 24th July, 2007 in the second middle part of the day, from Lomé not far from University of Lomé (Togo). The plant was authenticated at Department of Botany, by Professor Akpagana Koffi from Laboratory of Botanic and Plant Ecology (University of Lomé). The voucher specimen (TOGO 12671) was deposited in the herbarium of this Laboratory. The dried sample was extracted in water/ethanol mixture (1 : 1) for 72 h with manual discontinue agitation. The solution was filtered evaporated using a rotary evaporator (Buchi R120) set at 45°C to obtain a dry extract which contained alkaloids, flavonoids and tannin as revealed by previously phytochemical screening.

Animals

Wistar rats (150 to 200 g body wt.) of either sex were used for the experiments described. All animals were maintained on a standard laboratory chow and water *ad libitum*. They were kept in the Animal House of the Faculty of Sciences of University of Lomé (Togo). All experiment was done following bioethics committee of University of Lomé-Togo guidelines.

Total phenols determination

Total phenolic content of the extract was determined by the Folin-Ciocalteu reaction (Lawson-Evi et al., 2011). Briefly, a mixture of *I. coccinea* extract, Folin-Ciocalteu phenol reagent, and sodium carbonate was prepared and allowed to stand at room temperature for 30 min. After that, the mixture was centrifuged and the supernatant was measured at 760 nm. Gallic acid (0 to 250 mg/L) was used as the standard for the calibration curve. The phenolic contents were calibrated using a linear equation based on the calibration curve. The contents of phenolic compounds are expressed as mg gallic acid equivalent (GAE)/g extract.

Total flavonoids content of the extract

Total flavonoids content was determined according to aluminum chloride colorimetric method (Lawson-Evi et al., 2011). The extract (0.5 ml of 1:10 g m/L) in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was at room temperature for 30 min, the reaction mixture absorbance was measured at 415 nm with a double beam. Quercetin (5 to 100 µg/ml) was used as the standard for the calibration curve. The levels of total flavonoids contents were determined in triplicate and the result was expressed as mg quercetin equivalents (QE)/g extract.

DPPH radicals scavenging assay

Stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) solution was used to determine the free radical-scavenging activity of *I. coccinea* (Lawson-Evi et al., 2011). Different concentrations of the extract (50

to 2000 µg/ml) were added at an equal volume to methanolic solution of DPPH (100 µM) (Sigma, USA). After 15 min incubation at room temperature, the absorbance was read at 517 nm. The experiment was carried out in triplicate and quercetin was used as standard control. IC_{50} values which represent concentration required to scavenge 50% of DPPH free radicals was compared among *I. coccinea* extract and quercetin. The DPPH scavenging effect was calculated as follows:

% inhibition = (Ac – Ae) × 100 / Ac

Where: Ac = absorbance of DPPH without the sample (control), Ae = absorbance of the sample with extract or quercetin.

AAPH-induced hemolysis assay *in vitro*

Blood was collected from Wistar rats through retro-orbital sinus in heparinized tubes. The *in vitro* resistance of intact red blood cells to oxidation was evaluated with AAPH (Sigma-Aldrich, France) as described previously (Diallo et al., 2012). Erythrocyte and plasma were separated by centrifugation (3000 g for 10 min). The oxidation of rat erythrocyte (10% hematocrit with saline) was induced by AAPH at 37°C for 3 h under air. The extent of hemolysis was determined by measuring absorbance at 540 nm with a UV-visible recording spectrophotomer (UV-265FS, Shimadzu, Kyoto, Japan). The percentage of inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = [(A_{AAPH} - A_{\textit{I. coccinea}}) / A_{AAPH})] \times 100$$

Where $A_{\textit{I. coccinea}}$ is the absorbance of the sample containing *I. coccinea* extract and A_{AAPH} the absorbance of the sample without *I. coccinea.* L-ascorbic acid was used as a positive control. Four to five replicates were performed for each concentration.

Ex vivo* study of anti-hemolysis activity of *I. coccinea

Rats (n = 6) were given distilled water or extract (1 and 1500 mg kg^{-1}) orally after an overnight fast according to Zhu et al. (2002). Then the rats were anaesthetized with ether and blood was collected in heparinized tube 60 min after dosing. Erythrocytes from each rat were separated from the plasma by centrifugation at 1500 g for 20 min. The plasma was removed from the erythrocytes. After removing the buffy coat, the remaining erythrocytes were re-suspended in the plasma. 0.5 ml of the reconstituted blood was used for the haemolysis assay by adding 0.5 ml of AAPH solution and 0.5 ml of PBS followed by incubation at 37°C for 3 h. Then, 4 ml of PBS solution was added to the incubation mixture which was centrifuged at 1000 g for 10 min. The absorbance of the super-natant was measured at 540 nm. The percentage of inhibition was calculated as described earlier.

Induction of asthma in Wistar rats

The rats were actively sensitized by intraperitoneal (ip) injection of 20 mg ovalbumin with 100 mg $Al(OH)_3$ (chicken OVA, grade V, Sigma Chemicals Co., S^T Louis, MO) in physiological saline solution as described (Missebukpo et al., 2011). Control animals received ip saline with $Al(OH)_3$ on days 0, 3, 7 and 21 and intranasal (in) saline without $Al(OH)_3$ on days 24, 25, 26 and 27. Twenty-four hours after the last OVA challenge by intranasal administration of OVA, the rats were sacrificed and lungs were removed from the chest cavity.

