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ORIGINAL PAPER

SELENIUM CONCENTRATIONS IN TWO KNOWN EFFECTIVE ANTI-MISCARRIAGE HERBAL MIXTURES

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Abstract

Inadequate consumption of selenium is one of the most pertinent issues related to human infertility. Miscarriage is an infertility problem that primarily occurs before 24 weeks of pregnancy, and affects about 15-22% pregnant women worldwide. While some herbal mixtures (HM) have been described as potent for miscarriage prevention in Nigeria, little is known about their elemental composition. Thus, this study aimed at evaluating the selenium concentrations in materials used for the formulations of two known effective anti-miscarriage HM. To further ascertain the possible link of selenium to miscarriage, selenium concentrations in plant materials of the selected HM, namely: 1) [St. Louis Sugar + *Enantia chlorantha* stem bark + fermented maize water (FMW)], and 2) [*Cissampelos owariensis* leaves + FMW], were determined by Buck Atomic Absorption Spectrophotometry. Derived concentrations from primary values were also evaluated and compared with those of World Health Organisation (WHO) daily recommended limits for adults. Spectrophotometric analysis revealed the highest selenium concentration ($mgkg^{-1}$) in White fermented maize (280.80±2.41), followed by Yellow fermented maize (200.80±0.62), *C. owariensis* leaves (168.32±0.58), *E. chlorantha* stem bark (163.34±0.79), White maize seeds (137.48±5.75), while the lowest selenium amount was found in Yellow maize seeds (77.54±0.52). The maximum combined selenium concentration, 449 $mgkg^{-1}$ found in HM 2, was slightly higher than the WHO daily upper tolerable dosage of 400 $mgkg^{-1}$ for adults. The results suggest the possibility of selenium overdose in pregnant women if white FMW is used. Combined selenium concentrations in the herbal materials might be the responsible factor for the potency of the HM.

Keywords: *Enantia chlorantha*, *Cissampelos owariensis,* herbal mixtures, miscarriage, selenium concentration.

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INTRODUCTION

Globally, the prevalence of infertility conditions including miscarriage has been on the rise (Sun et al. 2019). Miscarriage as an infertility problem primarily occurs before 24 weeks of pregnancy, and affects about 15-22% pregnant women worldwide (García-Enguídanos et al. 2002, Ageno, Bhattacharya 2015). In Nigeria, the rate of miscarriage stands at 45.5% (Anikwe et al. 2019). While great attempts have been made through conventional Western medications, this problem still persists. Reflecting on the solutions to some specific human ailments such as miscarriage, many Nigerians have taken a step back to the increasing use of traditional medicines which has proven effective. Interestingly, the use of herbal medications has been globally embraced beyond the developing countries as complementary and/or alternative medicines, becoming the mainstream in the UK, continental Europe, North America and Australia (Nissen, Evans 2012, Ekor 2013, Konieczynski et al. 2018). However, orchestrated alarms by the orthodox medicine practitioners are a national challenge that threatens the usage of traditional medicines in Nigeria, especially among the people with high socio-economic profiles.

Several studies have been conducted regarding the use herbal formulations for treating female related infertility problems across the globe (Coulson, Jenkins 2005, de Wet, Ngubane 2014, Kaadaaga et al. 2014, Tsobou et al. 2016, James et al. 2018, Lans et al. 2018, Jaradat, Zaid 2019, Jan et al. 2020). Most of these studies, including those from Nigeria (Akaneme 2008, Soladoye et al. 2014, Ogwu et al. 2017, Sharaibi et al. 2017), focused on a broad survey of herbal plants used for infertility (de Wet, Ngubane 2014, James et al. 2018, Jaradat, Zaid 2019), and phytochemical analyses (Akaneme 2008, Sharaibi et al. 2017) and pharmacological assessments (Lans et al. 2018). Lu et al. (2021) reported the efficacies of herbal medicines for the treatment of threatened miscarriage to be between 79.22% and 100% success in China. Such an optimistic study suggesting the potential to prevent miscarriage using herbal medicine is lacking in Nigeria.

The plants (*Enantia chlorantha*, *Cissampelos owariensis* and maize) reported in this study were used to prepare herbal mixtures with proven efficacy as an anti-miscarriage product in at least 3 people known to the authors (n=2) after Western orthodox medications and care had failed. It has been highlighted that human infertility among other factors is influenced by the body's selenium richness (Barrington et al. 1996, Mirnamniha et al. 2019, Chiudzu et al. 2020). Thus, the inclusion of selenium rich foods/supplements in human diets has received an overwhelming attention in the last two decades with the understanding that its deficiency in a diet can cause infertility problems (Combs 2001, Ejezie et al. 2012, Abdulah et al. 2013, Ceko et al. 2015, Pieczynska, Grajeta 2015). In Nigeria, there are few studies that have focused on selenium concentrations in maize (Zarmai et al. 2019) but none in *E. chlorantha* and *C. owariensis*.

