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Research article

Heavy metal and phytochemical screening of anti-jaundice and anti-malaria concoctions and genotoxicity assessment of the concoctions using *Allium cepa* assay

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Abstract: Increasing population world-wide is depending on herbal medicine as a source of primary health care. However, many plants used in traditional and folk medicine have been shown to be potentially toxic, mutagenic, and carcinogenic. In this study, the cytotoxic and genotoxic potentials of anti-jaundice and anti-malaria concoctions commonly used in Nigeria in treating jaundice and malaria were investigated using *Allium cepa* assay. The concoctions were also analysed for the presence of some heavy metals and phytochemicals. The roots of *Allium cepa* were exposed to 10%, 50% and 100% of both concoctions respectively for 96 hours for macroscopic and microscopic analysis and *Allium cepas* exposed to tap water served as control. The anti-jaundice and anti-malaria concoctions significantly ($p<0.05$) inhibited the root growth of *Allium cepa* when compared with control with EC50 values of 5% and 6% respectively. Both concoctions also caused concentration dependent decrease in mitotic index in *Allium cepa* cells and induced chromosomal aberrations like vagrant chromosome, multipolar anaphase, c-mitosis, sticky chromosomes and binucleus but there were no chromosomal aberrations in *Allium cepas* exposed to tap water. Phytochemical screening of both concoctions revealed the presence of alkaloids, saponins, cardenolides, flavonoids, tannins and anthraquinones and heavy metals like cobalt, cadmium and nickel were detected in the concoctions. The results of this study show the cytotoxic and genotoxic effects of the anti-malaria and anti-jaundice concoctions on *Allium cepa*.
Keywords: *Allium cepa* - Genotoxicity - Cytotoxicity - Anti-jaundice - Anti-malaria.

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INTRODUCTION

Medicinal plants are used by over 70% of the world population for livelihood, primary health care, self-medication and national health services (Akerele *et al.* 1991, Heinrich 2010, Mehra *et al*. 2014). Some of the factors responsible for this include better accessibility, affordability, acceptability and the belief that medicinal plants work (Heinrich 2010). However, the potential toxicity of herbs has not been recognized by the general public or by professional groups of traditional medicine (Soetan & Aiyelaagbe 2009). Most of the traditional herbal products have never been the subject of comprehensive toxicological investigations, which is required for modern pharmaceutical products (Subramanion *et al*. 2013). Based on their traditional use for long periods of time, they are often assumed to be safe.

Despite the profound therapeutic advantages possessed by some of the medicinal plants, some constituents of medicinal plants have been found to be potentially toxic, mutagenic, carcinogenic and teratogenic. Interaction of plant extracts with cells may produce unwanted cellular damage. This interaction may vary depending on the

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active ingredient present in the extract, as it may occur on the cell surface, within the cell body, DNA, or in the tissues beneath as well as at the extracellular matrix. It has been shown that while some medicinal plants may suppress the effects of mutagens, others may contain toxic substances or provoke mutagenic effects (Vicentini *et al*. 2001).

Reports of unwanted side effects and potential toxicity of phytomedicines have been on the increase in recent years (Effraim *et al.* 2001, Ruffa *et al*. 2002, Amida *et al*. 2007, Konan *et al*. 2007, Sowemimo *et al*. 2007, Nwafor *et al*. 2007). Many researchers have shown that numerous herbal products, which are used as food ingredients or in traditional medicine, have in-vitro mutagenic or toxic properties (Cardoso *et al.* 2006, Déciga-Campos *et al*. 2007, Mohd-Fuat *et al*. 2007). The use of some plants has also been correlated with high rate of tumour formation in human (Ames 1986, Nguye *et al*. 1989, Brito *et al*. 1990). The genotoxic and mutagenic potentials of some herb extracts have also been reported by several workers (Barcelos *et al*. 2007, Konan *et al*. 2007, Sowemimo *et al*. 2007, Patterson *et al*. 1987, Magda 2001, Gadano *et al*. 2000, 2002, 2006, Paes-Leme *et al*. 2005).