Determination of lipid peroxidation (LPO)

Lung LPO was determined by estimating levels of malondialdehyde (MDA) using the thiobarbituric acid test (Satoh, 1978; Odabasoglu et al., 2006). Briefly, 150 mg of lung tissue were collected from each experimental rat, homogenized in 1 ml of Tris-HCl 10 mM (pH 7.4). The homogenate or standard MDA (175 µl) at 25, 31, 62.5, 125, 250, 500, 1000 ng ml^{-1} was added to a solution containing 250 µl of HCl 1 M, 100 µl of sodium dodecyle sulphate (SDS) 9.8%; 1 ml of thiobarbituric acid, 0.67% and 330 µl of distilled water. The mixture was incubated at 90°C for 1 h. Upon cooling, 2.5 ml of *n*-butanol was added. The mixture was centrifuged for 10 min at 3,000 rpm. The supernatant was measured at 535 nm (Spectra Max Molecular Device, Sunyval Corporation, California USA). The results were expressed as ng MDA/mg tissue.

Catalase activity

Catalase activity was measured based on the ability of the enzyme to break down H_2O_2. Decomposition of H_2O_2 in the presence of catalase was measured at 250 nm (Odabasoglu et al., 2006). Prior to the catalase measurement, 150 mg of lung of control and sensitized rats were homogenized in 1 ml of Tris-HCl 10 mM (pH 7.4). The homogenate was centrifuged and supernatants were diluted with phosphate buffer (1:20). At 25°C, 100 µl H_2O_2 (0.66 M) were added to 120 µl supernatant. The rapid decomposition of H_2O_2 was followed during 7 s from the decrease in absorbance at 250 nm. Catalase (CAT) activity was calculated by $K = \Delta DO/\Delta t \times 1000/\varepsilon$ tissue, where ε = 43.6 M^{-1}/cm molar extinction coefficient at 25°C. The results were expressed as enzymatic unity/mg lung tissues.

Statistical analysis

The data are expressed as the mean ± SEM. The statistical significance of any difference was performed by one-way analysis of variance (ANOVA) followed by Tuckey's significant difference test. A significant value was defined as $p < 0.05$. All statistical analysis were carried out using the Instat statistical package (GraphPad prism 5.0 software, Inc. USA)

RESULTS

Total phenolic and flavonoid content

The total phenolic content of ICE was 243 mg GAE/g extract and the total flavonoid content was 72.5 mg QE/g extract. The results show that *I. coccinea* has relatively high flavonoid content.

DPPH radical scavenging activity

Table 1 presents the results of DPPH radical scavenging activity of ICE. This assay provided information on the reactivity of the samples with a stable free radical. Because of the odd electron, DPPH shows a strong absorption band at 517 nm in visible spectroscopy. As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes and the

Table 1. IC_{50} values of hydro-alcoholic extract of the leaves of *I. coccinea* for antioxidant tests *in vitro* by AAPH and DPPH and those of quercetin and ascorbic acid.

Parameter	AAPH IC50 (µg/ml)	DPPH IC50 (µg/ml)
I. coccinea	72.92	283.3
Quercetin	-	20.00
Ascorbic acid	52.08	-

resulting decolorization is stoichiometric with respect to the number of electrons taken up. The DPPH• scavenging ability of the ICE is lower than quercetin (IC_{50} value of 20 µg/ml) enough to remove the DPPH• (IC_{50} of 283.3 µg/m), which may answer for its medicine use.

Effect of ICE on AAPH induced-hemolysis

After 3 h of incubation with AAPH, erythrocytes were lysed. The protective effects of ICE and ascorbic acid on the hemolysis induced by AAPH are shown in Figure 1 and Table 1. IC_{50} of the ICE and ascorbic acid were 72.92 and 52.08 µg/ml, respectively. The extract had a maximum inhibitory effect of 88.72 ± 1.79%.

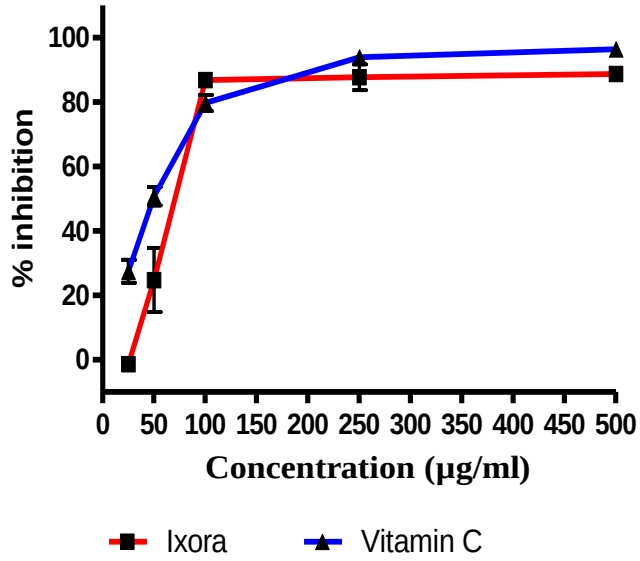

Figure 1. AAPH radical scavenging activity of hydro-alcoholic extract of the leaves of *I. coccinea* and ascorbic acid. AAPH induced in vitro Red cells membrane lipid peroxidation which leads to membrane lyse. The absorbance is a function of the color of the solution itself depends on the concentration of hemoglobin released. Inhibition percentage was calculated using mathematic formula described in the text. Values are expressed as mean ± S.E.M (n = 4).