With respect to the pharmacological role of fermented maize (*ogi*) water (FMW) in traditional medicines, FMW usage as an extractant has been known since ancient times. It has been reported to contain probiotic lactic acid bacteria (LAB) with a variety of biological activities (George-Okafor, Anosike 2011, Omeiza et al. 2020). *Enantia chlorantha*, popularly called *Awopa* (Yoruba, Nigeria), is a medicinal tree species native to Nigeria and other African countries (Olivier et al. 2015). *E. chlorantha*, especially its stem bark, has been reported to exhibit several noteworthy biological activities (Olivier et al. 2015). More specifically, it has been considered to be useful in treating malaria during pregnancy (Babalola et al. 2020) and improving male sperm motility (Salman, Adesokan 2008). Excellent biological actions of *E. chlorantha* stem bark formulations at lower doses have been documented (Batista et al. 2009, Olivier et al. 2015, Kuissu et al. 2020). Hepasor, a commercial formulation from *E. chlorantha* stem bark is an effective popular medicine for the treatment of viral hepatitis in Cameroon (Fokunang et al. 2011).

Cissampelos owariensis (Jokoje, in Yoruba Nigerian language) is native to Nigeria but widely distributed in Africa (Semwal et al. 2014). *C. owariensis* is a creeping/climbing plant occurring in secondary vegetation or disturbed/ cultivated lands. This plant is popularly used in folk medicine to treat infertility related problems, especially miscarriage (Elujoba 1995, Efunsayo et al. 2018), among other human ailments. Crude leaf extracts and isolated compounds of this plant have been reported to exhibit several potent pharmacological properties for miscarriage prevention such as tocolytic activity (Efunsayo et al. 2018), antioxidant activity (Habila et al. 2011, Ojo et al. 2020), antimicrobial activity (Habila et al. 2011), anti-diabetic activity (Ekeanyanwu et al. 2012) and anti-ulcerogenic activity (Omotoso et al. 2019). Similarly, a reduced dose of leaves from another representative of the *Cissampelos* species, *Cissampelos mucronate*, has been reported to effective against diabetes (Tanko et al. 2007).

These plants were investigated for selenium concentrations due to their inclusion in herbal preparations currently used by 3 people known to two of the authors after 2-4 recurrent miscarriages under Western orthodox pregnancy care. The results caused by these two herbal mixtures (HM), namely: 1) [Sugar + *E. chlorantha* stem bark + FMW], and 2) [*C. owariensis* leaves + FMW], seem promising as they could be beneficial to individuals and concerned authorities engaged in pregnancy management and miscarriage prevention.

MATERIALS AND METHODS

Collection and preparation of plant materials

Dried yellow and white maize seeds were purchased on Odeda market in Ogun State. *E. chlorantha* stem bark was purchased on Itoku market in Ogun State. *C. owariensis* leaves were harvested from Halleluyah Farm via Odeda, Ogun State. The plants' identification and authentication was done by Dr. Adekunle T. Oladele, a plant botanist at the herbarium unit in the Department of Forestry and Wildlife Management, University of Port Harcourt, Nigeria. The plants' samples were deposited at the same herbarium with voucher numbers UPFH 139 and UPFH 140 for *Enantia chlorantha* and *Cissampelos owariensis*, respectively. Yellow and white fermented maize (*Ogi*) was purchased in Port Harcourt, Nigeria. The seeds, stem bark and leaves were washed with distilled water and air dried for 7 days while the *Ogi* was Freeze dried. All the materials were ground separately and saved in zip locked bags and transported for analysis.

Preparation of herbal mixtures

Herbal mixture 1) included one pack of sugar placed first inside a 5 litre keg plus about 600 g of chipped *E. chlorantha* stem bark and then enough FMW added to fill the keg to the brim. The mixture was left for 24 hours before usage. About 30 cl of the mixture should be administered to patients once daily after a meal for two months. FMW was added periodically as the extract volume was reduced. Herbal mixture 2) contained about 25 g *C. owariensis* green leaves plus about 35 cl of FMW. The leaves were squeezed thoroughly with the added 35 cl of FMW and filtered, and the filtrate taken once a day after a meal for two months.

