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A Safety Assessment of the Antimalarial Herb *Artemisia annua* During Pregnancy in Wistar Rats

Amos O. Abolaji,[1,2]* Mbeh U. Eteng,[3] Patrick E. Ebong,[3] Ebiamadon Andi Brisibe,[4] Ahsana Dar,[1] Nurul Kabir[1] and M. Iqbal Choudhary[1]

[1]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[2]Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Oyo State, Nigeria
[3]Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, P.M.B. 1115, Calabar, Cross River State, Nigeria
[4]Department of Genetics and Biotechnology, Faculty of Science, University of Calabar, P.M.B. 1115, Calabar, Cross River State, Nigeria

***Artemisia annua* is a Chinese antimalarial herb that has been used for more than 2000 years. The maternal and foetal safety of the ethanolic leaf extract of therapeutically active *Artemisia annua* (EAA), with previously determined artemisinin yield of 1.098% was evaluated in Wistar rats. Twenty pregnant rats, divided into four study groups of saline treated (control), and test groups administered orally with 100, 200 and 300 mg/kg body weights of EAA, respectively, from gestation days (GD) 8 to 19. Following overnight fast, animals were sacrificed on GD 20, and maternal blood was collected to evaluate biochemical and haematological markers. Foetuses were carefully removed, weighed, and observed for any possible malformation. Biochemical and haematological studies revealed that EAA did not result in maternal hepatotoxicity, haematotoxicity, and hyperlipidemia. While litter size significantly decreased ($p < 0.05$) at 100 mg/kg EAA, maternal estrogen levels decreased in all the EAA-treated groups. Non-viable (21%) and malformed (31%) foetuses were observed at the 300 mg/kg dose of EAA, which implies that although consumption of the leaf extract may not predispose users to hepatotoxicity, haematotoxicity, and hyperlipidemia, it should be taken with caution during pregnancy due to possible risk of embryotoxicity at concentrations higher than the therapeutic dose. Copyright © 2012 John Wiley & Sons, Ltd.**

Keywords: Artemisia annua; artemisinin; embryotoxicity; biochemical indices; haematological indices.

INTRODUCTION

Artemisia annua L. asteraceae is a medicinal herb that has been used in China for more than 2000 years for the treatment of malaria and other disorders. Presently, it is cultivated in Nigeria, Uganda, Kenya, India, Afghanistan, Argentina, Australia, Bulgaria, and France. It is found ubiquitously in Iran, Italy, Malaysia, Romania, Spain, Turkey, the United States, Hungary, and Vietnam (WHO, 2006). *Artemisia annua* possesses antibacterial, anti-inflammatory, and cytotoxicity (antitumour) activities in addition to its antimalarial activity (Hasheminia *et al*., 2011; Efferth *et al*., 2011). Artemisinin, the active antimalarial constituent of *A. annua*, has been isolated and characterised in 1971 (Klayman, 1985).

Apart from artemisinin, other sesquiterpenoids isolated from *A. annua* include artemisinin I, artemisinin II, artemisinin III, artemisinin IV, artemisinin V, artemisic acid, artemisilactone, artemisinol, and epoxyarteannuinic acid (WHO, 2006). In addition, *A. annua* also contains biologically active monoterpenoids, flavonoids (luteolin, apigenin, and peduletin), coumarins (scopoletin and tomentin), steroids, phenolics, purines, terpenes (costunolide), lipids, and aliphatic compounds (Bhakuni *et al*., 2001, 2002). Moreover, *Artemisia annua* contains essential oils such as, artemisia ketone (Holm *et al*., 1997; Lari *et al*., 2002; Brown, 2010), linalool, 1,8-cineol, *p*-cymene, thujone, and camphor (Carnat *et al*., 1985). Some of these essential oils have been reported to be toxic because of their high liposolubility property that enables them to cross biological membranes. For instance, camphor induces excitation on the central nervous system (Perazzo *et al*., 2003). Certain flavonoids such as casticin, artemetin (Elford *et al*., 1987), chrysosplenol D, and chrysoplenetin (Stermitz *et al*., 2002) contribute to the antimalarial activity of artemisinin in the crude extracts of the plant.