In vivo antioxidant activity of ICE

Oral administration of the ICE reduced the extent of AAPH-induced hemolysis. The extract at 1.5 g/kg had an inhibitory effect of 47.202 ± 7.57% (Figure 2). Figure 3 shows the results of the level of the control, Ova-sensitized and treated rats. MDA, a marker for the oxidant stress, was significantly increased in sensitized rats com-pared with saline ($p < 0.05$) (Figure 3). ICE at 1.5 g/kg significantly reduced the MDA level of the lung ($p < 0.05$). Activity of catalase in tissue homogenate did not show any significant change in OVA-sensitized and challenged rats compared with control. Figure 4 illustrates that the activity of catalase in treated rats demonstrated twice higher levels of lung catalase activity (0.467 ± 0.042 µcat/mg lung) compared with saline rat (0.203 ± 0.032 µcat/mg lung).

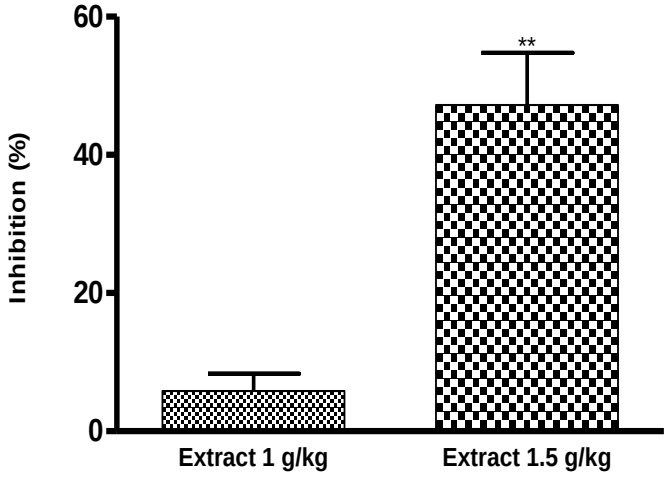

Figure 2. Inhibitory effect of hydro-alcoholic extract of the leaves of *I. coccinea* on AAPH-induced lysis of rat red blood cells. Rats were treated before blood collection. Test was done as described above in figure 1. The results are expressed as means ± SEM (n = 6) **p < 0.001 as compared with control group (0% inhibition).

DISCUSSION

Asthma is a chronic inflammatory disease of the respiratory tract where inflammation is often associated with an increased generation of ROS (Nadeem et al., 2008; Caramori and Papi, 2004). A wealth of studies identifies that ROS and loss of antioxidant defenses participate in the pathogenesis of asthma. Therefore, radical scavengers or antioxidants could play a useful role in therapy because antioxidants can mobilize and up-regulate the anti-oxidative capacity of cells to annihilate excessive ROS formation. This can be achieved through

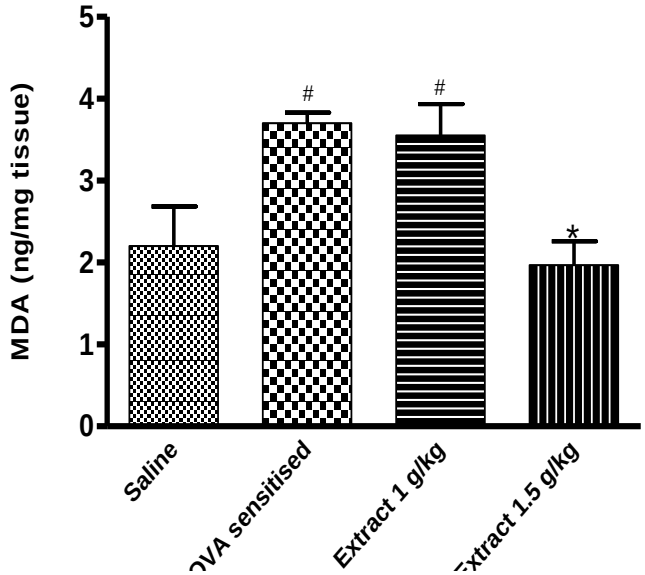

Figure 3. Content of malondialdehyde in lung homogenate of control (saline), Ova-sensitized and treated groups. Rats were made asthmatic using 28 days murine model. After 28 days rats were sacrificed and lungs were removed, 150 mg of tissues were homogenized to perform the test. Each point represents the mean ± S.E.M. (n = 4). $^{\#}p < 0.05$ vs saline rats; $^{*}p < 0.05$ vs Ova sensitized rats.

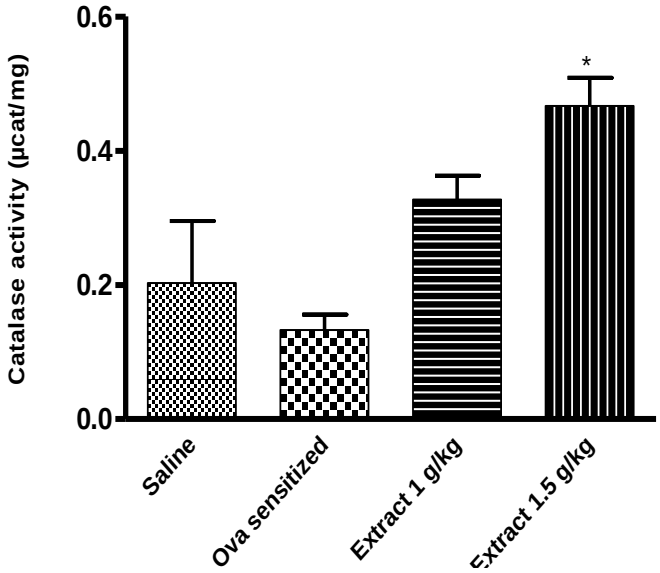

Figure 4. Catalase activity in control (saline), Ova-sensitized and treated groups. 150 mg of lung homogenized tissues were used to perform catalase activity essay. Catalase activity is expressed as enzymatic unity/mg lung. Each point represents the mean ± S.E.M. (n = 4). $^{*}P < 0.05$ compared with OVA-sensitized group.