Determination of selenium concentrations

Doses of 0.5 g of dried plant samples, previously ground in a Willey mill to 80 mesh were weighed into 250 mL beakers, 10 mL solution of 3:1 $V(HNO_3)/V(HClO_4)$ was added and swirled to mix (Sun et al. 2007). Samples were boiled at 75°C for 2 h in covered beakers under a fume cupboard, the digests and blank were allowed to cool at room temperature. The solutions were filtered with Whatman No. 42 filter paper and then transferred quantitatively to 25 ml standard volumetric flasks and replenished to mark by adding distilled water. Se content was determined directly in the filtrates on a Buck Scientific Atomic Absorption Spectrophotometer (VGP 210/211) at 196.0 nm. Concentrations of Se in samples in mg/kg were evaluated using the formula:

$$\text{Metal concentration in sample (mg kg}^{-1}) = \frac{X - Y \times Z - 1000}{W},$$

where: X – concentration of the metal obtained for digested sample from AAS (mg L^{-1}),
Y – concentration of the metal obtained for blank from AAS (mg L^{-1}),
Z – volume of the extract from which was made up to mark (L),
W – weight of the sample digested for analysis (g).

Determination of derived selenium concentrations

Six derived concentrations from primary values were also evaluated for the two types of the HM (Tables 1 and 2). ID i for HM 1) as white fermented maize (*Ogi*) + *E. chlorantha* stem bark, ID ii as yellow fermented maize (*Ogi*) + *E. chlorantha* stem bark, and ID iii as [white fermented maize (*Ogi*) + yellow fermented maize (*Ogi*)]/2 + *E. chlorantha* stem bark (Table 2). ID i for HM 2) as white fermented maize (*Ogi*) + *C. owariensis* leaves, ID ii as yellow fermented maize (*Ogi*) + *C. owariensis* leaves, and ID iii as [white fermented

Table 1

Comparison of Se concentrations in the plant materials

Plant Materials	Se concentrations mg kg^{-1} (mean±SD, n=3)	t-value	P-value
White fermented maize (*Ogi*)	280.80 ± 2.41^{aA}	55.74	0.000
Yellow fermented maize (*Ogi*)	200.80 ± 0.62^{bB}		
C. owariensis leaves	168.32 ± 0.58^{cA}	8.79	0.001
E. chlorantha stem bark	163.34 ± 0.79^{dB}		
White maize seeds	137.48 ± 5.75^{eA}	17.97	0.000
Yellow maize seeds	77.54 ± 0.52^{fB}		

Means with same small case letters are not significantly different along the column while means with same upper case letters are not significantly different within each plant material pair at $\alpha\leq0.05$; P-value is significant at $p\leq0.05$.

Table 2

HM 1) possible Se mixture combination concentration

ID	Herbal material combination	Se concentration combination (mg kg^{-1})
i.	white fermented maize (*Ogi*) + *E. chlorantha* stem bark	280.80±2.41 + 163.34±0.79 = 444.14±3.20
ii.	yellow fermented maize (*Ogi*) + *E. chlorantha* stem bark	200.80±0.62 +163.34±0.79 = 364.14±1.41
iii.	mixture of white and yellow fermented maize (*Ogi*) + *E. chlorantha* stem bark	(280.80±2.41 + 200.80±0.62)/2 + 163.34±0.79 = 385.41±2.52

maize (*Ogi*) + yellow fermented maize (*Ogi*)]/2 + *C. owariensis* leaves (Table 3). The derived concentrations were then compared with those of FAO/WHO daily recommended limits for adults (Table 3).

Table 3

HM 2) possible Se mixture combination concentration

ID	Herbal material combination	Se concentration combination (mg kg^{-1})
i.	white fermented maize (*Ogi*) + *C. owariensis* leaves	280.80±2.41 + 168.34±0.58 = 449.14±2.99
ii.	yellow fermented maize (*Ogi*) + *C. owariensis* leaves	200.80±0.62 +168.34±0.58 = 369.14±1.20
iii.	mixture of white and yellow fermented maize (*Ogi*) + *C. owariensis* leaves	(280.80±2.41 + 200.80±0.62)/2 + 168.34±0.58 = 390.39±2.10

Statistical analysis

Data were expressed as the mean values ± standard deviation (SD), analyzed using the single factor Analysis of Variance (ANOVA) and *t*-test, and the means were separated by Least Significant Difference (p<0.05).

RESULTS

Analytical figures of merit

The calibration curve for total Se solutions is linear from 0-4 mg L^{-1} as shown in Fig 1. 1000 mg l^{-1} stock Se solution (109118 gm $NaHSeO_3$ in 1.0 L DDW) was used to prepare a series of standard solutions (1.0, 2.0, 3.0 and 4.0 mg/l Se) and used for the AA calibration curve. The detection limit is defined as the concentration corresponding to the triple value of the standard deviation of the blank plus the blank signal. In the selenium determination assays, the detection limit was 0.50 mg L^{-1} of selenium and correlation coefficient (R) was 0.975, as shown in Table 4.