In Nigeria, a large percentage of the populace cannot afford to pay for orthodox medical service (Fasola & Egunyomi 2005, Obi *et al*. 2006) and therefore depend on herbal concoctions for treatment of most ailments most of the time, even though there is little information on the potential risk to health of such herbs. These extracts are prepared as a single herb or as concoctions of herbs. Some of them have genotoxic potential, cytotoxic and mitodepressive effects as reported by several workers (Heinrich *et al.* 2010, Akintonwa *et al.* 2009, Oyedare *et al.* 2009, Akinboro & Bakare 2007, Williams & Omoh 1996). Investigation of cytotoxic and mutagenic potentials of medicinal plants is necessary to ensure their safe use. Assessment of the potential cytogenotoxicity of traditional medicines is indeed an important issue as damage to the genetic material may lead to critical mutations and therefore also to an increased risk of cancer and other diseases. The present study determined the levels of heavy metals and phytochemicals in anti-malaria and anti-jaundice concoctions and also evaluated the genotoxic potential of the concoctions using *Allium cepa* chromosomal aberration assay. These concoctions are among the Nigerian folk medicinal plants frequently used in the treatment of malaria and jaundice in infants, especially among the less privileged. Malaria is a prevalent disease that affects about 60% of Nigerians annually (Ekwebene 2012) and most people depend on herbs for the treatment of malaria. Brinkmann & Brinkmann (1991) reported that only 8.25% of people with malaria visit health services, indicating that the rest uses alternative medicine which involves the use of medicinal herbs with anti-malarial properties. Jaundice is also prevalent especially in children; therefore it is necessary to investigate the genotoxic potential of these concoctions. This is essential as a guide towards standardization, safety administration and an understanding of mechanism of action of the concoctions to see if they could be used in alternative medicine to treat malaria and jaundice.

MATERIALS AND METHODS

Source of Allium cepa and herbs for the concoctions

Allium cepa, along with leaves and barks of other medicinal plants used for the preparation of the concoctions were obtained from Bode Market in Ibadan, Oyo State, Nigeria.

Preparation of Anti-malaria and Anti-jaundice concoctions

The concoctions were prepared according to the traditional use in Nigeria. The anti-malaria concoction was made up of mango leaves, pawpaw leaves, cashew leaves, bitter leaves, neem bark and morinda lucida root while anti-jaundice concoction was made up of lemon grass, lime leaves, yellow pawpaw leaves and pods of capsicum leaves. The leaves and barks were rinsed with water and heated together in water until the colour of the water turned brown; the mixture was then filtered to remove particulate matter. The mixture was cooled and stored in a refrigerator until use.

Allium cepa assay

The outer scales of the onion bulbs and the brownish bottom plate were first removed. The rings of the root primordial were left intact. The onion bulbs were first sprouted in water to initiate the growth of the onions with base of the onions touching the water as described by Friskesjo (1987). After 24hours, the best in terms of root growth were selected. Thirty five onion bulbs were divided into seven groups with five onions in each group. The onion bulbs were then placed on top of beakers each filled with 10%, 50% and 100% anti-malaria and anti-

jaundice concoctions respectively and fresh tap water for control for 96hours. The beakers were kept in a dark cupboard and the test samples were changed every 24hours, the root length of each bulb was also measured daily. The EC50 values (effective concentration producing 50% growth inhibition) for the two concoctions were determined by plotting a graph of concentration against root length. After 48hours, one root tip from each bulb was harvested for cytological study. The root tips were fixed and macerated in 45% acetic acid-1M HCl (9:1) solution and heated for 5 min at 50°C (Odeigah *et al*. 1997a). Subsequently, the roots were placed on a slide and the terminal root tips were cut off and used for further preparation. The rest of the material was removed from the slide and the excess liquid was sucked up with a piece of blotting paper. Two drops of fresh filtrated 2% orcein solution was added and mixed with the root tips by stirring and knocking with a blunt stick of stainless steel. A cover slip was placed on the root cells and allowed to absorb stain for 5-10 min afterwards; the cells were squashed by placing layers of blotting paper on the cover slip and pressing slightly down with the thumb. The cover slip was fixed carefully to the slide with nail cortex. The slides were observed under the light microscope and data on total cells, total dividing cells and cells carrying chromosomal aberrations were taken on at least 5 slides prepared for each of the different concentration of the extracts and the control. The mitotic index was calculated as, (No. of dividing cells / Total no. of cells) × 100.

Phytochemical screening of the concoctions

The phytochemicals tested for are alkaloids, cardenolides, anthraquinones, saponins and tanins. The presence or absence of these phytochemicals was carried out using standard methods; dragenduff's standard method for alkaloid test (Oguyemi 1979), killer-killiani standard procedure for cardenolides test (Trease & Evans 1989), chloroform or ammonia test for anthraquinones (Trease & Evans 1989), frothing test for saponin (Sofowora 1993), ferric chloride test for tannins (Jigna & Sumitra 2007) and ammonia test for flavonoids (Trease & Evans1989, Sofowora 1993).