The scourge of malaria in pregnancy is devastating not only to the mother, but also to the unborn child. On the average, there are more than 50 million pregnancies every year in malaria endemic societies such as those in sub-Saharan Africa. Indeed, malaria is associated with spontaneous abortion, stillbirth, or premature delivery (Robert *et al*., 2001; Snow *et al*., 2005; Abdullah *et al*., 2007; Menendez *et al*., 2007). Apart from pregnant women, about 40% of the world's population is at risk of malaria in poor countries of the world (WHO, 2001). Importantly, malaria drains the economy of Africa alone to the tune of 12 billion US dollars every year (Mboera *et al*., 2007). Some intervention strategies against malaria include: (i) the use of insecticide-treated bed nets, (ii) development of vaccines, and (iii) use of potent antimalarials. Moreover, the development of resistance in parasites, and in vector against most of the available drugs, is the major challenge of malaria treatment. For instance, chloroquine was once considered as safe and

* Correspondence to: Amos O Abolaji, Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Oyo State, Nigeria.
E-mail: amos_abolaji@yahoo.com

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affordable, but now, it is not very useful because of resistance, and the reported antifertility effect (Ebong *et al.*, 1999). As a result of this, artemisinin-based combination therapies (ACTs) have been recommended by the WHO for the treatment of uncomplicated malaria (WHO, 2010).

Toxicity of artemisinin compounds has been reported during pregnancy in different animal models despite the fact that there is no reported evidence of teratogenic or mutagenic effects in humans (Gordi and Lepist, 2004; Amos *et al.*, 2003). For instance, foetal resorption in both rats, and rabbits even at low concentrations (Longo *et al.*, 2006), as well as toxicity at different stages of pregnancy in animal models have been reported White *et al.*, 2006; Alkadi, 2007; Boareto *et al.*, 2008; Schmuck *et al.*, 2009; El-Dakdoky, 2009).

Artemisinin-based antimalarials have several advantages over the quinines in that, they have faster parasitemia clearance (Barnes and White, 2005). Despite the promising breakthrough with ACTs, the increase in demand, which has compelled producers to push up the cost (Cyranoski, 2004), and the fear of its safety in pregnant women, resulted in the use of natural products such as *A. annua* by pregnant women in malaria endemic regions. It is estimated that 80% of the population of many developing countries still use plant-based traditional medicines. Plant-based medicines are believed to have innate affinity for biological receptors and provide different constituents against the overall disease state of the users in addition to fighting the causative agent (Willcox and Bodeker, 2000; Ginsburg and Deharo, 2011). Also, whole plants or mixtures of plants are usually preferred to the isolated compounds, because there is indication that they possess higher *in vitro* and/or *in vivo* antiplasmodial activity than the isolated constituents at comparable concentrations (Rasoanaivo *et al.*, 2011). As a result of the foregoing therefore, pregnant women living in malaria endemic regions of the world resort to the use of *A. annua*, and or other natural products as treatment regimen for malaria. Our hypothesis was that EAA may not be toxic during pregnancy. We believed that the secondary metabolites in the crude plant extract may mitigate the reported embryotoxicity of the active antimalarial constituent, artemisinin, through the phenomenon of natural balance, and therefore served as an alternative drug for the treatment of malaria during pregnancy. Although, *A. annua* herbal preparation was reported to be safe and well tolerated in adults, published data on pregnant women are not currently available (Willcox, 2010; Willcox *et al.*, 2011; Carbonara *et al.*, 2012). This study was carried out, therefore, to assess the maternal and foetal safety of *A. annua* in a mammalian rat model.

MATERIALS AND METHODS

Plant material and identification. *Artemisia annua* L was cultivated and its dried leaves provided courtesy of Molecular Bio/Sciences Ltd, 124 MCC Road, Calabar, Cross River State, Nigeria. The plant was carefully identified at the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria, where a voucher specimen (U. Cal 01/110) was preserved.

Preparation of extract for animal administration. *A. annua* leaves (400 g) were freed of dust and air-dried under natural conditions. Air-dried leaves of *A. annua* previously analyzed using reversed phase high performance liquid chromatography to contain 1.098% of artemisinin (Abolaji *et al.*, 2010) were pulverized and subjected to Soxhlet extraction at 17–20^{o}C for 48 h with 98% ethanol. After filtration and evaporation under vacuum, the extract was left in the fume hood until the solvent was completely evaporated yielding 56 g of a greenish sticky extract representing 14% yields.

