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African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Determination of some mineral and heavy metals in Saudi Arabia popular herbal drugs using modern techniques

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The contents of 22 essential mineral elements and trace, heavy and toxic metals in 14 herbal drugs collected from the local markets of the western province of Saudi Arabia have been determined. All investigated elements were detected using inductively coupled plasma–atomic emission spectrometer (ICP-AES) after pretreatment of the tested samples with microwave digestion system. The levels of the most dangerous heavy metals Cd and Pb in the samples were below the maximum permitted levels reported by World health Organization (WHO) standards. K and Ca were present at high levels in samples 2 (Chamomile) and 11 (Becham), respectively. Ca and Mg were the most abundant mineral elements in all herbal samples. Moreover, it is observed that the concentrations of most of the tested toxic metals in the investigated herbal plants are found below the permitted levels reported by the international regulatory standards of the medicinal plants.

Key words: Herbal drugs, mineral elements, toxic metals, inductively coupled plasma–atomic emission spectrometer (ICP-AES), microwave digestion.

INTRODUCTION

Herbal plants are important and widely used in folk therapeutic treatments worldwide. It is well known that about 75% of the world's population relies on non-conventional medicines or herbal plants. These plants are sometimes contaminated with toxic heavy metals depending on their nature and origin, which impose serious health risks to consumers (Xudong et al., 2011). Herbal drugs may be contaminated easily during growing and processing. Consequently, recent developments have been reported in the environmental pollution control and treatment to reduce its undesired effects on the human health (Pesavento et al., 2009; Sitko et al., 2012).

The unexpected and undesirable effects arise from damage caused by various toxic constituents of beneficial herbs themselves, such as heavy and toxic metals. Therefore, WHO and European Pharmacopeia (*Ph. Eur.*) has developed guidelines and tolerance limits for assessing quality of herbal medicines with reference to contaminants and residues (WHO Guidelines, 2007; Gasser et al., 2009). On the other hand, the essential mineral constituents represent important elements as macronutrients or micronutrients in the herbal drugs. However, these essential elements can also have harmful effects when their in-takes exceed the recommended

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Table 1. List of the investigated herbal drugs samples.

Sample No.	Plant name (Common name)	Latin name (Genus, Species)	Family	Origin
1	Mentha (Peppermint)	*Mentha piperita*	Labiatae	Egypt
2	German Chamomile (Chamomile)	*Matricaria chamomile*	Compositae	Syria
3	Foenugreek	*Trigonella foenum graecum*	Leguminosae	Yemen
4	Juniper	*Juniperus communis*	Cupressaceae	Saudi Arabia
5	Curcuma (Turmeric)	*Curcuma domestica*	Zingiberaceae	India
6	Sweet Basil (*Ocimum*)	*Ocimum basilicum*	Labiatae	Saudi Arabia
7	Hibiscus	*Hibiscus sabdariffa*	Malvaceae	Sudan
8	Cumin	*Cuminum cyminum*	Umbelliferae	Syria
9	Thyme	*Thymus vulgaris*	Labiatae	Syria
10	Half-Bar (*Cymbopogon*)	*Cymbopogon proximus*	Graminae	Egypt
11	Becham	*Commiphora opobalsamum*	Burseraceae	Saudi Arabia
12	Santonica (*Artemisia*)	*Artemisia cina*	Compositae	Egypt
13	Fennel	*Foeniculum vulgare*	Umbelliferae	India
14	Black seed (*Nigella*)	*Nigella sativa*	Ranunculaceae	Syria

quantities significantly in herbs (Mustafa et al., 2004). It is important to have a good quality control for herbal medicines in order to protect consumers from contamination (Chwan-Bor et al., 2003). Therefore, it is a critical challenge to determine the concentration of essential elements, heavy and toxic metals in herbal plants in order to ensure that their levels meet the related standards or regulations limiting their concentration in herbal drugs. Toxic and heavy metals such as Pb, Cd, As, and Cr are widely considered as potential contaminants in our environment due to their toxicities to human. Consequently, their quantification in such popular medicinal plants is essential for herbal drugs quality control purposes.

In order to enhance the awareness about the toxicity in medicinal plants, several authors all across the world have recently reported many studies on the assessment of inorganic constituents of the herbal drugs (Sumontha et al., 2006; Remigius et al., 2003; Slavica et al., 2006; Alwakeel 2008; Lasisi et al., 2005; Galia et al., 2010; Bushra et al., 2011; Sembratowicz et al., 2009; Pharidhavi and Agrrawal, 2007; Muhammad et al., 2010a, b; Ram et al., 2010; Shazia et al., 2010; Munish and Jaspreet, 2012; Jinyu et al., 2011; Mahwash et al., 2011; Archana et al., 2011; Petenatti et al., 2011; Ragavendran et al., 2012; Amirah 2008). Prior to the metal detection, the mineralization procedure is of great interest for obtaining accurate, precise and reliable results in the metallic analysis of medicinal plants. Many procedures have been reported for the pretreatment step, namely, the wet method, the dry ashing procedure and the microwave digestion method (Sumontha et al., 2006; Remigius et al., 2003; Slavica et al., 2006; Alwakeel 2008; Lasisi et al., 2005; Galia et al., 2010; Bushra et al., 2011; Sembratowicz et al., 2009; Pharidhavi and Agrrawal 2007; Muhammad et al., 2010a, b; Ram et al., 2010; Shazia et al., 2010; Munish and Jaspreet, 2012; Jinyu et al., 2011; Mahwash et al., 2011; Archana et al., 2011; Petenatti et al., 2011). Microwave digestion method has many advantages including rapid, efficient and has reproducible results (Sumontha et al., 2006; Remigius et al., 2003; Slavica et al., 2006; Alwakeel, 2008; Lasisi et al., 2005; Galia et al., 2010). In order to assess the essential and toxic heavy metals in herbal plants, several techniques have been used after the dissolution step, such as: atomic absorption spectroscopy (Remigius et al., 2003; Slavica et al., 2006; Alwakeel, 2008; Lasisi et al., 2005; Galia et al., 2010; Bushra et al., 2011; Sembratowicz et al., 2009; Pharidhavi and Agrrawal, 2007; Muhammad et al., 2010a, b; Ram et al., 2010; Shazia et al., 2010; Mahwash et al., 2011; Archana et al., 2011; Petenatti et al., 2011), neutron activation analysis (Jinyu et al., 2011; Amirah, 2008), inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Xudong et al., 2011; Sumontha et al., 2006; Alwakeel, 2008; Galia et al., 2010; Petenatti et al., 2011), scanning electron microscopy-energy dispersive X-ray (SEM-EDX) analyzer (Xudong et al., 2011; Ragavendran et al., 2012) and inductively coupled plasma–mass spectroscopy (ICP-MS) (Qing-hua et al., 2012; Rao et al., 2007).

The purpose of the present work was to estimate the concentration of some macronutrients, micronutrients and trace toxic metals in some marketed herbal plants in the western province in Saudi Arabia using ICP-AES after pretreatment by microwave digestion system.

MATERIALS AND METHODS

Sampling

A total of 14 different herbal plant samples were collected from local markets in western province of Saudi Arabia (Table 1). The investigated herbal samples were cut into small pieces, washed 3 times with de-ionized water and dried overnight at 100±5°C. Five grams quantities of the plants were stored in clean and dry Petri-

dishes at room temperature until analysis.