two approaches: Either by increasing the endogenous antioxidant enzyme defenses or by enhancing the non-enzymatic defenses through dietary or pharmacological means (Kirkham and Rahman, 2006). Thus, we investigated the possible radical scavenging activity of hydro-alcohol extract of *I. coccinea* leaves by use of series of *in vitro* and *in vivo* experiments with some new methods applied to evaluate antioxidant activities of this extract.

Free radical scavenging activity was evaluated *in vitro* using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical and AAPH. In this study, ICE possessed *in vitro* antioxidant activity when tested with DPPH radical scavenging assay. The IC_{50} for the extract was 283.3 µg/ml compared with quercetin (IC_{50} = 20 µg/ml). Thus, it can act as moderate radical-scavengers which can reduce the auto-oxidation in body system or even food product that contains unsaturated lipid when compared with quercetin (Rao et al., 2007). Recently, Idowu et al. (2010) also reported that some of the phytochemicals isolated from the leaves of *I. coccinea* are effective in DPPH scavenging effects *in vitro* with gallic acid as control. These results corroborate those obtained by other researchers (Banerjee et al., 2011; Bose et al., 2011) who showed antioxidant activities of different parts of *I. coccinea* using different methods of evaluation of antioxidant activities *in vitro* like: superoxide anion scavenging activity assay, hydroxyl radical scavenging activity assay, Nitric oxide scavenging activity assay, Fe^{2+} chelating activity assay, hydrogen peroxide scavenging activity assay and reducing power assay.

The excessive peroxidation of biomembranes is accepted as one of the processes by which tissues can be damaged during inflammation (Zhu et al., 2002). The peroxidation of erythrocyte membranes and hemolysis induced by AAPH has been extensively studied as a model for membrane-peroxidative damage (Yoshida et al., 2004). In the present study, we investigated the antioxidative activity of *I. coccinea* extract using AAPH induced hemolysis. A dose-dependent protection was demonstrated toward the hemolysis of red blood cells *in vitro* with IC_{50} = 72.92 µg/ml. The inhibitory effect of ICE was nearer to ascorbic acid (IC_{50} = 52.08 µg/ml), which has been shown to act as an antioxidant against human low-density lipoprotein oxidation and acts as a primary defense against aqueous radicals in the blood (Ma et al., 1994). To explore mechanism of action of *I. coccinea* extract in the protection with bronchic epithelial mem-brane cells in asthma, we have evaluated antioxidant effect of extract *in vivo*. For *in vivo* assays, ICE was found to increase the levels of antioxidant in plasma. The increase in plasma antioxidant capacity observed following ICE administration is suggested by our erythrocyte hemolysis data. When we mixed erythrocytes from rats given ICE with plasma collected, the degree of hemolysis inhibition was related to the concentration of extract in the plasma.

Oxidative stress is a hallmark of asthma and increased

levels of oxidants are considered markers of the inflammatory process. Most studies to date addressing the role of oxidants in the etiology of asthma were based on the therapeutic administration of antioxidants (Reynaert et al., 2007). In the current study, OVA-sensitized rats with inflammation characteristics (Missebukpo et al., 2011), had an increment in pulmonary malondialdehyde (MDA) when compared with control group (non sensitized rats). This implies that rats during sensitization are exposed to a considerable degree of lipid peroxidation. This finding is consistent with other observation (Bulani et al., 2011). The increase in ROS during sensitization, as demonstrated by significant elevation of MDA, may overwhelm endogenous anti-oxidant defenses. This is illustrated in the present work by the decrease of MDA level in the lung in treated group, accompanied by increased catalase activity. In line with our findings, Bulani et al. (2011) showed that ovalbumin significantly increased the level of lipid peroxidation and decreased the level of GSH, SOD and catalase in the OVA sensitized rats when compared with non-sensitized group.

Catalase represents an important component of the endogenous antioxidant defense system of the lung, one of the major antioxidant enzymes that prevent the biological macromolecules from oxidative damage (Zhang et al., 2003). Increased ROS lead to modification of proteins and alterations in their function that are biologically relevant to the initiation and maintenance of inflammation, among which is the loss of antioxidant capacity of catalase (Comhair and Erzurum, 2010). However our results reveal no significant decrease in catalase activity in OVA-sensitized rats compared with non-sensitized. But Ghosh et al. (2006) demonstrated the oxidative inactivation of catalase in a murine model of allergic airway disease as well as decreased catalase activity in lungs of patients with asthma.

Several epidemiological studies have been undertaken which have established a beneficial link between polyphenol intake and lower disease risk with many of the clinical benefits being attributed to both the antioxidant and anti-inflammatory properties of polyphenols (Arts and Hollman 2005). Phenolic and flavonoid compounds are recognized as material base of the antioxidant activity of plant extract (Adedapo et al., 2009). Therefore, the chemical constituents present in the extract, which are responsible for this activity, need to be investigated. High total phenolic and flavonoids content values found in the extract (243 mg/g GAE and 72.5 mg/g QE) imply the role of phenolic compounds in contributing these activities.