Animals and animal care. Animal studies were carried out after the approval of institutional animal care and use committee in accordance with the declaration of Helsinki and European Community guidelines for the ethical handling of laboratory animals. Forty albino Wistar rats of either sex weighing between 220 and 260 g were obtained from the Animal House Facility of the International Center for Chemical and Biological Sciences, University of Karachi, Pakistan, where this research was conducted. The animals were housed for one week prior to experiment under controlled conditions with 12 h light/dark cycle, temperature 22 ± 2^{o}C, and free access to feed and water. For mating, one female was placed together with one male overnight. The day when there was an evidence of mating (vaginal smear with sperm cells) was recorded as gestational day 0.

Animal treatment. Pregnant animals were randomly assigned five rats per group to control (saline), and test groups administered orally with 100, 200, and 300 mg/kg from the 8th day of gestation to the 19th (Rath *et al.*, 2004), approximately equivalent to 5, 10, and 15 times higher than the therapeutic dose of 9 g *A. annua*/day, respectively, using a yield of 14% with average adult weight of 65 kg. The 9 g *A. annua*/day was chosen as a reference dose because ethanol was only used to facilitate extraction, and all ethanol was removed from the final concentration. All ingredients in a concentrated ethanolic extract were also present in at least the same concentration in the dried leaves and the extract (Rezelman and Goris, 2008). Following last dose administration, the rats were fasted for 18 h, and on the 20th day of gestation, animals were anaesthetized using *i.p.* pentothal sodium (60 mg/kg, Abbot Laboratory, Pakistan). The blood and various organs were collected for subsequent studies.

Blood and organ collection. Blood was collected by cardiac puncture using sterile syringes and needles into anti-coagulant free serum separator tubes, allowed to clot for 1 h and centrifuged at 3000 rpm for 10 min using Eppendof 5810R centrifuge. Serum was transferred into plastic tubes and stored at -20 ^{o}C till further use. The blood for the glucose determination was collected into tubes containing anti-coagulant. The following organs of the rats were carefully removed and weighed: ovaries, liver, kidneys, spleen, hearts, and lungs. The liver was stored in formalin for histopathological study.

Biochemical analyses. Serum was used to determine the levels of creatinine, total bilirubin, direct bilirubin, total protein, albumin, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), cholesterol, triacylglycerol, high density lipoprotein (HDL), low density lipoprotein (LDL), sodium (Na^{+}), potasium (K^{+}), chloride (Cl^{-}), and

bicarbonate (HCO_3^-) ions, while the glucose levels were determined in the plasma collected using Hitachi 902 automated analyzer (Roche Diagnostics, Germany).

Hormonal analyses. The levels of estrogen and progesterone were determined in serum using Elecsys 2010 automated analyzer (Roche Diagnostics, Germany).

Haematological analyses. The following haematological parameters were determined in the whole blood of pregnant rats collected in EDTA bottles using Beckman Coulter HMX analyzer (USA): haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC), and white blood cell (WBC).

Histopathology. Biopsies from maternal liver were fixed in 10% formalin and processed for histology. Briefly, liver specimen was fixed in 10% neutral-buffered formaldehyde solution (pH 7.2 to 7.4). After dehydration procedures, the samples were blocked in paraffin. Sections of 4–5 μm were made using a microtome and stained with hematoxylin and eosin. Mounted slides were examined under a light microscope and photographed (Wu *et al.*, 2006). All slides were coded before examination with light microscope by investigators who were blinded to control and treatment groups.

Foetal examination. Following collection of blood and organs, uterus was carefully removed and weighed with the foetuses (gravid uterine weight). The pups and placenta were carefully removed from the membrane and individually weighed and observed for viability and malformation, if any (Stacy, 2004).

Statistical analysis. The data were evaluated by using one-way analysis of variance with a post hoc Dunnett's test. Values were presented as means ± standard error. Statistical analysis was performed using SPSS10 software (SPSS Inc., Chicago, IL, USA). Probability value (p) < 0.05 was considered to be significant.