Reagents

All reagents used were of analytical reagent grade unless otherwise stated. Hydrogen peroxide (35% w/v) and nitric acid (69%) were purchased from Avonchem (UK) and Sigma-Aldrich (Germany), respectively. De-ionized water with conductivity <0.2 μS/cm obtained from a Milli-Q water system (Millipore, France, Elix 10) was used to prepare standard samples and washing all glassware throughout. All plastic and glassware were cleaned by soaking in dilute HNO_3, rinsed with de-ionized water and air dried before use. Mixed working standard solutions of the investigated mineral and toxic heavy metal ions prepared by appropriate stepwise dilutions of certified stock atomic spectroscopy standards (5% HNO_3, 3 to 500 mg/kg, Perkin Elmer, USA) were used for ICP-AES validation measurements.

Apparatus

CEM (model Mars, USA) microwave digestion system (maximum power, 1600 W; maximum pressure, 800 psi; maximum temperature, 300°C) equipped with closed vessel (EasyPrep) of Teflon reaction vessels was used in all the digestion procedures of plants samples. The reaction vessels were cleaned using 5 ml of concentrated nitric acid and thoroughly rinsed with de-ionized water before each digestion.

The simultaneous determination of the investigated mineral and heavy metal were carried out using a Perkin-Elmer (Optima 2100 DV, Norwalk, CT, USA) ICP-AES instrument connected with an AS 93 Plus auto-sampler. The 40-MHz free-running generator was operated at a forward power of 1300 W; the outer, intermediate and Ar carrier gas flow rates were 15.0, 0.2 and 0.8 L/min, respectively. The pump flow rate was 1.5 ml/min. The carrier gas flow rate was optimized to obtain maximum signal-to-background ratios.

Microwave digestion pretreatment

One gram of each herbal sample was digested with 4 ml of H_2O_2 (35% w/v) and 10 ml of nitric acid (69%) in the microwave digestion system via temperature ramping (ramped to 120°C for 10 min, held for 5 min, then ramped to 200°C over 10 min, then held for 15 min). A two blank digests were carried in the same way. The resulting clear digested solutions were quantitatively diluted with de-ionized water before analysis by ICP-AES.

Assessment of mineral and heavy metals using ICP-AES

The investigated mineral and toxic heavy metal ions were analyzed using ICP-AES under optimized plasma condition. Using the auto-sampler, the measured samples were nebulized downstream to the plasma and the concentrations were automatically determined using the standard calibration graph. The ranges of standard concentrations used varied between 0.03 (e.g. Pb) and 50 (e.g. Ca) mg/L depending on the levels in the matrix of the investigated metal ions. The system was adjusted to measure the samples in triplicates and the relative standard deviation was automatically calculated. The relative standard deviation (RSD) was <2% and the correlation coefficient was >0.99998.

RESULTS AND DISCUSSION

Safety and efficacy of herbal medicines are two main issues of a drug therapy to which, the source and quality of the raw materials play an important role. For this purpose, the concentration of 22 elements of macronutrients, micronutrients and trace toxic heavy metals were determined in different 14 herbal plants using ICP-AES under the optimized conditions and after microwave digestion. Using highly sophisticated ICP-AES in combination with the microwave digestion system provides accurate, precise and reliable measurements of the investigated elements (Slavica et al., 2006). The precession is on average lower than 3% (RSD). The variation in elemental concentrations is mainly attributed to the difference in botanical structure and the plant origin as well as the preferential absorbability of the plant.

Macronutrients

Metals play a vital role as structural and functional components of proteins and enzymes in cells. Each mineral plays a number of different functions in the body. The most important pathway of metals to transport into human is from soil to plant and from plant to human. The levels of the mineral essential elements (Ca, Mg, Na, K and Fe) detected in the investigated herbal drugs are presented as mg/kg in Table 2. The data revealed that all analyzed elements were accumulated by the plants species at different concentration. Levels of the essential elements were found to be higher than those of the non-essential metals.

The results indicate that samples 1, 11, 2, and 13 contained the highest (Fe and Mg), Ca, K and Na, respectively, among the 14 herbal drugs studied. There are no international limits for the macronutrients in the herbal drugs. However, the results obtained agreed with the earlier studies of elemental distribution in herbal plants species (Sumontha et al., 2006; Lasisi et al., 2006; Shazia et al., 2010; Munish and Jaspreet, 2012). The results obtained reveal that Fe levels in herbs did not exceed the physiological limits for plants. The abundance of Ca, Mg, Na and K in the present study was also in agreements with the previous studies, which indicate that these elements were the most abundant elements in many herbal drugs.

Micronutrients

Some types of metals such as Se, Cr, Mn, Zn, Cu, and Ni are natural essential micronutrients. The levels of these metals at studied herbal drugs are presented in Table 3. The results indicated that samples 1, 3, 5, and 12 contained the highest Cu, Se, (Cr, Mn and Ni) and Zn, respectively. In all cases, the results were similar to, or lower than the case of Cr, those reported in several studies (Mustafa et al., 2004; Chwan-Bor et al., 2004; Sumontha et al., 2006; Slavica et al., 2006). The

Table 2. Macronutrients contents (µg/g) in the tested herbal species (mean), n=3.

Sample No.	Plant name	Fe	Ca	Mg	K	Na
1	Mentha	1068	15375	5725	23160	958.9
2	German Chamomile	466.5	6141	1867	25365	2485
3	Foenugreek	72.7	2043	1055	10428	373.1
4	Juniper	315.1	11164	730.9	3591	137.5
5	Curcuma	726.0	1851	2062	24120	132.7
6	Sweet Basil	225.0	14136	2697	15825	190.0
7	Hibiscus	132.0	16335	3636	18765	905.4
8	Cumin	134.1	7849	2556	12391	344.7
9	Thyme	274.1	20160	2380	14185	937.2
10	Half-Bar	937.8	3552	1113	2400	96.4
11	Becham	54.4	25364	482.2	5034	836.1
12	Santonica	863.9	13215	5265	15810	27.0
13	Fennel	104.7	13654	3037	14164	2664
14	Black seed	59.3	6486	2007	7270	37.8

Table 3. Micronutrients contents (µg/g) in the tested herbal species (mean), n=3.

Sample No.	Plant name	Se	Cr	Mn	Zn	Cu	Ni
1	Mentha	2.01	2.44	102.88	18.46	22.63	1.33
2	German Chamomile	1.74	1.32	43.06	31.41	14.26	1.24
3	Foenugreek	3.36	0.70	16.15	34.45	8.23	0.340
4	Juniper	1.26	0.72	29.67	4.33	3.48	0.310
5	Curcuma	1.60	2.68	570.6	37.33	6.01	3.45
6	Sweet Basil	0.66	0.61	20.02	21.93	7.38	0.135
7	Hibiscus	1.29	0.525	180.0	30.10	4.69	1.62
8	Cumin	3.04	0.78	17.43	27.09	7.83	0.750
9	Thyme	1.08	1.24	22.29	19.56	8.83	1.87
10	Half-Bar	0.225	2.38	44.31	14.16	3.36	1.12
11	Becham	0.255	0.240	2.55	0.495	2.17	0.045
12	Santonica	0.780	2.02	69.46	56.37	15.60	1.41
13	Fennel	0.765	0.750	69.69	29.56	13.90	1.08
14	Black seed	0.525	0.525	20.85	41.14	13.80	2.59

concentration range detected for Mn (2.55 to 570.6 mg/kg) was relatively high. However, the concentration of Mn was within the same range as previous studies (824.8 mg/kg) (Sumontha et al., 2006; Slavica et al., 2006). It was reported that a variety of medicinal plants show great Mn accumulating ability (Sembratowicz et al., 2009).