Selenium concentrations

ANOVA results showed that white fermented maize had the highest selenium concentration in mg kg^{-1} (280.80±2.41), followed by yellow fermented maize (200.80±0.62), *C. owariensis* leaves (168.32±0.58), *E. chlorantha* stem bark (163.34±0.79), white maize seeds (137.48±5.75), while the lowest concentration was in yellow maize seeds (77.54±0.52). *T*-test results between

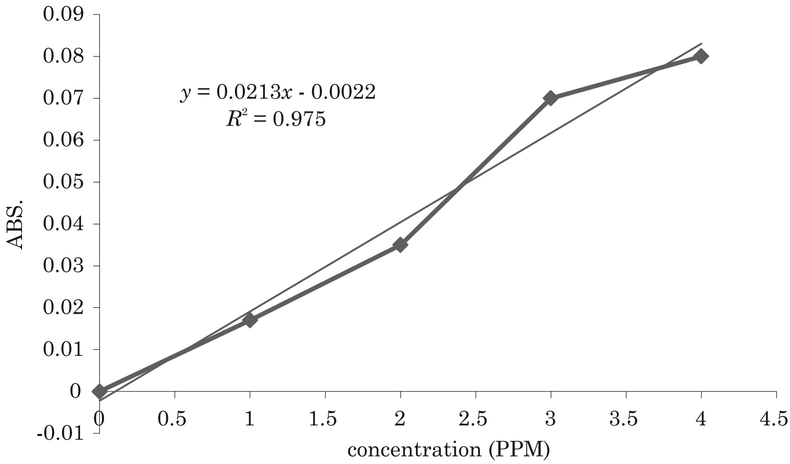

Fig. 1. The calibration curve for total Se solutions.

Table 4

Analytical figures for total Se determination

Linear range (mg L^{-1})	From 0 to 4 total Se
Regression equation	$y = 0.0213x - 0.0022$
Correlation coefficient (*R*)	0.975
Detection limit	0.50 mg L^{-1}

white and yellow fermented maize concentration were highly significant (t value, 55.74), between the forest plants *C. owariensis* leaves and *E. chlorantha* stem bark concentration looked similar but significant (*t* value, 8.79), and the selenium concentration between white and yellow maize seeds was also significant with a *t* value of 17.97 (Table 1).

Derived selenium concentrations

Selenium derived from combining ID i of HM 2) (Table 3) had the highest concentration followed by ID i of HM 1) (Table 2), ID iii of HM 2) (Table 3), ID iii of HM 1) (Table 3), ID ii of HM 2) (Table 3), and the minimum was ID ii of HM 1) (Table 2). ID i of both HM 1 and 2 were higher than FAO/WHO daily upper limit recommended dosage for adults.

DISCUSSION

Several recent studies have provided an increasing body of evidence on the role of selenium in human fertility and reproductive health. While the cases of the authors' relatives are personal testimonies that instigated this study, selenium concentrations in herbal materials were evaluated and used as a gauge in determining the anti-miscarriage potential of the herbal mixtures. Analysis of variance has revealed statistically significant differences among the investigated samples due to the variation in plant species. Higher concentrations of selenium in *Ogi* compared to those in maize seeds basically reflect the importance of fermentation in enhancing a selenium concentration. Respectively, Ajayi et al. (2017) and Di Nunzio et al. (2018) demonstrated that enhanced selenium of *Acacia nilotica* seeds and sourdough flatbread was influenced by fermentation. Closely related results were also reported suggesting that fermentation caused an increase in the protein and improved mineral bioavailability in fermented maize meals, (Onyango et al. 2004, Cui et al. 2012, Nwokoro, Chukwu 2012) and in fermented sesame seed meal (Hajimohammadi et al. 2020). White fermented maize (*Ogi*) produced a stronger effect on the selenium concentration. This was not unexpected as white maize seeds contained a higher concentration of selenium than yellow maize seeds (Table 1). The selenium content in *Ogi* and its concentration in *Ogi* water are closely connected. Hence, it is clearly indicated that enhanced selenium in *Ogi* caused by fermentation justified the use of *Ogi* water as an extractant of herbs for pregnancy management and miscarriage prevention. A serious warning was echoed not to use any other eluent except *Ogi* water. Both white and yellow maize seeds contained good amounts of selenium, although yellow maize seeds which are currently most widely used for *Ogi* contained significantly less Se. The results are within the range previously reported by Zarmai et al. (2019). The results are also consistent with ones reported in South Africa on yellow and white maize seed samples (Courtman et al. 2012). While *Ogi* is the primary fermented product, *Ogi* water being a secondary product is often more popular in administration of traditional medicines. This study indicates that maize seeds are good substrates for fermentation with enhanced selenium-rich products. The use of *Ogi* water in traditional medicines extends beyond it being an extractant, but also contributing essential health benefits (George-Okafor, Anosike 2011).