Determination of Heavy metals

The concentration of the following heavy metals: lead, chromium, cadmium, nickel and cobalt were determined in both anti-malaria and anti-jaundice concoctions using atomic absorption spectrophotometer.

Statistical analysis

Results were expressed as Mean ± Standard Deviation. Differences between groups were determined. One way ANOVA was performed using microcal origin software (Drug Discovery & Development magazine 2008) and p-values < 0.05 were considered significant.

RESULTS

Table 1. Root lengths of *Allium cepa* exposed to various concentrations of anti-malaria and anti-jaundice concoctions.

Concentration		Mean root length after				Root length % of control
		24hrs	48hrs	72hrs	96hrs	
Control		0.2±0.18	1.3±0.5	2.8±0.4	5.8±0.8	100.0
Anti-malaria Concoction	**10%**	0.2±0.08	0.4±0.1	0.6±0.2**	0.7±0.2*	12.1
	50%	0.3±0.08	0.4±0.1*	0.5±0.2**	0.3±0.1**	5.2
	100%	0.3±0.14	0.4±0.1*	0.5±0.2**	0.5±0.1**	8.6
Anti-jaundice Concoction	**10%**	0.3±0.09	0.4±0.05*	0.5±0.2**	0.7±0.1*	12.1
	50%	0.3±0.07	0.3±0.08*	0.4±0.1**	0.4±0.1*	6.9
	100%	0.1±0.04	0.2±0.05*	0.2±0.07**	0.2±0.05**	3.4

Note: Significantly different from control *P<0.05, **P<0.001

There were significant (p<0.05, p<0.001) reductions in root lengths of *Allium cepa* after 24hours, 48hours, 72hours and 96hours exposure to 10%, 50% and 100% of anti-malaria and anti-jaundice concoctions respectively when compared with control (Table 1), the root lengths of *Allium cepa* exposed to 10%, 50% and 100% of anti-malaria concoction after 96hours were 12.1%, 5.2% and 8.6% respectively of the root length of control while the root lengths of *Allium cepa* exposed to 10%, 50% and 100% of anti-jaundice concoction after 96hours were 12.1%, 6.9% and 3.4% respectively of the root length of control (Fig. 1). The anti-malaria and anti-jaundice concoctions altered phenotypic indices and also induced chromosomal aberrations in *Allium cepa* cells (Table 2). Phytochemicals detected in the anti-malaria and anti-jaundice concoctions are alkaloids, tannins,

cardenolides, saponins and flavonoids (Table 3). Heavy metals detected in the anti-malaria and anti-jaundice concoctions are cobalt, cadmium and nickel (Table 4).

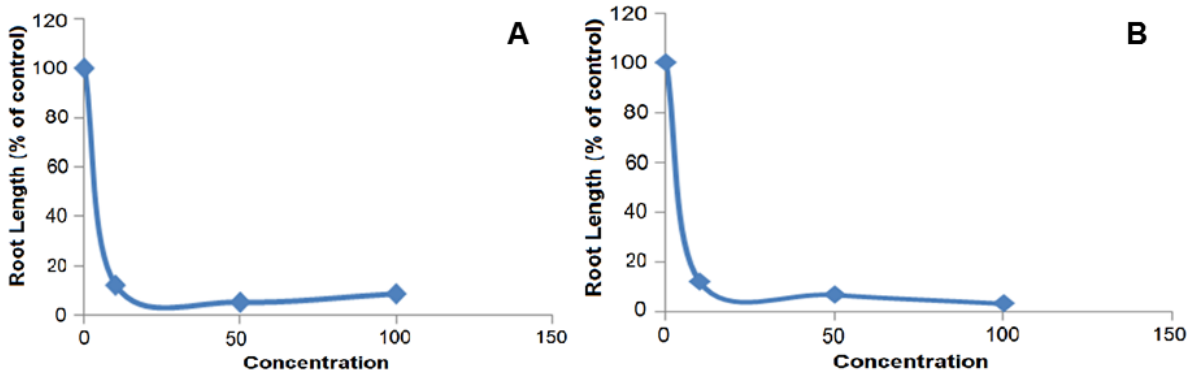

Figure 1. Growth curve of *Allium cepa* roots exposed to various concentrations of: **A**, anti-malaria and **B**, anti-jaundice concoction.

Table 2. Phenotypic indices and chromosomal aberrations in *Allium cepa* exposed to various concentrations of anti-malaria and anti-jaundice concoctions.