Trace toxic heavy metals

Contamination of herbal drugs with chemically toxic substances can be attributed to environmental pollution, soil composition and fertilizers. In addition, pesticides containing arsenic and mercury are widely used in some countries (WHO Guidelines, 2007). It was reported also, that the accumulation of heavy metals in herbal plants depends on climate factors, plant species, air and soil pollution (Lasisi et al., 2005). Trace toxic elements of Pb, Be, Cd, As, Ag, Sb, Al, Ba, Tl, Co, and V were determined in the investigated herbal plants using ICP-AES under the optimized conditions and after microwave digestion. The results obtained are summarized in Table 4. The results obtained indicated that Be, As, Ag and Tl were not detected in all investigated herbal drug samples (not presented).

Indeed, the analyzed herbal plants are widely used in Saudi Arabia as traditional drugs. However, the toxic metals present in high concentration in species are of particular importance in relation to WHO standards for Pb and Cd as toxic metals. The maximum permissible levels (MPL) for Pb and Cd are 10 and 0.3 mg/kg, respectively (Mustafa et al., 2004; Lasisi et al., 2005), while our values are below the WHO MPL for Pb and Cd for all investigated herbal plants. Moreover, the levels of Pb and Cd in

Table 4. Trace elements contents (µg/g) in the tested herbal species (mean), n=3.

Sample No.	Plant name	Pb	Cd	Sb	Al	Ba	Co	V
1	Mentha	0.825	0.075	1.08	685.5	26.23	0.09	1.99
2	German Chamomile	1.03	0.120	0.870	670.8	3.42	ND	0.28
3	Foenugreek	1.59	0.195	2.05	28.47	ND	ND	ND
4	Juniper	ND	ND	0.405	497.2	4.42	ND	ND
5	Curcuma	1.54	0.360	0.930	731.9	60.42	0.87	0.66
6	Sweet Basil	ND	ND	0.045	245.9	6.34	ND	ND
7	Hibiscus	1.18	0.180	0.285	227.4	39.34	ND	ND
8	Cumin	ND	0.030	0.975	181.3	8.88	ND	ND
9	Thyme	1.36	0.120	0.465	496.8	12.67	ND	0.330
10	Half-Bar	0.405	0.030	0.195	1237	6.64	0.060	2.14
11	Becham	ND	ND	ND	97.42	32.20	ND	ND
12	Santonica	0.345	0.105	0.045	695.9	192.9	ND	1.51
13	Fennel	ND	0.045	0.105	83.4	5.50	ND	ND
14	Black seed	ND	0.060	0.045	31.84	3.10	ND	ND

ND: No detection.

all investigated herbal plants come with a good agreement (or lower) with those reported in similar studies (Mustafa et al., 2004; Chwan-Bor et al., 2003; Sumontha et al., 2006; Slavica et al., 2006; Bushra et al., 2011).

The highest concentration of antimony is 2.05 mg/kg and was detected in *Foenugreek*. Although, this value is slightly higher than the antimony levels reported in previous studies in tea products and *Nigella sativa* seeds (Sumontha et al., 2006; Amirah, 2008); this value is still lower than those reported for antimony levels in earlier studies in some herbs (He et al., 2012; Müllera et al., 2009). The range of Al concentration in the investigated herbal plants varied between 28.47 mg/kg in sample 3 and 731.9 mg/kg in sample 5. Although the concentration of Al is relatively high in most of the investigated herbal plants, our values are below the averages levels reported for Al in tea products (2014 mg/kg) (Sumontha et al., 2006). Ba, Co, and V contents detected in all investigated samples (Table 4) are considerably lower and comparable with those reported in previous studies (Sumontha et al., 2006; Galia et al., 2010; Ram et al., 2010; Muhammad et al., 2010; Shazia et al., 2010).

Conclusion

Twenty-two elements of macronutrients, micronutrients and toxic heavy metals have been determined in 14 popular herbal plants which are widely used in Saudi Arabia using ICP-AES under optimized conditions and after microwave digestion. Based on the obtained results, the herbal plants tested in this work are good source of important elements, and therefore, they could serve as supplements of macro and micro nutrients elements in the body. The concentrations of the essential and nonessential elements were found safe in most of the investigated herbal plants. The results of this study may provide a useful reference for analysis of essential and toxic elements in herbal plants.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of the anti-ulcer property of aqueous extract of unripe *Musa paradisiaca* Linn. peel in Wistar rats

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This study investigated the antiulcer activity of aqueous extract of unripe *Musa paradisiacal* (plantain) peels in rats using ethanol, aspirin, indomethacin and pyloric ligation-induced ulcer models. Fresh peels of *M. paradisiaca* were extracted in hot water and the yield lyophilised. Distilled water, standard drugs and extract were injected intraperitoneally before inducing ulcer. Lethality test and quantitative phytochemical analyses were also carried out using standard techniques. Results showed that the extract at 50, 100 and 200 mg/kg wt offered 73.87, 80.18 and 81.98% protection, respectively against ethanol-induced ulcer, whereas cimetidine (50 mg/kg) produced 72.07% ulcer protection. There was no significant difference ($p > 0.05$) between the various groups. The extract also inhibited aspirin-induced ulcer whereas omeprazole (20 mg/kg) significantly enhanced aspirin-induced ulcer. Similar to cimetidine, the extract did not inhibit indomethacin-induced ulceration. Extract (50, 100, and 200 mg/kg) and cimetidine (50 mg/kg) inhibited pyloric ligation-induced ulcer by 100 and 75%, respectively. Findings suggest antiulcerogenic potentials of the extract, thereby supporting its ethnomedicinal use as antiulcer agent. Up to 5000 mg/kg of extract did not cause mortality of the animals, indicating safety of the extract. The extract was rich in flavonoids (1.40 ± 0.02 mg/100 g).

Key words: *Musa paradisiaca*, peptic ulcer, antiulcerogenesis, aspirin-induced ulcer, medicinal plants, flavonoids.

INTRODUCTION

Ulcer is amongst the common defects of the gastric or intestinal walls, clinically presented as abdominal stress, most often in the upper part of the abdomen and epigastric region. It may manifest as superficial, deep or perforated erosions of the mucosal lining of the stomach (a gastric ulcer), or the small intestine (a duodenal ulcer). Both types of ulcerations are commonly known as peptic ulcer (Dhasan et al., 2010). Peptic ulcer, which is a non-malignant type of ulceration, arises as a result of the distortion of the balance between the endogenous aggressive factors (HCl, pepsin, bile, reactive oxygen species, etc) and the intestinal cytoprotective components (prostaglandins, nitric oxide, mucus bicarbonate system, surface active phospholipids, endogenous antioxidants, some growth factors, etc) (Raju et al., 2009; Wasman et al., 2010).

Epidemiological evaluations indicate that gastric and peptic ulcers are high ranking global health challenges

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effecting approximately 8 to 10% of the population (Kelly et al., 2009). Factors that predispose to the development of this disorder include stress, alcohol consumption, smoking, use of non-steroidal ant-inflammatory drugs (NSAIDS), *Helicobacter pylori* infections and drugs that stimulate the secretion of gastric acid and pepsin (Bandyopadhyay et al., 2001). A wide range of synthetic anti-ulcer agents commonly employed in the treatment of ulcer such as the H_2 – blockers (cimetidine, ranitidine, etc), M_1 – blockers (pirenzepine, telenzepine, etc) and proton pump antagonists (omeprazole, lansaprazole etc) decrease gastric acid secretion, whereas sucralate, carbenoxolone and like-drugs enhance mucosal defences. Although these drugs have improved ulcer therapy, the problems associated with their use (side actions), tolerance and possible incidence of relapse are still a great concern.