Some of phenolic constituents have already been isolated from this plant and some have antioxidant properties *in vivo* model (Bose et al., 2011; Sen et al., 2011; Versiani et al., 2012; Idowu et al., 2010; Lee et al., 2010). Hence, the observed antioxidant activity may be due to the presence of any of these constituents. In unpublished results, we have shown that ICE contains chlorogenic acid, caffeic acid, and scopoletin which are strong antioxidants (Sato et al., 2011; Shaw et al., 2003).

Conclusion

From the above results, it is evident that hydro-alcohol extract of *I. coccinea* possessed both *in vitro* and *in vivo* antioxidant activity. It was not only able to enhance the plasma antioxidant level, but was also able to enter into living cells in the organ and protect them from oxidative damage after 5 days of consumption. It can be used in compensating the decrease in total antioxidant capacity in lung and enhance the Catalase activity in organ and thereby reduces the risks of lipid peroxidation in asthma. This is evident with the highest total phenolic and flavonoid content.

Abbreviations

ICE, Extract of Ixora coccinea; **OVA,** ovalbumin.

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Full Length Research Paper

Effect of oral ingestion of an *Arctium lappa* extract on the biodistribution of the radiopharmaceutical sodium pertechnetate in rats

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The aim of the present study was to assess the effect of the oral ingestion of an extract of the *Arctium lappa* (burdock) on the bio-distribution of the radio-pharmaceutical (radio-biocomplex) sodium pertechnetate ($Na^{99m}TcO_4$) in rats. Male *Wistar* rats (3 to 4 months of age, 329 ± 16 g) were treated with a burdock extract (1 ml, 20 mg/ml, n = 5) or 0.9% NaCl solution (control: n = 5) for 7 days. After this period of time, $Na^{99m}TcO_4$ (3.7 MBq, 0.3 ml) was injected through the ocular plexus. After 10 min, the rats were sacrificed, the organs isolated and counted in an automatic gamma counter. The percentage of radioactivity was calculated per gram of tissue (%ATI/g) or per whole organ (% ATI/organ). Alteration in $Na^{99m}TcO_4$ uptake was observed in liver from 1.72 ± 0.38 to 0.27 ± 0.07 (% ATI/organ, $p < 0.05$) and % ATI/g in lung (from 0.45 ± 0.40 to 1.02 ± 0.15 % ATI/g), in testis (from 0.12 ± 0.01 to 0.18 ± 0.02 % ATI/g), in tooth (from 0.24 ± 0.08 to 0.06 ± 0.13 % ATI/g), in tongue (from 0.38 ± 0.06 to 0.08 ± 0.16 % ATI/g) and in liver (from 1.07 ± 0.06 to 0.56 ± 0.15) after treatment with burdock. These findings could result from the interaction between components of the *A. lappa* extract and the radio-biocomplex which may influence the uptake of $Na^{99m}TcO_4$ in some organs of rats. Therefore, precautions are suggested in the interpretation of nuclear medicine results in patients using burdock.

Key words: *Arctium lappa* (Burdock), biodistribution, sodium pertechnetate, radiobiocomplex.

INTRODUCTION

Herbal products uses are increasing in most countries of the world, as part of a resurging belief in efficacy and safety of natural and traditional remedies (Simões et al., 2010). *Arctium lappa* L. (burdock) has been cultivated as

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a vegetable for a long time in orient, especially, Taiwan and Japan (Gentil et al., 2006). Its roots are widely used as food, whereas the seeds are used in traditional Korean medicine as a diuretic, anti-inflammatory or detoxifying agent (Preds et al., 2011), for hypertension and arteriosclerosis treatment (Neves et al., 2007; Liu et al., 2012). Its anti-diabetic property may be attributed to arctiin fraction (Lu et al., 2012). Jian-Feng et al. (2012) have reported a study using an aqueous extract of *A. lappa* L. roots (1,200 mg/kg) administered for a duration of 3, 7 and 15 days and they observed an enhancement of the sexual behavior in male rats. Further, Huang et al. (2010) suggest that burdock extract (100 mg/kg) administration for 8 days can prevent intestinal damage and decrease inflammatory cytokines in mice with ulcerative colitis.

Some investigations have demonstrated that burdock extract possesses hepatoprotective action that could be attributed, at least in part, to its anti-oxidative activity (Cunha et al., 2003; Song-Chow et al., 2005; Predes et al., 2012). This study evaluated the anti-bacterial activity of a phytotherapeutic agent prepared from an ethyl acetate fraction (AcOEt) extracted from *A. lappa* (Gentil et al., 2006). An infusion of the leaves is useful to impart strength and tone to the stomach, for some forms of long-standing indigestion (Song-Chow et al., 2005).