Concentration		No. of cells	No. of dividing cells	Mitotic index	Binucleus	Attached	C-Mitosis
Control		425	56	13.17	-	-	-
Anti-malaria	**10%**	180	5	2.78	4	-	1
	50%	120	-	-	-	-	-
	100%	78	-	-	-	-	-
Anti-jaundice	**10%**	189	10	5.29	3	1	3
	50%	124	6	4.84	3	3	-
	100%	106	-	-	-	-	-

Table 3. Phytochemical screening of anti-malaria and anti-jaundice concoctions.

S.No.	Phytochemical test	Anti-malaria Concoction	Anti-jaundice Concoction
1.	Alkaloid	Positive	Positive
2.	Cardenolides	Positive	Positive
3.	Anthraquinones	Negative	Negative
4.	Saponins	Positive	Positive
5.	Tannins	Negative	Positive
6.	Flavonoid	Positive	Positive

Table 4. Heavy metal analysis of anti-malaria and anti-jaundice concoctions.

S.No.	Heavy metals	Anti-malaria Concoction	Anti-jaundice Concoction
1.	Chromium	ND	ND
2.	Lead	ND	ND
3.	Cobalt	0.117	0.116
4.	Cadmium	0.083	0.052
5.	Nickel	0.232	0.377

Note: ND, Not detected

DISCUSSION

Allium cepa assay is a simple, cheap, reproducible and effective model for evaluating and monitoring cytotoxicity and genotoxicity of chemicals and mixture substances (Fiskesjo 1993, 1997, Hoshina & Marin-Morales 2009).

The results of this study showed that the concoctions significantly ($p<0.05$, $p<0.001$) inhibited root growth in *Allium cepa* when compared with control. The anti-malaria concoction inhibited root growth with an EC50 value of 5% while anti-jaundice concoction inhibited root growth with EC50 value of 6%. This is an indication that the concoctions are cytotoxic. The cytotoxic effects of some medicinal herbs such as *Azadirachta indica, Carica papaya, Cymbopogon citratus*, , *Mangifera indica, Ocimum gratissimum, Morinda lucida* , *Terapluera*

tetraptera, *Plumbago zeylanica*, *Xylopia aethiopica*, *Newbouldia laevis*, *Alstonia boonei*, *Enantia chlorantha* and *Rauvolfia vomitoria* have been reported as well (Oyedare *et al*. 2009, Akintonwa *et al.* 2009, Akinboro & Bakare 2007). Root growth in *Allium cepa* like other plants is due to expansion of cells in the elongation zone of the root tip where cellular differentiation occurs (Cordoba-Pedrosa *et al.* 2004). The observed inhibition of root growth by the concoctions suggests that they contain toxic substances that impaired one or more other biological processes which mediate cell expansion and differentiation at the elongation region of *Allium cepa* root tip. Heavy metals such as lead, chromium, cobalt, cadmium and nickel detected in the concoctions might be responsible for this effect.

The results of this study also show that the concoctions possess inhibitory, mito-depressive effects on cell division and chromosome behaviour of *Allium cepa* which would have prevented DNA synthesis that resulted in reduction in the number of dividing cells. The two concoctions caused reduction in mitotic index. The mitotic index in *Allium cepa* exposed to 10% anti-malaria concoction was 2.78 compared with 13.17 of control; while the mitotic index in those exposed to 10% and 50% anti-jaundice concoction were 5.29 and 4.89 respectively compared with 13.17 of control. The decline of mitotic index below 22% in comparison to control can have lethal impact on the organism (Antonsiewiez 1990). Reduction in the mitotic activity could be as a result of inhibition of DNA synthesis or blocking of the G2 phase of the cell cycle, preventing the cell from entering mitosis (Sudhakar 2001). There were no dividing cells in *Allium cepa* exposed to 50% and 100% anti-malaria concoction and those exposed to 100% anti-jaundice concoction. This shows that the concoctions totally inhibited cell division at these concentrations which is a sign of blockage of the cell cycle at the interphase stage. Mitotic index is a measure of the total number of dividing cells in cell cycle; it gives a measure of the proportion of cells in the mitotic phase of the cell cycle and its inhibition could be interpreted as cellular death or a delay in the cell proliferation kinetics (Rojas 1993). Therefore, the decrease in the mitotic index of *Allium cepa* cells exposed to anti-malaria and anti-jaundice concoctions shows that exposure of *Allium cepa* cells to the concoctions resulted in cellular death. This shows direct genotoxic effect of these concoctions. The reduction of the mitotic index might be as a result of the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis. The presence of alkaloids, tannins, cardenolides, saponins and flavonoids in the concoctions might be responsible for their mitodepressive effects. This is similar to the reports of Bernice *et al*. (2009) who also attributed the mitodepressive effects of *Citrus medica*, *Ocimum Gratissimum* and *Morinda lucida* to the presence of alkaloids, tannins, flavonoids, anthraquinones and saponoside. The mitodepressive effects of some plant extracts and their ability to block of DNA synthesis have also been reported (Akinboro & Bakare 2007, Soliman, 2001, Askın & Aslanturk 2007, Mercykutty & Stephen 1980, Schulze & Kirscher 1996).

Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Chromosome aberrations observed in *Allium cepa* exposed to the two concoctions are vagrant chromosomes, binucleus, attached and c-mitosis. These aberrations show that the concoctions affected spindle formation which resulted in cell division disturbances. Vagrant chromosomes increase the risk for aneuploidy (Leme & Marin-Morales 2009). Vagrant chromosomes may be caused by unequal distribution of chromosomes with paired chromatids which could have resulted from non-disjunction of chromatids in anaphase. Vagrant chromosomes and C-mitosis are usually attributed to the failure of the spindle apparatus to organize and function in a normal way. Although c-mitosis may be regarded as indicative of a weak toxic effect which may be reversible. However c-mitosis may induce the formation of polyploid cells when not reversed (Odeigah *et al*. 1997b). Binucleated cells usually arise as a consequence of the inhibition of cell plate formation, which could be as a result of the suppression of phragmoplast formation in the early telophase (Soliman 2001). Induction of binucleated cells consequently leads to the formation of multinucleate cells in succeeding generations (Oyedare *et al*. 2009). Phytochemical screening of anti-malaria and anti-jaundice concoctions revealed the presence of alkaloids, tannins, cardenolides, saponins and flavonoids. These phytochemicals, especially alkaloids may be responsible for chromosomal aberrations induced by the concoctions. Ene & Osuala (1990) reported that alkaloids such as vincristins and vinblastine were responsible for chromosome aberration observed in the water extracts of *Borreria filiformis* and *Vinca rosea.* Alkaloids inhibit mitosis by binding with tubulin and prevent the formation of the mitotic spindle (Khakdan & Piri 2012). Maruta *et al*. (1995) reported that *Arctium lappa roots* contained various alkaloids, tannins, saponins, anthraquinones and cardiac glycosides and Khakdan & Piri (2012) also reported that spindle disturbances observed in *Allium cepas*

exposed to *Allium lappa* root extracts were due to the presence of alkaloids in the extracts. Oyedare *et al*. (2009) also attributed chromosomal aberration (binucleated cells) observed in the cells of *Allium cepa* treated with an aqueous extract of *Ocimum gratissimum* to the presence of alkaloids in the extract. Akinboro & Bakare (2007) and Williams & Omoh (1996) also reported that the flavonoids (quercetin, rutin and kaempferol), tannins and other phenolic compounds (hydrocinnamic acid) play roles in mitodepression and chromosomal aberrations in *Allium cepa* exposed to extracts of *Inula viscosa*, *Morinda Lucida*, *Carica papaya* and *Ocimum gratissimum.* Studies have shown that both tannins and flavonoids evoke free radical generation by complexing with cellular proteins and DNA, which at the expense of a depleted antioxidant system could cause lipid peroxidation, DNA damage and cell death (Iwalokun *et al*. 2011). However, the ability of tannins and flavonoids to induce genotoxicity is based on their chemical composition and structure since some species of tannins and flavonoids lack genotoxic effects. Heavy metals in the concoctions could also be responsible for the genotoxic effects of the concoctions. Various heavy metals are known to induce chromosome breaks, fragments and micronucleus formation in plants and mammalian test systems (Knasmuller *et al*. 1998), and their effects were emphasized to be the result of formation of DNA–DNA and DNA–protein cross-links (De Flora *et al*. 1990, Costa 1991, Costa *et al*. 1994). Chromium and nickel have been reported to affect the mitotic spindles thereby causing aberrant mitotic stages (Anderson 1985).

CONCLUSION

The results of this study show the cytotoxic and genotoxic effects of the anti-malaria and anti-jaundice concoctions on *Allium cepa*. Heavy metals and some phytochemicals detected in these concoctions as well as other undetected toxicants in the concoctions could be responsible for these effects. Therefore, the use of these concoctions in herbal medicine should be discouraged. However, there is need for evaluation of the genotoxic effects of these concoctions in experimental animal models.

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