Medicinal plants, on the other hand, have equally contributed tremendously in the management of diseases and maintenance of health. This could be as a result of their availability, higher safety margin, and cheaper cost. Several herbal preparations have reportedly been employed in ulcer treatment. Included in this list are extracts of *Momordica* species (Vijayakumar et al., 2011; Dhasan et al., 2010; Alam et al., 2009), extracts of *Moringa oleifera* (Devaraj et al., 2007; Kansara and Singhal, 2013), extracts of the bark and leaves of *Sesbania* species (Bhalke et al., 2010; Sertie et al., 2001). Different parts of *Musa paradisiaca* Linn. (*Musaceae*) such as fruits, leaves, peels, roots and stalks have been reported to possess haemostatic effect (Obadomi and Ochuko, 2002), antidiabetic activity (Ojewole and Adewunmi, 2003), antihypertensive effect (Mohammad and Saleha, 2011; Jaiprakash et al., 2006), and hypolipidaemic activity (Mohammad and Saleha, 2011). In addition, Prabha et al. (2011) reported the antiulcer activity of the edible part of *Musa sapientum* on peptic ulcer, whereas Surabhi, (2011) reported the antiulcer potential of the aqueous leaf extract of *M. paradisiaca*. The thrust of this study is to evaluate the efficacy of the peels of unripe *M. paradisiaca* as a remedy against peptic ulcer using various experimentally-induced ulcer models.

MATERIALS AND METHODS

Plant

Freshly cut bunches of unripe plantain (*M. paradisiaca* Linn.) were harvested from Nanka, Orumba North Local Government Area of Anambra State, Nigeria, in February, 2012. The plant was identified and authenticated by Mr. P.O. Ugwuozor of The Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria.

Animal

Apparently healthy male Wistar albino rats weighing between 100.0 to 112.0 g were used for the studies. All the rats were purchased from the Animal house of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka and were housed in standard animal cages in The Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka. The animals were kept on animal pellet diet (Guinea Feeds Limited, Ewu, Edo State, Nigeria) and drinkable water *ad libitum* for one week before the experiments, to allow for acclimatization.

METHODOLOGY

Preparation of the aqueous extract

The fresh unripe *M. paradisiaca* fingers were washed and peeled, avoiding the edible part, and the peels cut into very small pieces. Known quantities (1.0 kg) of the peels were boiled in distilled water at the ratio of $1{:}2^{w}/_{v}$ for $1^{1}/_{2}$ h. The extract was filtered using cheese cloth and a membrane filter paper of 47.0 mm diameter and pore size approximately 0.45 µm, with the aid of a suction pump. The filtrate was concentrated using rotary evaporator, and the extract stored in the refrigerator prior to use.

Ethanol-induced ulcer study

The effect of *M. paradisiaca* extract on ethanol-induced ulcer was investigated using the method of Mbagwu et al. (2011). Thirty rats (100 to 110 g body wt.) were starved for 18 h and grouped into five groups (A to E) of six rats each based on their body weights. One hour before ethanol administration, animals in group A received distilled water, 2.0 ml/kg body wt. whereas rats in group B received cimetidine (50.0 mg/kg wt.). The extract-treated rats in groups C, D, and E received 50.0, 100.0 and 200.0 mg/kg body wt. doses of the extract, respectively. One millitre per kilogramme weight of ethanol (90%) was then administered to all the animals. Distilled water, cimetidine, extract and ethanol were all given intraperitoneally (i.p). Cimetidine and extract were dissolved in distilled water. One hour after ethanol injection, the rats were anaesthetized with chloroform and dissected. The stomachs were excised and carefully opened along the line of greater curvature to expose the walls. The stomachs' contents were then washed off and the stomach walls viewed with the aid of hand lens to determine the ulcer scores using the method of Raju et al. (2009). The ulcerative lesions were counted and scored as follows: Normal stomach – 0; spot ulceration – 1.0; haemorrhagic streaks – 1.5; ulcer – 2.0; and perforations - 3.0.

Aspirin-induced ulcer study

The effect of the extract on aspirin-induced ulcer model was studied according to the method of Ubaka et al. (2010). Thirty rats (100 to 110 g) were sorted into five groups (n = 6) according to their weights. Group A (negative control) received 2.0 ml/kg body wt. of distilled water, while group B (positive control) received the reference drug, omeprazole (20.0 mg/kg body wt.). Animals in the test groups C, D, and E received *M. paradisiaca* peel extract (50.0, 100.0, and 200.0 mg/kg body wt., respectively). Distilled water, omeprazole and the extract were administered intraperitoneally (i.p). After 1 h, all the animals were given aspirin (200.0 mg/kg body wt.) injection (i.p). Four hours later, the animals were anaesthetized with chloroform and dissected. Their stomachs were removed and treated as mentioned and the ulcers scored.

Indomethacin-induced ulcer study

The method of Ubaka et al. (2010) was adopted. Thirty male Wistar rats (105 to 115 g) were starved for 18 h and grouped into five groups (n = 6) according to their weights. Groups A, B, C, D and E received intraperitoneal injections of distilled water (2.0 ml/kg body wt.), cimetidine (50.0 mg/kg body wt.), extract (50.0 mg/kg body wt.), extract (100.0 mg/kg body wt.), and extract (200.0 mg/kg body wt.), respectively. Distilled water and cimetidine served as negative and positive controls, respectively. One hour later, indomethacin injection (i.p) was given to all the rats at a dose of 40.0 mg/kg body wt. Cimetidine, extract and indomethacin were solubilized in distilled water. Eight hours after indomethacin injection, the rats were anaesthetized with chloroform and dissected. The stomachs were isolated and treated as previously mentioned and the ulcer scores determined.

Pyloric ligation – induced ulcer study

Anti-ulcerative effect of the extract was also studied using the pyloric ligation method of Raju et al. (2009). Thirty male Wistar albino rats used were subjected to a 24 h – fast and grouped into five groups A to E (n = 6). Their abdomen were slightly opened under mild chloroform anaesthesia, and their pylorus carefully lifted and ligated, avoiding any damage to the vascular tissues. After ligation, the stomachs were quickly replaced and the abdomen sutured. Immediately after suturing, the animals in groups A, B, C, D and E received intraperitoneal injections of distilled water (2.0 ml/kg body wt.), cimetidine (50.0 mg/kg body wt.), extract (50.0 mg/kg body wt.), extract (100.0 mg/kg body wt.) and extract (200.0 mg/kg body wt.), respectively. Cimetidine and extract were dissolved in distilled water. Distilled water and cimetidine served as negative and positive references, respectively. Four hours after treatment, the animals were anaesthetized with chloroform, their abdomen dissected and duodenal portion ligated. The stomachs were then opened along the line of greater curvature and gastric content extracted. Gastric volumes were determined after centrifuging the gastric content at 4000 rpm for 20 min. Ulcer scores were also estimated as mentioned previously.

Calculation of ulcer index and percentage inhibition

Ulcer index (UI) = Mean of ulcer scores per rat

Percentage ulcer inhibition = UI (control) – UI (treated) × 100 /UI (control)

Acute toxicity study

Lorke (1983) method was adopted for the determination of the median lethal dose (LD_{50}) of the aqueous *M. paradisiaca* extract. In the pilot study, nine male Wistar rats weighing between 80 to 85 g were used. The rats were randomly divided into three groups (A, B, and C) of three rats each and were administered *M. paradisiaca* extract (i.p) 10.0, 100.0 and 1000.0 mg/kg body wt., respectively. The animals were then observed for behavioural changes and mortality for 24 h. When no death was observed in any of the groups, five other groups (I to V) were given 1250, 1500, 2000, 2500 and 5000 mg/kg body wt. of the extract (i.p) and monitored for 24 h for changes in behaviour and mortality. The LD_{50} is usually calculated as the geographic mean of the least lethal dose that killed a rat and the highest dose that did not kill a rat.

Analysis of phytochemical constituents

The amount of phenolic constituents present in the extract was determined using the method of Kahkonen et al. (1997).