Phytochemistry analysis carried out by some authors has demonstrated that the species *A. lappa* contains inulin (45 to 60%), sesquiterpenical lactones, phenol acids, essential oils, poliacetilenes, tannins (Simões et al., 2010), flavonoid (baicalin), lignans (arctigenin), vitamins B and C, calcium and phosphorus (Gomes et al., 2011). Lignans, tannins and flavonoids have properties of anti-tumor, anti-oxidant, anti-inflammatory, anti-hepatotoxic, anti-coagulant (Rotblat and Ziment, 2002). Although the toxicity of *A. lappa* extract is not known, cases of allergy due to burdock have been reported as contact dermatitis resulting in anaphylaxis (Rodriguez et al., 2006). Meanwhile, tannic acid, a specific substance found in certain tannin-containing herbs, can be a gastrointestinal irritant when taken in large amounts (Rotblat and Ziment, 2002). It has been related that plants of Asteraeae family as *A. lappa* possess anti-leukemic properties and induce cells death via apoptosis (Wegiera et al., 2012). Haghi et al. (2013) related the presence of chlorogenic acid (5-CQA) and 1,5-dicaffeoylquinic acid (1,5-DCQA) as main compounds, total phenolic, which are caffeoyl esters present in wild and cultivated *A. lappa* L. (Haghi et al., 2013).

Radiopharmaceuticals (radiobiocomplexes) (Moreno et al., 2005) are radioactive tracers employed in nuclear medicine for the investigation of several morphological and physiological conditions, such as blood flow and absorption, biodistribution and metabolism in target and non-target organs. These considerations are highly relevant in early detection of a disease and the images obtained are denominated metabolic images. This fact permits proper clinical action in the beginning of the disease increasing the possibility of a successful intervention (medication, surgical) (Saha, 2010).The incorporation of a radionuclide into a drug formulation permits the determination of the biodistribution kinetics and the release sites of the latter (Owunwanne et al., 1996). Technetium-99m (^{99m}Tc) has been widely used in nuclear medicine due to its optimal half-life 6.0 h and energy characteristics, providing images with high efficiency with the administration of low doses to the patient (Moreno et al., 2005). Radio-biocomplexes such as sodium pertechnetate ($Na^{99m}TcO_4$) are tracers widely employed in scintigraphic studies (single-photon emission computed tomography - SPECT) mainly of the thyroid but also of the brain and stomach (Saha, 2010).

Natural and synthetic products have been reported to affect the biodistribution of different radiobiocomplexes (Owunwanne et al., 1996; Bernardo-Filho, 2005; Saha, 2010). PubMed (www.ncbi.nlm.nih.gov/sites/entrez) is a service of National Library of Medicine US that includes over 22 million citations from MEDLINE and other health sciences, among others. Scielo (Scientific Electronic Library Online) is an important index (www.scielo.org) of scientific publications. Review of the literature available in this data base did not show any reference about of the effect of *A. lappa* extract on the bioavailability of sodium pertechnetate ($Na^{99m}TcO_4$). This finding, as well as the possibility of human beings that are undertaking burdock may need a nuclear medicine procedure, the aim of this investigation was to evaluate the effect of the oral ingestion of an extract of the burdock on the biodistribution of the radiobiocomplex sodium pertechnetate ($Na^{99m}TcO_4$) in rats.

MATERIALS AND METHODS

Preparation of extract

The burdock extract was prepared with 2 g of leaf, stem and flowers of *A. lappa* (Estrella da Terra Produtos Naturais LTDA, Brazil, lot 003) in 100 ml of NaCl 0.9% solution at room temperature. It was triturated with a domestic electric extractor. This mixture was filtered (Schleicher and Schulle filter paper Lot number K 932, Size 11 cm) and the filtered solution was considered to be 20 mg/ml or 100%. The absorbance spectrum (Spectrophtometer, Analyzer Comércio e Indústria Ltda, Brazil) was determined, in the range of 400 to 700 nm as described by Neves et al. (2007). The value of the absorbance at 500 nm (0.754 ± 0.002) was considered as a marker of the reproducibility of the conditions of the extract at the highest concentration (Neves et al., 2007). As there is not a defined dosage of the extract (Huang et al., 2010; Jian-Feng et al., 2012) that is administered to the animals, as well as the time of the treatment, we decided to use in our investigation the dosage of 70 mg/kg during 7 consecutive days. The protocols of the experiments were performed without sacrificing of the animals and was approved by the Ethical Committee of the Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro with the protocol number CEA/141/2006.

Strategy adapted for survey of literature in the PubMed and Scielo data base

It was performed in PubMed (www.ncbi.nlm.nih.gov/sites/entrez)

and Scielo (www.scielo.org) a search (April 18th, 2013) using the keywords "Arctium lappa" and $Na^{99m}TcO_4$, "burdock" and "$Na^{99m}TcO_4$, "burdock" and "radiopharmaceutical", "*A. lappa*" and "radiopharmaceutical", " burdock" and "sodium pertechnetate"; "*A. lappa*" and "sodium pertechnetate ".

Treatment of animals

Adult male Wistar rats (n = 5), 3 to 4 months of age, 329 ± 16 g of weight following the Ethical Guidelines of the Institution were used in all the experiments. They were obtained from the Laboratório de Radiofarmácia Experimental (Departamento de Biofísica e Biometria, Universidade do Estado do Rio de Janeiro, UERJ, RJ, Brazil). The animals were used after an acclimatization period of 7 days and maintained under controlled room conditions corresponding to 22 ± 5°C, 12 h of light/dark cycle with water and a normal diet *ad libitum* during the experimental period. The *A. lappa* preparation (20 mg/ml, 70 mg/kg) was administered (1 ml) to the animals (n = 5) using a metal oropharyngeal cannula, daily doses for 7 days. The control group received 0.9% NaCl solution. $Na^{99m}TcO_4$ radiobiocomplex (0.3 ml, 3.7 MBq; Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, São Paulo, SP, Brazil) was administered (after 7 days) through the ocular plexus and the animals were sacrificed 10 minutes later.