The two herbal plants (*E. chlorantha* stem bark and *C. owariensis* leaves) had better concentrations of selenium though with similar values, but *C. owariensis* leaves had statistically significantly higher Se content. These concentrations are substantial evidence that *E. chlorantha* stem bark and *C. owariensis* leaves are good sources of an anti-miscarriage element. With respect to chemistry, the literature on essential elements, especially selenium, in *E. chlorantha* and *C. owariensis* parts is too scarce for comparison. Dawodu et al. (2014) investigated proximate composition of *E. chlorantha* stem bark and found 72.3% crude fibre, 10.8% crude protein among others. Individually, all the materials are selenium-rich and considered to be adequate to supply the daily requirement of selenium for adults with selenium-deficiency.

Herbal preparations, either in powder, paste or a liquid form, are often offered in mixtures. The results of assays of the samples were combined to account for the range of selenium concentrations in the HM types (Table 3). The FMW extracts of the HMs, particularly HM 1), were highly effective against recurrent miscarriage in 3 relatives of the authors of this study. Selenium doses recommended for different categories of patients and human diseases are numerous and varied, with higher doses recommended to pregnant and lactating women (Thomson 2004, Hanson et al. 2015, Kieliszek, 2019). The WHO recommended dietary selenium for adult women in the range of 60-400 mg kg^{-1} (Kieliszek, Blazejak 2016). The consumption of HM presumably containing selenium concentrations ranging from 364 to 449 mg kg^{-1} without any complication until giving birth suggests that the herbal formulations are safe for miscarriage prevention. Interestingly, ID i of HM 1) and 2) being above the WHO upper limit recommendation in terms of Se content is in support of increasing the body of evidence that intakes of selenium above the normal nutritional range could provide further benefit (Rayman 2002). However, the activity of selenium has been reported to impact negatively when the concentration is 900 mg kg^{-1} and above (Combs Jr 2001, Whanger 2004, Letavayova et al. 2006). The FMW extracts from HM have excellent anti-miscarriage activity, implying that a large scale commercial product can be developed to effectively prevent miscarriage.

Conservation and future research considerations

While *E. chlorantha* is considered a species threatened with extinction (Olivier et al. 2015), *C. owariensis* is not listed on the IUCN red list. These plants are significant national commodities, largely collected from the wild and with industrial potential for sustainable prevention of human ailments, including miscarriage. *E. chlorantha* stem bark can still be found in local markets but fresh leaves of *C. owariensis* are scarce. The scarcity of this plant's fresh leaves has limited their more frequent use in the preparation of anti-miscarriage mixtures that will be administered on a daily basis for one to two months. The frequent daily use of these plants, especially stem bark harvested from *E. chlorantha*, is the major factor of their endangered status (Olivier et al. 2015). There is an urgent need to establish mass plantations of medicinal plants that would include these two plants in strategic locations, and to engage research institutions in Nigeria in order to obtain HMs for sustainable prevention of some specific ailments. Considering the fact that selenium concentrations were quantified from the materials used for the preparation of HM extracts, future research should be directed towards quantification of selenium in FMW and HM extracts in order to determine the actual bioavailability selenium present in the effective herbal formulations.

CONCLUSIONS

Maize seeds, fermented maize (*Ogi*), *E. chlorantha* stem bark and *C. owariensis* leaves are all good sources of selenium for preparation of effective anti-miscarriage formulations, with 100% of the mixtures containing high but adequate Se concentrations. The study has demonstrated HMs to be effective sources of medicines against recurrent miscarriage that Western orthodox care cannot effectively manage. However, the use of herbal preparations is unpopular among the people with high socio-economic profiles as well as Christians. Efforts should be made to demystify the doctrines among these groups of people in Nigeria against the use of effective herbal medicines for some specific ailments that Western medications cannot effectively manage.

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Availability of data:

The data that support the findings of this study are available on request from corresponding author.

Authors' contributions:

AAA and AGA initiated the study; AGA and CLE collected the materials; IOD and TOE were responsible for the laboratory work; AGA and CLE wrote the initial manuscript; AAA, IOD and TOE proof read the manuscript; All the authors approved the final manuscript.

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