Total alkaloids, cyanogenic glycosides, saponins, tannins and phytates were determined according to the method of AOAC (1984), whereas the quantity of flavonoids present was estimated using the method of Bohamam and Kocepal (1974).

Statistical analysis

All the data presented are means ± standard errors of means. Comparative analysis between the various groups was performed using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The pathogenesis of peptic ulcer is the distortion of the equilibrium existing between the aggressive factors (gastric acid, pepsin, etc) and the gastrointestinal components responsible for the maintenance of the integrity of gastrointestinal mucosal membrane (Raju et al., 2009; Wasman et al., 2010). Consequently, any agent that possesses the capacity to restore the equilibrium by enhancing the activity of the cytoprotective agents or diminishing the secretion of gastric acid could serve as an antiulcerative agent. Mbagwu et al. (2011) reported that ethanol induces ulceration due to the stimulation of lipid peroxidation resulting to increased generation of reactive oxygen species which cause serious damaging effect on the cells and cellular membranes of the mucosal epithelium.

The results from our studies indicate that intraperitoneal administration of ethanol produced severe ulcers and haemorrhagic streaks on the gastric mucosa of the experimental animals. Pre-treatment of the animals with 50.0 mg/kg body wt. of aqueous peel extract of *M. paradisiaca* prior to ethanol injection protected against ulcerogenesis by 73.87% (from UI of 1.11 ± 0.31 to 0.29 ± 0.05). Higher doses of the extract, 100.0 and 200.0 mg/kg body wt. inhibited ulcer formation by 80.18% (that is, 1.11 ± 0.31 to 0.22 ± 0.05) and 81.98% (1.11 ± 0.31 to 0.20 ± 0.07), respectively (Table 1). The differences between the groups were not statistically significant at $p < 0.05$. These observations also validate the ethnomedical use of concoctions containing *M. paradisiaca* extracts as effective antiulcerogenic ingredients (Swathi et al., 2011; Vadilevan et al., 2006; Mbagwu et al., 2011).

Results also show that 11.25% of the damage caused by ethanol appeared as red colouration of the gastric mucosa. The implication of this is that all the lesions (perforations, ulcers, or haemorrhagic streaks) developed as a result of the disruption of the vascular tissues of the gastric mucosal endothelium (Nagaraju et al., 2012). The *M. paradisiaca* extract effect was not statistically different ($p > 0.05$) from that of the standard antiulcer agent, cimetidine

Table 1. Effect of aqueous *M. paradisiaca* peel extract on ethanol-induced ulcer in rats.

Treatment	Ulcer Index	Percentage ulcer inhibition
Distilled water, 2.0 ml/kg	1.11 ± 0.31	-
Cimetidine, 50.0 mg/kg	0. 31 ± 0.09	72.07
M. paradisiaca, 50.0 mg/kg	0.29 ± 0.05	73.87
M. paradisiaca, 100.0 mg/kg	0.22 ± 0.05	80.18
M. paradisiaca, 200.0 mg/kg	0.20 ± 0.07	81.98

Results are presented as means ± standard errors of means, n = 6. *M. paradisiaca* extract protected against ethanol-induced ulceration of rat stomach. No significant difference at $p<0.05$ was observed between groups.

Table 2. Effect of aqueous *M. paradisiaca* peel extract on aspirin – induced ulcer in rats.

Treatment	Ulcer index	Percentage ulcer inhibition
Distilled water, 2.0 ml/kg	0.10 ± 0.05	-
Omeprazole, 20.0 mg/kg	0. 52 ± 0.09*	+420.00
M. paradisiaca, 50.0 mg/kg	0.10 ± 0.03**	0.00
M. paradisiaca, 100.0 mg/kg	0.00 ± 0.00**	100.00
M. paradisiaca, 200.0 mg/kg	0.01 ± 0.01**	90.00

Results are presented as means ± standard errors of means, n=6. * is significant at $p < 0.05$, when compared with the control group. ** is significant at $p<0.05$, when compared with omeprazole. **+**represents increase in ulcer index. *M. paradisiaca* peel extract (≥ 100.0 mg/kg) decreased the incidence of aspirin – induced ulcer in rats.

(50.0 mg/kg body wt.), which offered 72.07% protection. The non-steroidal anti-inflammatory drugs (NSAIDs), otherwise known as aspirin and aspirin-like drugs, such as indomethacin, adversely affect the gastroduodenal mucosa causing ulcerative lesions on the mucus membrane. This may result through several mechanisms, including suppression of the synthesis of gastric cyto-protective prostaglandin E_2 (via cyclooxygenase enzyme inhibition), decreased blood supply to gastric mucosa, increased gastric acid secretion, as well as inactivation of the growth factors involved in mucosal defence and repair (Wallace, 2000).

Results presented in Table 2 also revealed that the extract at all doses tested inhibited aspirin-induced ulcer in rats. Maximal inhibition (100%) was recorded at 100.0 mg/kg body wt. of extract, although this observation was not statistically significant at $p < 0.05$. Conversely, the reference drug, omeprazole (20.0 mg/kg body wt.) significantly ($p < 0.05$) aggravated aspirin-induced ulcer by 420% when compared with the negative control. This effect of omeprazole was statistically ($p<0.05$) different from that of *M. paradisiaca* extract at all the doses tested. The extract may have countered the effect of aspirin by reactivating prostaglandin synthesis that was inhibited by aspirin, or the processes essential for the regeneration of gastrointestinal mucosa. On the other hand, omeprazole could be blocking gastric ulcers through a mechanism or mechanisms different from the cyclooxygenase pathway. In an earlier report (Biswas et al., 2003), omeprazole inhibited gastric ulcer by virtue of its antioxidant property (the scavenging of hydroxyl radicals).

Result of the investigation of the effect of *M. paradisiaca* extract on indomethacin-induced ulcer is presented in Table 3. It indicates that extract at 50.0, 100.0, and 200.0 mg/kg body wt. did not offer any protection against gastric ulcer induced by indomethacin admini-stration. Rather, the extract (100.0 mg/kg body wt.) increased the severity of the ulcer by 159.26%. The effect of the extract was similar to that of cimetidine (50.0 mg/kg body wt.) which promoted ulcer formation by 97.0%. Differences between the various groups were non-significant ($p > 0.05$). This result corroborates earlier report by Kauffman et al. (1979), that cimetidine did not inhibit indomethacin-induced bowel ulceration. The results also suggest that the extract, just like cimetidine, synergized with indomethacin in the enhancement of mucosal damage. This synergistic combination could be between indomethacin and any of the phytochemicals present in the extract. Moreover, Clayton et al. (2006) had reported that cimetidine and ranitidine (short-acting inhibitors of acid secretion) were very much weaker at inhibiting indomethacin-induced ulcers.

Table 3. Effect of aqueous *M. paradisiaca* peel extract on indomethacin – induced ulcer in rats.

Treatment	Ulcer index	Percentage ulcer inhibition
Distilled water, 2.0 ml/kg	0.54 ± 0.22	-
Cimetidine, 50.0 mg/kg	1.51 ± 0.26	+97.00
M. paradisiaca, 50.0 mg/kg	1.04 ± 0.26	+92.59
M. paradisiaca, 100.0 mg/kg	1.40 ± 0.20	+159.26
M. paradisiaca, 200.0 mg/kg	0.96 ± 0.18	+77.78

Results are presented as means ± standard errors of means, n=6. +represents increase in ulcer index. Like cimetidine, *M. paradisiaca* extract enhanced indomethacin – induced ulceration of rat stomach. No significant differences at p<0.05 were observed between groups.

Table 4. Effect of *M. paradisiaca* peel extract on pyloric ligation – induced ulcer in rats.