Heparinized whole blood was rapidly obtained by cardiac puncture. The organs (brain, liver, duodenum, heart, kidney, lung, spleen, stomach, pancreas, testis, bone, muscle, thyroid, right upper incisor tooth and tongue) were isolated and weighed and the radioactivity was counted in a well counter (Automatic Gamma Counter, Packard Instrument Co, Illinois, USA). The samples were put in specific and appropriated tubes the conditions were always the same and the well counter was adjusted to the photonic energy of the ^{99m}Tc (gamma emission, 140keV). After that, the % of radioactivity (%ATI) was calculated in relation to the total dose that was injected. As some authors have already published, we used in our investigation two ways to assess the %ATI (Moreno et al., 2007a; Saha, 2010). The percentage of radioactivity per organ (%ATI/organ) was determined dividing the activity in each organ by the total activity administered to the animals (Moreno et al., 2007a; Saha, 2010). The percentage of radioactivity per gram of tissue (%ATI/g) was calculated dividing the %ATI /organ by the mass of each organ (Moreno et al., 2007a; Saha, 2010).

Statistical analysis

Analysis involved one-way analysis of variance (ANOVA), followed by the Turkey-Kramer multiple comparisons test, with the significance level being $P < 0.05$. InStat Graphpad software was used to perform statistical analysis (GraphPad InStat version 3.01 for Windows 95/NT, GraphPad Software, San Diego Ca, USA).

RESULTS

Some publications were found in the PubMed and Scielo following the used strategy: "*A. lappa*" and/or burdock (153 in PubMed and 11 in Scielo). When the keywords were "burdock", "*A. lappa*" and $Na^{99m}TcO_4$, "burdock" and "$Na^{99m}TcO_4$, "burdock" and "radiopharmaceutical", "*A. lappa*" and "radiopharmaceutical", "burdock" and "sodium pertechnetate" or "*A. lappa*" and "sodium pertechnetate" no item was found. The results in Table 1 show the relationship between the percentage of radioactivity per organ (% ATI/organ) of the radiobiocomplex $Na^{99m}TcO_4$ in the experimental group treated with *A. lappa* extract and the control group. Results indicate a significant increase in the uptake of the $Na^{99m}TcO_4$ in stomach, from 2.46 ± 0.70 (control) to 3.82 ± 0.54 (treated, p = 0.010) and tooth from 0.05 ± 0.02 (control) to 0.16 ± 0.08 (treated, p = 0.0001). A significant decrease in the uptake of the $Na^{99m}TcO_4$ in liver from 1.72 ± 0.38 (control) to 0.27 ± 0.07 (treated, p = 0.0011) was also found. No significant changes in the uptake of this radiobiocomplex in the brain, duodenum, heart, kidney, spleen, pancreas, lung, blood, thyroid, testis, muscle, tongue and bone (% ATI/organ) were found.

Table 2 shows the percentage of radioactivity per gram of tissue (% ATI/g) of the radiobiocomplex $Na^{99m}TcO_4$ in the treated animals with burdock extract and in the control group. Increase in the uptake of the $Na^{99m}TcO_4$ in the stomach, from 1.87 ± 0.56 (control) to 2.75 ± 0.76 (treated, p = 0.07, not statistically significant) was observed. Significant decrease in the uptakes in liver from 1.07 ± 0.06 (control) to 0.56 ± 0.15 (treated, p = 0.0001), in tooth from 0.24 ± 0.08 (control) to 0.06 ± 0.13 (treated, p = 0.029) and in tongue from 0.38 ± 0.06 (control) to 0.08 ± 0.16 (treated, p = 0.0299) were found.

DISCUSSION AND CONCLUSION

Much of the medical literature on medicines suggests that the safe or toxicity of medicinal plants is based on suboptimal evaluations of the available data (Rotblat and Ziment, 2002; Simões et al., 2010). The results obtained indicate that the burdock extract may affect the biodistribution of $Na^{99m}TcO_4$ in specific organs. Moreno et al. (2005) reported that *Ginkgo biloba* extract altered the uptake of $Na^{99m}TcO_4$ in rats. *Nectandra membranacea* extract altered the radioactivity uptake in heart, thyroid, kidney and muscle (Moreno et al., 2007a). *Uncaria tomentosa* extract altered the uptake of the $Na^{99m}TcO_4$ in the heart, pancreas and muscle after the treatment orally (Moreno et al., 2007b). Souza et al. (2011) demonstrated that natural products such as senna extracts can also induce changes in the biodistribution of $Na^{99m}TcO_4$. Jankovic and Djokic (2005) reported the alteration of the organ uptake of several radiobiocomplexes labeled with ^{99m}Tc induced by the administration of the cytotoxic drugs methotrexate sodium and cyclophosphamide using this same experimental model (Jankovic and Djokic et al., 2005). Rebello et al. (2008) described an alteration in the radioactivity uptake of the $Na^{99m}TcO_4$ in the duodenum, spleen, pancreas, stomach and blood when the animals were treated with *Passiflora flavicarpa* extract (Rebello et al., 2008).

When the drug interaction with radiobiocomplexes is unknown, the consequences of the procedure are the possibility of misdiagnosis and/or repetition of the

Table 1. Shows the effect of the *Arctium lappa* extract on the biodistribution os ^{99m}Tc (% ATI/organ) in the male Wistar rats which had received (20 mg/ml) or not (control group) the extract.