RESULTS

Maternal data

Dams treated with EAA did not show any sign of maternal toxicity. As shown in Fig. 1A and B, dams in the 100 mg/kg EAA group consumed more water and had the lowest feed intake compared to the other groups. Expected weight gain occurred in all the groups. Groups dosed with 200 and 300 mg/kg of EAA increased significantly compared to the control group. The weights of the liver and heart were elevated at 200 mg/kg ($p < 0.05$). There was reduction in spleen weights at 100 and 200 mg/kg of EAA ($p < 0.05$) compared to the control group. There were no changes in the weights of the ovaries, kidneys, and lungs of EAA-treated rats compared to the control group (Table 1). Furthermore, no clinical or behavioural changes were observed in dams treated with EAA.

Effects of EAA on maternal biochemical biomarkers

Maternal biochemical biomarkers determined after EAA treatment are shown in Table 2. There were significant decreases in creatinine, total bilirubin, and total protein levels at 200 and 300 mg/kg ($p < 0.05$). Direct bilirubin decreased significantly ($p < 0.05$) in all the three groups. While glucose levels decreased at 100 and 300 mg/kg of EAA ($p < 0.05$), there were decreases in the levels of all the electrolytes in the EAA-treated groups; this difference was significant only at the 300 mg/kg of EAA

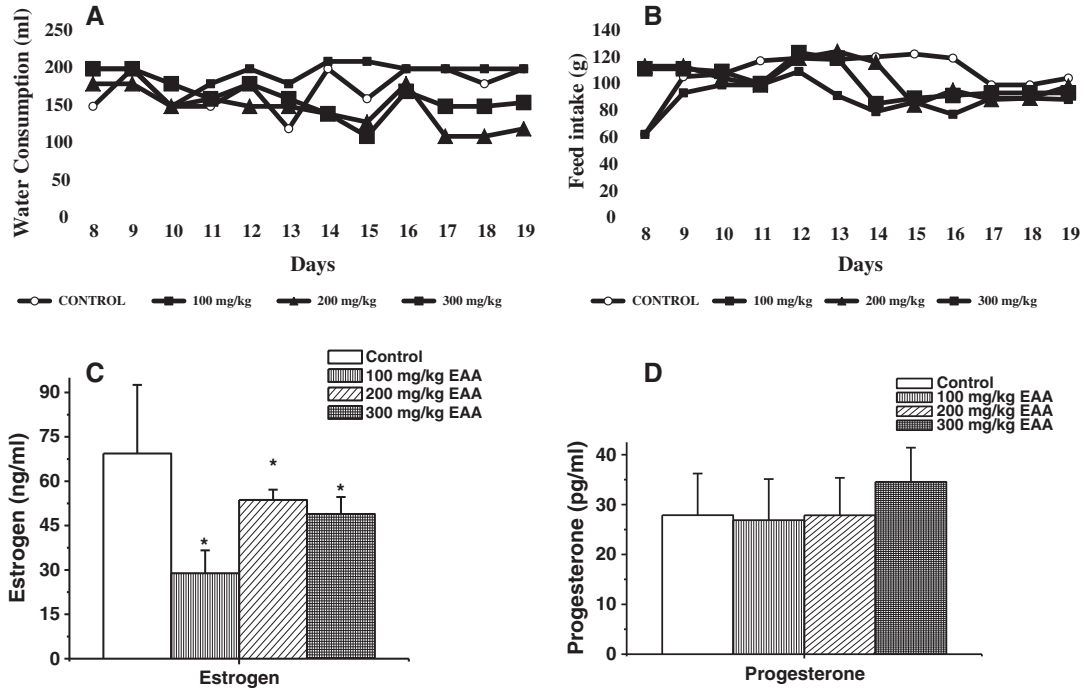

Figure 1. Maternal daily average water (**A**) and feed (**B**) intake, estrogen (**C**) and progesterone (**D**) levels after administration of *A. annua* during gestational period 8–19 days. Data presented as Mean + SEM, $n = 5$. $p^* < 0.05$.