Treatment	Ulcer index	Gastric juice Vol. (ml/100 g)	Percentage ulcer inhibition
Distilled water, 2.0 ml/kg	0.50 ± 0.14	1.60 ± 0.30	-
Cimetidine,50.0 mg/kg	0.10 ± 0.02	1.00 ± 0.04	75.00
M. paradisiaca,50.0 mg/kg	0.00 ± 0.00	0.10 ± 0.01	100.00
M. paradisiaca,100.0 mg/kg	0.00 ± 0.00	0.17 ± 0.03	100.00
M. paradisiaca,200.0 mg/kg	0.00 ± 0.00	0.13 ± 0.08	100.00

Results are presented as means ± standard errors of means, n=6. Like cimetidine, *M. paradisiaca* extract inhibited pyloric ligation – induced ulceration of rat stomach. No significant difference at p<0.05 was observed between groups.

The reference drugs, omeprazole, and cimetidine, used in this study, are conventional antisecretory drugs (gastric acid pump inhibitors) that are employed in the treatment of peptic ulcer. Pyloric ligation stimulates the secretion of gastric juice in experimental animals, though the mechanism is still unclear. The antisecretory effect of the extract was then studied using pyloric-ligation-induced ulcer model. As shown in Table 4, the extract remarkably prevented pyloric ligation-induced gastric ulcer, at all the tested doses. Doses of the extract above 50.0 mg/kg body wt. were more efficacious (produced 100.0% protection, that is UI 0.50 ± 0.14 to UI 0.00 ± 0.00) than the standard drug, cimetidine (50.0 mg/kg body wt.), which decreased the ulcer index by 75.0%. At 50.0 mg/kg body wt., the extract non-significantly (p > 0.05) reduced the volume of gastric secretion from 1.60 ± 0.30 to 0.10 ± 0.10. The effect of the extract increased with the dose. However, it is quite obvious from the results presented, that unripe *M. paradisiaca* peel extract mimics the actions of cimetidine in ameliorating experimental ulcers.

Findings from the 24 h–acute toxicity testing in rats showed that doses of *M. paradisiaca* peel extract as high as 1000.0 mg/kg body wt. did not cause any behavioural change or mortality in the animals. Phytochemical screening of the constituents of the extract showed that it contained phenolic substances (281.00 ± 0.82 mg/100 g), flavonoids (1.40 ± 0.02 mg/100 g), alkaloids (0.09 ± 0.01 mg/100 g), cyanogenic glycosides (6.25 ± 0.58 mg/100 g), saponins (0.73 ± 0.01 mg/100 g), tannins (1.25 ± 0.01 mg/100 g) and phytates (3.51 ± 0.02 mg/100 g). The antiulcer effect of *M. paradisiaca* extract could be ascribable to the presence of any of the detected chemical agents. Since plant flavonoids have been found to demonstrate gastroprotective and gastric ulcer healing properties (Kelly et al., 2009), these natural products may also play a role in the antiulcer activity of the aqueous, flavonoid-rich, unripe *M. paradisiaca* peel extract. Furthermore, screening for active antiulcer agents in *M. paradisiaca* extract using high performance liquid chromatography (HPLC), Lewis and Shaw (2001) reported the presence of a monomeric flavonoid, leucocyanidin, which protected the gastric mucosa from aspirin-induced erosions by increasing mucus thickness.

Conclusion

Findings from our studies indicate that the aqueous peel extract of *M. paradisiaca* Linn. protected against ulcerative lesions induced by ethanol, aspirin and pyloric ligation. Similar to the action of cimetidine standard, the extract exacerbated indomethacin-induced ulcer. *M. paradisiaca* peel extract mimics the actions of cimetidines in ameliorating ulcers in all the experimental models adopted, therefore it may possess an antisecretory property.

CONFLICT OF INTEREST

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Effect of co-administration of artemether and nevirapine on haematological parameters in normal and immunosuppressed Wistar rats

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Artemether and nevirapine are effective drugs used in the treatment of malaria and as part of antiretroviral therapy, respectively in many parts of the world. However, concomitant use of these drugs may pose an adverse drug interaction that may alter their pharmacological effects. In this study, possible effect(s) of artemether-nevirapine co-administration on haematological indices in both normal and immune-compromised rats were investigated. Animals were divided into 6 groups of 6 rats per group. Groups 4, 5 and 6 received 30 mg/kg of nevirapine daily for 21 days. In addition, groups 5 and 6 received 5 and 10 mg/kg artemether, respectively while rats in groups 2 and 3 received artemether 5 and 10 mg/kg, respectively from day 15, for 7 days. This was repeated in immunosuppressed rats with an additional group receiving only dexamethasone and 3% v/v Tween 80. Rats in all groups received dexamethasone (20 mg/kg) and booster doses of 10 and 5 mg/kg on days 8 and 15, respectively, except the control group. All drugs administration were through intraperitoneal route. Data were analysed using analysis of variance (ANOVA), followed by Dunnett's post hoc test. P-values less than 0.05 were considered statistically significant. Artemether-nevirapine co-administration caused a significant decrease ($P < 0.05$) in packed cell volume (PCV), red blood cell (RBC), haemoglobin, lymphocyte as well as an increase in neutrophils in both normal and immunosuppressed rats. Findings from this study showed that concomitant administration of artemether and nevirapine altered PCV, RBC, haemoglobin, WBC, neutrophil and lymphocyte of both normal and immunosuppressed rats and this may induce some adverse effects on blood parameters.

Key words: Artemether, nevirapine, haematology, immunosuppression, rats.

INTRODUCTION

Concurrent use of two or more drugs is sometimes essential in order to achieve therapeutic goal(s) (Nwonu et al., 2008). However, when two or more drugs are administered together, there may be a possibility that one drug will alter the pharmacological effects of the other(s) (Perucca, 2006). With the increasing availability of new drugs, for example, artemether and their concomitant use with other drugs e.g.nevirapine), there has been a rise in

the potential for adverse drug interactions (Kuhlman and Muck, 2001). Artemether is a derivative of artemisinin, used as an effective drug in the treatment of malaria (Akomolafe et al., 2011). Nevirapine is one of the drugs routinely given as part of antiretroviral therapy in Nigeria (Idigbe et al., 2005) and other parts of the world (Boulle et al., 2008). However, concomitant use of these drugs may pose an adverse drug interaction that may alter their pharmacological effects. There exist pharmacokinetic interaction between artemether and nevirapine (Byakika-Kibwika *et al*., 2011) but there are no published studies on pharmacodynamic interaction involving these drugs, although the potential exists since nevirapine is a known inducer of human CYP3A4 and CYP2B6 isoenzymes, which metabolise a range of drugs and other zenobiotics. In addition, it was reported that the sub-class of antiretroviral drugs known as non-nucleotide reverse transcriptase inhibitors to which nevirapine belongs are metabolized to some extent by P_{450} enzymes, therefore exposing them to clinically significant drug interactions (Soyinka *et al*., 2009). Moreover, nevirapine has been reported to affect the pharmacokinetic profiles of artemether (Kredo *et al*., 2011), establishing the need for more clinical investigation. On the other hand, artemether is metabolized to dihydroartemisinin via cytochrome P450 CYP3A4, CYP2B6 and possibly CYP2A6 (Djimde and Lefevre, 2009). The presence of almost the same isoenzymes for artemether and nevirapine creates the potential for drug interaction on co-administration. The present study therefore aims at investigating the effect of artemether and nevirapine co-administration on haematological parameters in both normal and immunosuppressed Wistar rats.