Organ	Control (% ATI/organ)	Treated (% ATI/organ)
Brain	0.07±0.01	0.19±0.27
Liver	1.72±0.38	0.27±0.07*
Duodenum	0.15±0.03	0.18±0.09
Heart	0.33±0.09	0.35±0.06
Kidney	0.57±0.16	0.59±0.06
Stomach	2.46±0.70	3.82±0.54*
Pancreas	0.18±0.08	0.22±0.54
Lung	0.05±0.02	0.06±0.02
Testis	0.17±0.02	0.20±0.01
Bone	0.19±0.36	0.12±0.07
Muscle	0.06±0.01	0.08±0.01
Thyroid	1.64±0.16	1.49±0.14
Spleen	0.23±0.02	0.21±0.53
Blood	1.12±0.17	1.17±0.16
Tooth	0.05±0.02	0.16±0.08*
Tongue	0.22±0.02	0.22±0.01

Data are reported as mean ± SD for 5 animals in each group.
After 7 days of treatment with extract of *Arctium lappa* (burdock) by intragastric via, once a day, (20 mg/mL), male *Wistar* rats received 0.3 mL $Na^{99m}TcO_4$ by the intravenous route. The animals were sacrificed, the organs isolated and %ATI/organ determined. Asterisks indicate significant differences ($p<0.05$).

examination, with an increase in the radiation dose administered to the patient (Bernardo-Filho, 2005). The knowledge about this phenomenon may contribute for proper clinical decisions and correct diagnosis.

Tsai et al. (2011) have enfaced that the protective effect on hepatocytes and the inhibition of interleukin-2 in primary human T lymphocytes might be attributed to the arctigenin bioactive component of *A. lappa*. It is possible to speculate that the alteration in the uptake of the studied radiobiocomplex (Tables 1 and 2) could be associated with the action described by Tsai et al. (2011). As tannins-containing herbs can be a gastrointestinal irritant (Rotblat and Ziment, 2002), the increase of radiobiocomplex uptake in stomach (Table 1) of animals treated with burdock extract could be associated with the presence of tannins in this extract. Moreover, the alteration of radiopharmaceutical uptake in liver (Tables 1 and 2) and stomach (Table 1), are in accordance with the literature, that also have described hepato-protective and gastro-protective action promoted by burdock extract (Song-Chow et al., 2002; Lima et al., 2006).

An interesting finding is related with the alteration of the uptake in the testis (Table 2) and this fact could be associated with the enhancement of the sexual behavior in male rats as reported by Jian-Feng et al. (2012). The aphrodisiac effects of the plant extract may be related to the presence of flavonoids, saponins, lignans and alkaloids, acting via a multitude of central and peripheral mechanisms. These results thus support the traditional use of *A. lappa* L. root extract for treating impotence and sterility. These considerations are described by Jian-Feng et al. (2012).

Meanwhile, precautions are suggested in the interpretation of nuclear medicine results in patients using the burdock since it alters the biodistribution of the sodium pertechenetate radiopharmaceutical in some organs and this fact could influence proper actions related to the diagnosis and therapy of some diseases.

Conclusion

The metabolization of the *A. lappa* (*in vivo)* could generate active metabolites with properties that could influence the biodistribution of the $Na^{99m}TcO_4$ radiobiocomplex in the treated animals with this extract.

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Table 2. Shows the effect of the *A. lappa* extract on the biodistribution os^{99m}Tc (% ATI/g) in the male Wistar rats which had received (20 mg/ml) or not (control group) the extract.

Organ	Control (% ATI/organ)	Treated (% ATI/organ)
Brain	0.04 ± 0.01	0.11 ± 0.05
Liver	1.07 ± 0.06	0.56 ± 0.15*
Duodenum	0.93 ± 0.57	0.89 ± 0.17
Heart	0.29 ± 0.05	0.25 ± 0.17
Kidney	0.53 ± 0.12	0.57± 0.09
Stomach	1.87 ± 0.56	2.75 ± 0.76
Pancreas	0.37± 0.01	0.36 ± 0.03
Lung	0.45 ± 0.40	1.02 ± 1.15*
Testis	0.12 ± 0.01	0.18 ± 0.02*
Bone	0.25 ± 0.36	0.15 ± 0.06
Muscle	0.11 ± 0.01	0.11 ± 0.15
Thyroid	5.71 ± 0.91	5.37 ± 0.93
Spleen	0.40 ± 0.01	0.39 ± 0.06
Blood	2.93± 0.17	2.92 ± 0.38
Tooth	0.24 ±0.08	0.06 ± 0.13*
Tongue	0.38 ± 0.06	0.08 ± 0.16*

Data are reported as mean ± SD for 5 animals in each group.
After 7 days of treatment with extract of *Arctium lappa* (burdock) by intragastric via, once a day (20 mg/ml), male *Wistar* rats received 0.3 mL $Na^{99m}TcO_4$ by the intravenous route. The animals were sacrificed, the organs isolated and %ATI/gram determined. Asterisks indicate significant differences ($p<0.05$).

ABBREVIATIONS

AcOE, Ethyl acetate fraction; **$Na^{99m}TcO_4$,** sodium perthecnetate; **MBq,** mega becquerel; **NaCl,** sodium chloride; ***A. Lappa*,** *Arctium lappa*; **Tc-99m,** technetium-99m.

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