Table 1. Effects of *Artemisia annua* on maternal and organ weights

		Artemisia annua (mg/kg)			
		Control	100	200	300
Body Weights (g)	Initial	249.60 ± 8.10	262.80 ± 12.00	240.40 ± 5.60	225.60 ± 4.50
	Final	282.40 ± 1.00	287.60 ± 11.80	310.80 ± 22.50	288.40 ± 7.30
Body Weight Changes (%)		11.57 ± 0.65	8.62 ± 1.62	21.04 ± 5.71*	21.72 ± 0.89*
Liver (g)		8.44 ± 0.39	8.28 ± 0.29	10.30 ± 0.44*	8.92 ± 0.55
Ovaries (g)		0.20	0.20	0.20	0.20
Kidneys (g)		1.44 ± 0.05	1.52 ± 0.10	1.60 ± 0.10	1.58 ± 0.03
Heart (g)		0.70 ± 0.03	0.70 ± 0.03	0.96 ± 0.08*	0.86 ± 0.04
Lungs (g)		1.48 ± 0.67	1.38 ± 0.10	1.38 ± 0.11	1.68 ± 0.11
Spleen (g)		0.74 ± 0.05	0.56 ± 0.02*	0.62 ± 0.04*	0.64 ± 0.02

Data presented as Mean ± SEM, $n = 5$. $p^* < 0.05$.

Table 2. Maternal biochemical indices following administration of *Artemisia annua* during gestational periods 8–19 days

Parameters and Groups	Control	100 mg/kg	200 mg/kg	300 mg/kg
Creatinine (mg/dL)	0.72 ± 0.11	0.66 ± 0.03	0.42 ± 0.03*	0.40 ± 0.02*
Total Bilirubin (mg/dL)	0.26 ± 0.01	0.25 ± 0.01	0.15 ± 0.04*	0.13 ± 0.01*
Direct Bilirubin (mg/dL)	0.49 ± 0.02	0.46 ± 0.01*	0.01 ± 0.02*	0.03 ± 0.01*
Total Protein (g/dL)	2.86 ± 0.17	2.85 ± 0.17	1.96 ± 0.18*	2.20 ± 0.07*
Albumin (g/dL)	2.88 ± 0.29	3.08 ± 0.13	3.06 ± 0.12	3.084 ± 0.03
Glucose (mg/dL)	67.2 ± 3.61	50.20 ± 1.07*	68.80 ± 3.28	52.00 ± 2.43*
HDL (mg/dL)	33.20 ± 0.73	37.00 ± 2.47	48.00 ± 1.38*	35.00 ± 2.84
Cholesterol (mg/dL)	38.40 ± 3.39	36.00 ± 2.47	32.60 ± 1.08	37.00 ± 3.18
Triacylglycerol (mg/dL)	185.00 ± 32.42	92.00 ± 14.99*	88.60 ± 12.56*	129.00 ± 8.25
LDL (mg/dL)	7.00 ± 1.61	3.80 ± 0.73	10.20 ± 1.16	9.20 ± 0.37
ALT (U/L)	45.00 ± 5.34	39.60 ± 3.34	56.00 ± 2.00*	65.00 ± 1.48*
AST (U/L)	166.4 ± 4.80	187.40 ± 2.00 *	200.80 ± 1.60 *	180.40 ± 4.40 *
Na^+ mEq/l	134.00 ± 0.71	132.60 ± 0.98	132.2 ± 0.92	131.4 ± 0.51*
K^+ mEq/l	5.60 ± 0.28	5.18 ± 0.26	5.48 ± 0.22	5.54 ± 0.11
CL^- mEq/l	99.00 ± 1.00	97.80 ± 0.86	98.20 ± 0.370	96.40 ± 0.51*
HCO_3^+ (mEq/l)	99.00 ± 1.00	97.80 ± 0.86	98.20 ± 0.37	96.40 ± 0.51*

Data presented as Mean ± SEM, $n = 5$. $p^* < 0.05$.

($p < 0.05$) for Na^+, HCO_3^-, and Cl^- ions. While AST levels were all significantly elevated, the levels of ALT were elevated at 200 and 300 mg/kg of EAA ($p < 0.05$) compared to the control group. The elevation was not up to twofold of the control level in both cases. While HDL was elevated in all the EAA-treated groups, there were no significant changes in the levels of LDL in all the EAA-treated groups compared to the control. In addition, there were reductions in the levels of triacylglycerol in all the EAA-treated groups compared to the control (Table 2).

Effects of EAA on maternal haematological biomarkers

There were significant increases in the levels of Hb, RBC, PCV, MCHC, and WBC at 200 and 300 mg/kg of EAA ($p < 0.05$). There was no change in the levels of MCV and MCH in the EAA-treated groups compared to the control group (Table 3).