METHODOLOGY

Experimental animals

Seventy-eight adult male and female Wistar rats (180 to 230 g) housed in a temperature-controlled environment with approximately 12 h light and 12 h dark cycle, with free access to standard rat feed and water *ad libitum* were used for this study. They were bred in the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The use and handling of the animals were in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals (UND/World Bank/WHO, 2001) and was approved by the Institutional ethical committee on the use of laboratory animals.

Drugs

Nevirapine (Hetero Drugs Limited, India) was dissolved in 3% v/v Tween 80 according to the required concentration for administration in rats and administered immediately. Artemether (Haupt Pharma Livron, France) was diluted using the same solvent. Dexamethasone (JinLing Pharmaceutical, China) was the immunosuppressive agent used. All drugs were freshly prepared before use.

Experimental design

Animals used were divided into 6 groups of 6 animals per group (males and females). Groups 4, 5 and 6 received 30 mg/kg of nevirapine daily for 21 days. In addition, groups 5 and 6 received 5 and 10 mg/kg artemether, respectively while rats in groups 2 and 3 received artemether 5 and 10 mg/kg, respectively from day 15 for 7 days. This experiment was repeated in immunosuppressed rats with an additional group that received only dexamethasone and 3% v/v Tween 80. Rats in all groups received dexamethasone (20 mg/kg) and booster doses of 10 and 5 mg/kg on days 8 and 15, respectively, except those rats in group 1 that received 3% v/v Tween 80. All drugs administration was through intraperitoneal route. On the 22nd day of the experiment, all the experimental rats were humane-killed under light chloroform anaesthesia and blood samples were collected into EDTA bottles for parametric estimation of CD_4^+ Count (using Partec Cyflow Counter, Japan), red blood cell (RBC), white blood cell, lymphocyte, neutrophil (Neut), packed cell volume (PCV), haemoglobin (Hb) and platelets (PLT) using Sysmex KX 21N Haematological Auto Analyser Machine (Japan). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were accordingly determined.

Statistical analysis

The research results were calculated as means ± standard error of mean (SEM). Test of significance was carried out using analysis of variance (ANOVA) with Dunnet's post-hoc test. Values of $P < 0.05$ were considered statistically significant and results were presented as tables and figures.

RESULTS

The result showed that artemether-nevirapine co-administration caused a significant decrease ($p < 0.05$) in PCV, RBC, lymphocyte and haemoglobin in both normal and immunosuppressed Wistar rats while NEU increased significantly in normal Wistar rats in a dose-related manner (Tables 1 and 2). WBC also decreased significantly in normal Wistar rats but decreased in immunosuppressed Wistar rats, In addition, platelets were also decreased in normal rats, though not statistically significant. Other haematological indices measured (MCV, MCH and MCHC) were also not statistically different ($P > 0.05$) from the control. Weekly

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Table 1. Effect of Co-administration of artemether-nevirapine on haematological Indices and CD4 count in normal rats

NVP	T_{80}	T_{80}	T_{80}	NVP	NVP	NVP
ART	**T_{80}**	**ART_5**	**ART_{10}**	**T_{80}**	**ART_5**	**ART_{10}**
CD4 (cells/µl)	4.0±0.8	4.0±0.6	3.2±0.5	4.2±0.6	3.7±0.4	3.7±0.3
WBC (x10^3/µl)	18.4±3.4	11.7±2.2[a]	12.2±2.0[a]	12.5±2.3[a]	16.1±1.2	12.2±1.2[a]
LYMP (%)	73.9±1.6	77.2±3.6	72.9±2.4	61.0±3.5[a]	60.9±4.9[a]	57.9±2.9[a]
NEUT (%)	19.2±3.9	20.0±3.5	23.8±1.9	35.2±3.4[a]	35.4±4.5[a]	37.7±3.3
RBC (x10^6/µl)	7.8±0.3	7.4±0.3	7.2±0.1	7.9±0.2	7.5±0.2	6.8±0.3[a]
PCV (%)	46.3±1.2	44.4±2.1	42.7±0.9	45.2±0.9	42.4±1.1	38.5±0.9[a]
Hb (g/dl)	14.2±0.3	13.7±0.4	13.4±0.3	13.9±0.3	13.4±0.3	12.5±0.3[a]
PLT (×10^3/µl)	982.8±45.5	111.5±26.5	992.5±57.5	971.8±1.4	113.8±96.9	138.9±54.7
MCV (fl)	58.4±1.3	60.4±1.3	59.1±0.9	57.5±1.6	56.9±0.7	56.7±0.9
MCH (pg)	18.1±0.4	18.6±0.1	18.5±0.3	17.7±0.0	18.0±0.3	18.5±0.4
MCHC (g/dl)	31.5±0.4	30.9±0.7	31.3±0.3	30.9±0.5	31.7±0.2	32.6±0.2

n= 6. Data above are means ± SEM, statistically significant at p<0.05, when subjected to Analysis of variance (ANOVA) followed by Dunnett's post-hoc test, WBC = White Blood Cell, LYMP = Lymphocyte, NEUT = Neutrophil, RBC = Red Blood Cell, PCV= Parked Cell Volume, Hb = Haemoglobin, PLT = Platelet, MCV =Mean Cell Volume, MCH = Mean Corpusular Haemoglobin, MCHC = Mean Corpusular Haemoglobin Concentration, ART_5 = Low Dose of Artemether (5 mg/kg), ART_{10} = High Dose of Artemether (10 mg/kg), NVP = Nevirapine (30 mg/kg), T_{80} = 3%v/v Tween 80.

Table 2. Effect of artemether on haematological Indices and CD4 Count in immunosuppressed rats receiving nevirapine.

DEX	T_{80}	DEX	DEX	DEX	DEX	DEX	DEX
NVP	**T_{80}**	**T_{80}**	**T80**	**T_{80}**	**NVP $_{30}$**	**NVP**	**NVP**
ART	**T_{80}**	**T_{80}**	**ART_5**	**ART_{10}**	**T_{80}**	**ART_5**	**ART_{10}**
CD4 (cells/µl)	4.2±0.6	3.4±0.6	4.2±0.4	4.5±1.6	4.6±0.7	4.3±0.5	10.3±4.7
WBC (×10^3/µl)	10.5±0.9	11.6±2.0	12.4±0.8	9.7±2.2	13.5±2.4	11.9±1.9	15.8±5.4
LYMP (%)	76.8±1.6	38.2±4.2[a]	56.9±4.2	54.9±3.8 [a]	46.1±7.7[a]	41.8±5.2[a]	41.9±0.7 [a]
NEUT (%)	14.3±2.5	16.7±9.6	28.3±8.9	22.5±11.4	7.6±1.1	17.6±3.5	16.5±1.6
RBC (×10^6/µl)	7.9±0.3	8.1±0.2	7.9±0.3	7.7±0.4	7.4±0.1	6.9±0.4	5.1±1.0 [a]
PCV (%)	45.4±1.0	47.2±1.5	46.6±1.5	45.3±2.0	43.9±0.9	39.5±2.1	27.6±5.6 [a]
Hb (g/dl)	13.7±0.3	14.4±0.4	14.0±0.4	13.9±0.6	13.6±0.4	12.0±0.7	9.3±1.7 [a]
PLT (×10^3/µl)	92.9±1.0	84.2±68.5	88.6±89.5	94.4±50.9	90.3±63.9	96.3±28.7	80.6±1.7
MCV (fl)	57.1±0.9	58.6±1.2	59.5±0.9	58.8±1.1	59.4±1.2	57.6±1.8	58.9±1.2
MCH (pg)	17.5±0.5	17.8±0.4	17.9±0.4	18.0±0.5	18.2±0.3	17.6±0.6	17.9±0.3
MCHC (g/dl)	30.5±0.4	30.5±0.2	30.1±0.2	30.7±0.3	30.5±0.1	30.8±0.1	30.5±0.3

n = 6. Data above are means ±SEM, statistically significant at p<0.05, when subjected to Analysis of variance (ANOVA) followed by Dunnett's post-hoc test. WBC = White Blood Cell, LYMP = Lymphocyte, NEUT = Neutrophil, RBC = Red Blood Cell, PCV= Parked cell volume, HB = Haemoglobin, PLT = Platelet, MCV =Mean cell volume, MCH = Mean Corpusular Haemoglobin, MCHC = Mean Corpusular Haemoglobin Concentration, DEX = Dexamethasone, ART_5 = Low dose of artemether (5 mk/kg), ART_{10} = High dose of artemether (10 mg/kg), NVP = Nevirapine (30 mg/kg).

changes in PCV of both normal and immunosuppressed rats were also recorded; this was statistically significant (P < 0.05) in both normal (day 21 only) and immunosuppressed Wistar rats (days 14 and 21) in artemether-nevirapine co-administered groups (Figures 1 and 2).