Effects of EAA on maternal liver histology

There was no detectable toxicity in the maternal liver of the treated groups as compared to the control group. The histology showed normal hepatocytes (Fig. 2).

Effects ofEAA on maternal hormonal changes and foetal data

Estrogen levels in pregnant rats reflected significant decreases in all the EAA-treated groups ($p < 0.05$) (Fig. 1C). Progesterone did not change in all the treated groups as compared to the control group (Fig. 1D). *Artemisia annua* did not cause any observable malformation in the foetuses at 100 and 200 mg/kg doses of EAA. However, at the 300 mg/kg dose, non-viable (death *in situ*; 21%) and malformed (31%) foetuses were observed. Further observation revealed that one of the rats had the uterus developed into a tumour-like mass (Fig. 3I, II, III, IV, and V). It was also observed that the litter size significantly reduced ($p < 0.05$) at 100 mg/kg EAA. There was no change in the gravid uterine, placental, and foetal weights of the treated rats compared to the control group (Table 4).

DISCUSSION

Depending on geographical location, cultivation conditions, and advances in breeding techniques, the artemisinin

Table 3. Maternal haematological changes following administration of *Artemisia annua* during gestational periods 8–19 days

Parameters and Groups	Control	100 mg/kg	200 mg/kg	300 mg/kg
Hb (g/dl)	7.68 ± 0.27	7.12 ± 0.26	10.64 ± 0.33*	11.00 ± 0.44*
RBC (million/μl)	3.95 ± 0.06	3.76 ± 0.16	5.33 ± 0.20*	5.46 ± 0.22*
PCV (%)	23.90 ± 1.29	21.06 ± 0.77	29.44 ± 0.81*	30.30 ± 1.62*
MCV (fl)	56.00 ± 1.56	56.12 ± 1.02	55.64 ± 0.59	55.16 ± 0.54
MCH (pg)	19.42 ± 0.53	19.02 ± 0.21	20.08 ± 0.24	20.08 ± 0.36
MCHC (g/dl)	34.58 ± 0.27	33.94 ± 0.77	36.14 ± 0.33*	36.26 ± 0.14*
WBC (10^9/L)	3.10 ± 0.47	4.18 ± 0.62	5.74 ± 0.52*	5.42 ± 0.34*

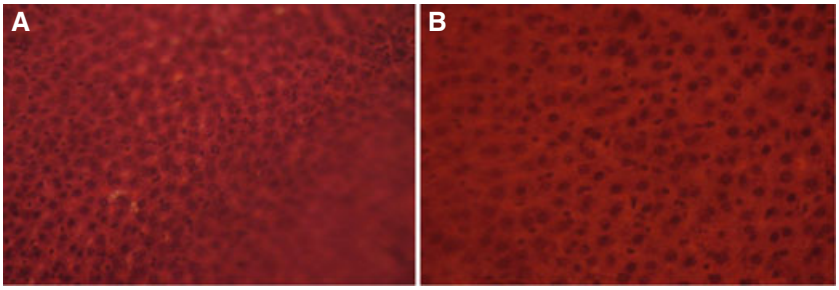

Figure 2. Effect of *A. annua* on maternal liver (A) control, and (B) 300 mg/kg of *A. annua* (Mag. 40×). This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