DISCUSSION

This study showed a dose-dependent decrease (P < 0.05) in some of the haematological parameters measured (RBC, PCV, Hb, WBC Lymp and platelets) in artemether and nevirapine co-administration. Decreased

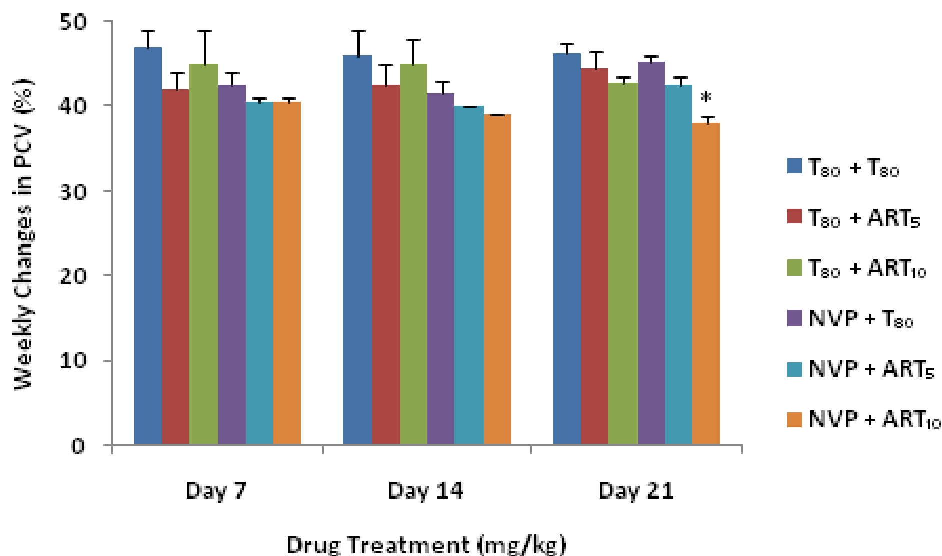

Figure 1. Weekly Changes in PCV on Co-administration of artemether and nevirapine in normal rats. n= 6, data are means ± SEM, *statistically significant at $p<0.05$ compared with the control (3% Tween 80) when subjected to Analysis of variance (ANOVA) followed by Dunnett's post-hoc test. ART_5 = lower dose of artemether (5 mg/kg), ART_{10} = Higher dose of artemether (10 mg/kg), ART = Artemether, NVP = nevirapine.

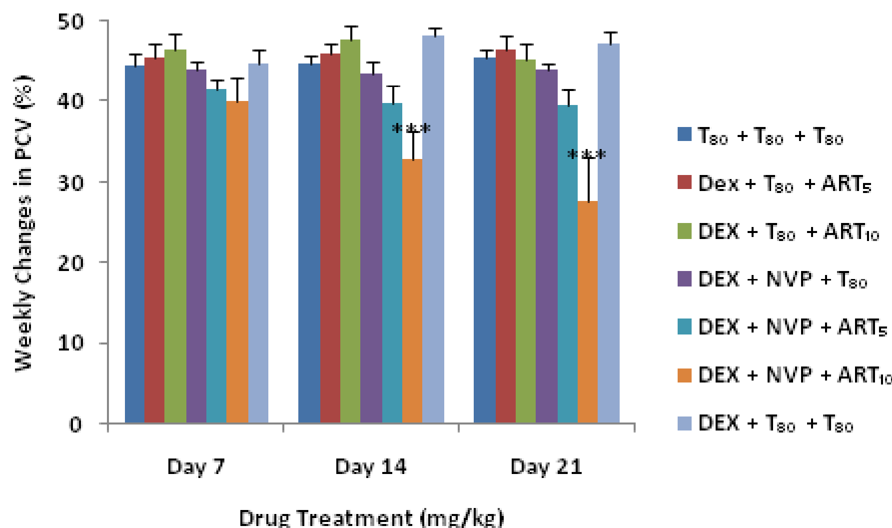

Figure 2. Weekly Changes in PCV on Co-administration of artemether and nevirapine in immunosuppressed rats. n= 6, data are means ±SEM, *statistically significant at $p<0.001$, $cp<0.0001$compared with the control (3% Tween 80) when subjected to Analysis of variance (ANOVA) followed by Dunnett's post-hoc test. DEX= Dexamethasone (Immunosuppressive agent), ART_5 = lower dose of artemether (5 mg/kg), ART_{10} = Higher dose of artemether (10 mg/kg), ART = Artemether, NVP = nevirapine.

in lymphocytes observed in both normal and immunosuppressed rats may be due to the fact that immune system is been compromised as a result of concurrent artemether and nevirapine administration in normal rats or may be due to the administration of dexamethasone in immunosuppressed Wistar rats.

Increased neutrophils observed is suggestive of increased infection as a result of drug administration. The significant decreased in RBC was an indication that there was a destruction of matured RBCs. Co-administration of artemether and nevirapine induced anemia as this effect was observed in decreased PCV. Haematological parameters are of diagnostic significance in routine clinical evaluation of the state of health (Sule et al., 2012); and therefore can be used to monitor several disease progression such as HIV/AIDS especially when viral load testing and CD4 cell count monitoring are not readily available (Chen et al., 2007). Blood has been re-ported to be the most easily accessible diagnostic tissue (Maina et al., 2010); therefore employed to evaluate the effect of concomitant use of artemether and nevirapine in this study. Several studies have reported the effects of nevirapine when used alone (Adaramoye et al., 2012) or in combination with other drugs (Odunukwe et al., 2005; Umar et al., 2008) such as pharmacokinetic effect of artemether and nevirapine (Byakika-Kibwika et al., 2011). A previous study by Cinque et al. (1993) showed that Non-nucleoside reverse-transcriptase inhibitor (NNRTI) such as nevirapine was associated with several complications including anaemia in a dose dependent manner. Thus, the present study where artemether was co-administered with nevirapine is in line with that report.

It has been reported that the use of artemether may potentiate haematological abnormalities such as anaemia (Osonuga et al., 2012), as observed when it was co-administered with nevirapine in this study. The increase in both WBC (leucocytosis) and lymphocytes (lymphocytosis) is a sign of immunological response to a trauma caused or induced by drugs (Guyton and Hall, 2006). Dexamethasone used in this study may also be attributed to the changes in immunological status observed. High dose artemether and nevirapine co-administration has been demonstrated in this study to induce anaemia and caused reduction in immunity in both normal and immunosuppressed Wistar rats. Therefore caution with close monitoring should be observed when artemether and nevirapine are co-administered especially in individuals that are susceptible to anaemia as well as immunocompromised patients.

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Conflict of interest

There is no conflict of interest as regard this study.

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