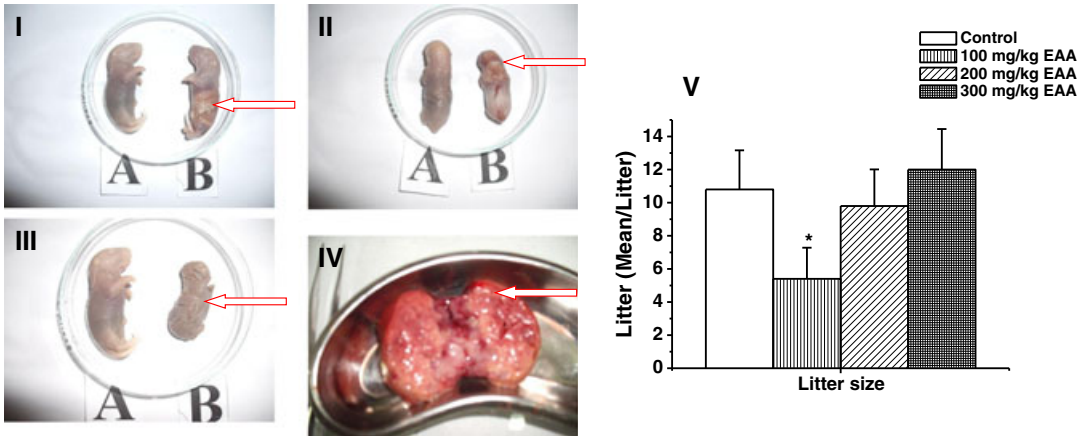

Figure 3. Effects of *A. annua* on foetal development and litter size in Wistar rats. Foetuses control (A), and after treatment with 300 mg/kg (B) with *A. annua* extract, are represented in (**I**), (**II**), and (**III**). In (**IV**), tumour found in the uterus. Litter sizes of control and after treatment with *A. annua* are presented in (**V**). Data presented as Mean + SEM, $n = 5$. $p^* < 0.05$. This figure is available in colour online at wileyonlinelibrary.com/journal/ptr.

Table 4. Foetal evaluation after oral administration of *A. annua* to pregnant Wistar rats

Parameters and Groups	Mated Female Rats	% Pregnant Rats	Gravid Uterine Weights	Placental Weights	Foetal Weights	Non-Viable Foetuses (%)	Foetuses with Malformation (%)
Control	5	100	53.40 ± 5.41	6.26 ± 0.91	4.058 ± 0.96	0	0
100 mg/kg	5	100	34.36 ± 25.07	4.22 ± 2.09	5.448 ± 1.55	0	0
200 mg/kg	5	100	46.64 ± 32.54	6.68 ± 3.04	4.0484 ± 1.34	0	0
300 mg/kg	5	100	59.6 ± 20.04	6.98 ± 1.00	4.5646 ± 0.97	21	31

Data presented as Mean ± SEM, $n = 5$.

yield of *A. annua* varies from 0.02% to 1.4% of the dry weight (Mueller *et al.*, 2000; Delabays *et al.*, 2001). In a previous study, we obtained a yield of 1.098% of artemisinin in the Nigerian grown *Artemisia annua* using reversed phase high perfomance liquid chromatography (Abolaji *et al.*, 2010). We quantified artemisinin level in the dry leaves of *A. annua* before commencement of study to be sure that the plant is therapeutically active.

Ethanolic extract of *A. annua* was used because the purpose is to administer the drug as an extract, since the extract will contain most of the artemisinin constituents of the dry plant (Rezelman and Goris, 2008).

In the present study, we investigated the maternal and foetal safety after oral administration of EAA to pregnant rats to know if is safe during pregnancy since it contains artemisinin, the active antimalarial ingredient. Due to a decrease in xenobiotic metabolizing enzyme activities during pregnancy, the endocrine environment of the developing foetus is exposed to harmful impact of the xenobiotic (Parvez *et al*., 1975; Tsutsumi *et al*., 2001; Randy and Ernest, 2004). Toxicities of herbal medicines have been reported (Veiga-Junior *et al*., 2005; Saad *et al*., 2006; Colson and De Broe, 2005). For example, some herbal medicines resulted in hepatotoxicity (Cheng *et al*., 2006) and nephrotoxicity (Tennant, 1997; Debelle *et al*., 2008). At present, detailed *in vivo* reports on the maternal and foetal safety assessment studies on *A. annua* in pregnant animal models are not currently available. Because of this reason, we could not compare our findings with previous results.

The usual markers of liver toxicity are total and direct bilirubin, ALT (E.C. 2.6.1.2), and AST (E.C.2.6.1.1). The liver, being a key organ involved in metabolism and detoxification of xenobiotics, is vulnerable to damage induced by several xenobiotics (Tennant, 1997). During pregnancy, its vulnerability is increased due to a decrease in hepatic metabolism and plasma albumin leading to an increase in the proportion of free drugs in maternal plasma (Grance *et al*., 2008). The observed increase in the levels of ALT as well as AST in some of the EAA-treated groups was not enough to conclude hepatotoxicity of *A. annua* in maternal liver. This is because the increase was less than two-fold of the control in each instance. In addition, the levels of total and direct bilirubin were reduced in all the treated groups. This was supported further by the normal hepatocytes observed in the liver at all the concentrations of EAA. In addition, the extract did not result in renal dysfunction at the 100 and 200 mg/kg EAA because the electrolytes were not affected by EAA.

The decreases in glucose concentrations at the 100 and 300 mg/kg EAA groups indicated its blood glucose lowering effect at these concentrations.

The clinical significance of lipids is primarily associated with their contribution to coronary heart disease (CHD) and various lipoprotein disorders. Increased LDL cholesterol is a factor in the cause of atherosclerotic diseases. Several studies have established that when total cholesterol and LDL cholesterol concentrations are high, the incidence and prevalence of CHD are also high (Nader *et al*., 2008). Since the levels of the LDL and HDL in the EAA-treated groups were observed to be normal, it implies that *A. annua* may not predispose to atherosclerosis.

The haematological profile reflects the general health status of an individual (RCOG, 2007). The haematological profile of the pregnant woman is one of the factors that affect pregnancy, and its outcome (Celik and Suzek, 2008). The elevated levels of Hb at 200 and 300 mg/kg of EAA correspond with the increased production of RBC in these groups of rats. The observed elevation of Hb, RBC, and PCV at the 200 and 300 mg/kg of EAA could be a reflection of increased iron supply resulting in the increased Hb production. From the observed values of WBC, it is clear that an increase in the number of WBCs is a normal reaction of rats to substances which alter their normal physiological processes. The leucocytosis observed in the present study therefore indicated an immune system that was triggered to protect the rats due to the presence of EAA (US EPA, 1991).

In order to evaluate the toxicity of EAA on the foetuses in this study, we will consider factors such as hormonal levels, presence of malformations, and mortality (US EPA, 1996). Estrogen has multiple functions during pregnancy. Apart from regulating production of progesterone, it also initiates foetal maturation. Without it, foetal tissues and organs will not mature. In addition, the placenta also provides oxygen and nutrition for the growth and development of the foetus, and estrogen helps in the maintenance of the foetal-placental well-being. Indeed, estrogen triggers the process of placental corticosteroid pathway so as to influence foetal adrenal glands (University of Maryland, 1997). The observed reduction in the levels of estrogen therefore is an indication of disturbance of the foetal placental well-being as well as hormonal imbalance. In addition, the significant decreases in the levels of estrogen in the EAA-treated groups could be due to the artemisinin content of EAA. Artemisinin has been reported to be effective in the treatment of breast cancer. Since estrogen plays important role in the induction of breast cancer, artemisinin decreases the level of estrogen receptors thereby blocking the ability of estrogen to induce breast cancer (Sundar *et al*., 2008). The decrease in estrogen level was more pronounced at the 100 mg/kg of EAA because the receptors may not be fully saturated at this dose compared with the groups at the higher doses of EAA.

The presence of malformations at a dose of 300 mg/kg of EAA could represent possible embryotoxicity of *A. annua* at high concentration. The initial teratogenic effect is associated with apoptosis or alteration in the rate of cell growth. The non-viable foetuses observed at 300 mg/kg of EAA further suggested its adverse effect on the foetuses. Mortality could be the result of direct action of EAA, or it may be secondary to maternal toxicity (Eduard *et al*., 2005). The malformations and non-viability observed could also be a direct action of artemisinin content of EAA at the stages of foetal erythropoiesis and vasculogenesis of the earliest developing RBC thereby resulting in cell death and severe anaemia in the embryos at high peak concentration of EAA (Qigui and Weina, 2010).

CONCLUSION

In conclusion, we have carried out investigation to assess maternal and foetal safety after administration of EAA from the second to the third trimester of pregnancy in Wistar rats. *A. annua* may not result in maternal hepatotoxicity, hyperlipidemia, and haematotoxicty. Although EAA may be safe to the mother even at concentrations higher than the therapeutic dose, users should, however, be aware of its possible hypoglycemic effect, which can be exacerbated during malaria infection. We advise that the plant should be taken with caution during pregnancy as there is the possibility of embryotoxicity at concentrations higher than the therapeutic dose. We recommend that detailed developmental toxicity study on EAA be carried out at different stages of pregnancy in animal models, after which further studies should be carried out

on healthy pregnant human volunteers at concentrations not exceeding the therapeutic dose.

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Conflict of Interest

The authors have no conflicting interests to declare.

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