Ovarian and Uterine Genes Transcriptional Responses to Artesunate in Female Wistar Rats

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ABSTRACT

__Background:__ This research was designed to investigate ovarian and uterine gene transcriptional responses to artesunate in female rats.
__Materials and Methods:__ Female rodents (120 – 140 g) numbering ten were designated for this study. Artesunate (1.43 mg/kg) was given by gavage to the rodents for 50 days. The method of RT-PCR was employed to investigate the expressions of p53, Bcl-2, SOD, aromatase and IL-1β in the ovaries and uteri. Histopathological analyses of the uteri and ovaries were also done. Graphics were generated as average +/- SEM with Graph-pad Prism (version 8.0).
__Results:__ The p53 and aromatase expressions were significantly (p<0.05) down-regulated, while the Bcl-2 and IL-1β expressions were up-regulated significantly (p<0.05) in the artesunate treated rats' ovaries relative to their controls. In addition, the p53, Bcl-2, SOD and IL-1β expressions were up-regulated significantly (p<0.05), but the aromatase expression was significantly (p<0.05) down-regulated in the artesunate treated rats' uteri relative to their controls. Furthermore, the histopathological analyses indicated severe congested (hemorrhagic) ovarian medulla as well as expanded lumens of the endometrial glands.
__Conclusion:__ Conclusively, this study has suggested that artesunate inhibited apoptosis and steroidogenesis, but induced inflammation and carcinogenesis in rats' ovaries. In addition, it probably inhibited apoptosis, superoxide radicals (oxidative stress) and steroidogenesis; but also induced inflammation and carcinogenesis in rats' uteri. Furthermore, it is also probably toxic to the ovaries and uteri at histological level.

Keywords: Artesunate; RT-PCR; Bcl-2; Aromatase; Rats

INTRODUCTION

Artesunate is a medication used to treat malaria [1]. It is a member of the artemisinin group of drugs. Frequently it is given in the form of combination therapy, for instance mefloquine combine with artesunate. The drug is not taken as a prophylaxis. It can be given via muscular injection, intravenous injection as well as through rectal and oral administrations [2].

It is well tolerated when consumed [3]. It is a drug of preference during the gestation period due its high safety index, but opposite results were suggested in animal studies [4]. Lactating mothers are also allowed to it. It was invented by Liu Xu in 1977 [5] and was listed by World Health Organization as an essential medicine [6]. There is also substantial evidence that it could also has beneficial effects on infection due *Schistosoma haematobium* [7], but this has not been proven convincinly.

It has a similar efficacy to arthemeter when used in treating adults' malaria caused by *P. falciparum* [8], but artesunate in combination with other medications has superior advantages when compared to artemether-based drugs, that is to say, via uptake and via the administrative routes and could be more efficacious in the treatment of serious malaria in children [9]. Medications that inhibit hepatic enzyme CYP2A6 must not be taken together with artesunate, examples of such agents are letrozole, amiodarone, isoniazid etc.

Artesunate effect on: kidneys toxicity in rats [10], rats' hypoglycemia and hemolysis induction [11] rats' and rabbits' developmental toxicities [12], rats' hepatic histopathological alterations [13], toxicokinetics and embryotoxicity in rats [14], rats' osteological development toxicity [15] female rats reproductive function [16] as well as on other neurological therapeutic effects [17] have been documented.

But due to limited information obtained from literature concerning artesunate effects on ovarian and uterine genes expressions in female rodents, hence, this study intends to obliterate this lacuna.

Experimental Animals (1.1)

Female rats numbering ten (120 – 140 g) raised in the ABUAD Animal Holding were used. They were housed in laboratory ambient with unhindered access to feed and water; the rodents were acclimatized to laboratory conditions for two weeks prior to the experimental commencement. Animal experiments were carried out according to the dictates of Helsinki on animal experimentation.

Drugs (1.2)

Artesunate (Green Energy, China) was bought from Danax Pharmacy, Ibadan, Nigeria. It (50 mg) was was solubilized in distilled water (10 ml) to give a concentration of 5.0 mg/ml. The artesunate dosage considered in this study was as prescribed by the manufacturing industry.

EXPERIMENTAL DESIGN

Female rodents numbering ten (five per group) used in this study received the following doses of artesunate and distilled water (control) by gavage for 50 days as follows:

Group I rodents (control group) were administered 0.5 ml/100 g of distilled water.

Group II rodents were administered 1.43 mg/kg of artesunate.

On the next day after the last treatment (day 51), the rodents were euthanized by overdosing with pentobarbital sodium (180 mg/kg, i.p); ovaries and uteri were harvested with the fatty tissue removed and transferred quickly into TRIzol reagent (ThermoFisher Scientific) for isolation of RNA.

Isolation of RNA (2.1)

Isolation of RNA from whole tissues was as described by [18]. In summary, the ovaries were homogenized in TRI reagent at cold 4 °C. Partitioning of Total RNA in chloroform was done by centrifuging at 15,000 rpm for 15 minutes. Supernatant containing RNA was removed from the solution with isopropanol of same volume. Ethanol (70%) was used to wash the extracted RNA twice which was then dehydrated for 5 minutes before being re-suspended in the buffer.

Conversion of cDNA (2.2)

Spectrophotometer was used to determine the purity and quantity of total RNA at an absorbance of A_{260}/A_{280} as described by [18].

Polymerase chain reaction (PCR)/Electrophoresis (2.3)

Bcl-2, p53, SOD, aromatase and IL-1β genes were amplified by PCR targeting primers highlighted in the table below. A software called Primer3 was used to design the primers. The PCR amplification process was carried out as described by [19].

Amplification products were electrophoresed in agarose gel (1.5%) using 0.5X TBE (Tris-borate EDTA,JHD chemicals, China) containing ethidium bromide at 100V for 60 minutes. The gel was visualized with UV light with the aid of a photo documentation system fitted with a camera. Gel images were analyzed using keynote platform as previously described by [19] and Image J software was used to quantify it. All

graphs were plotted as mean +/- SEM using graph-pad prism (version 8.0).

Table 1: List of primers

s/n	Gene	Accession	Forward Primer sequence (5'-3')	Reverse Primer sequence (5'-3')	Tm °C	Amplicon Size (bp)
1	SOD	NM_017050.1	GCGTCATTCACTTCGAGCAG	CCTCTCTTCATCCGCTGGAC	60	174
2	Aromatase	NM_017085.3	GCTTCTCATCGCAGAGTATCCGG	CAAGGGTAAATTCATTGGGCTTGG	60	192
3	IL-1β	NM_031512.2	GACTTCACCATGGAACCCGT	CAGGGAGGGAAACACACGTT	59	191
4	p53	NM_030989.3	TCGAGATGTTCCGAGAGCTG	GTCTTCGGGTAGCTGGAGTG	58	153
5	Bcl-2	NM_016993.1	GGGATGACTTCTCTCGTCGC	TGACATCTCCCTGTTGACGC	58	200

**Histological preparation of tissues (2.4)**

The histological processing of the ovarian and uterine tissues was as described by [20].

## RESULTS

Analysis of Gene Expression (3.1)

From (**Figure 1**), it was reported that p53 expression was down-regulated significantly ($p < 0.05$) in the artesunate treated rats' ovaries relative to the control. Furthermore, from the result presented in (**Figure 2**), the Bcl-2 expression was significantly ($p < 0.05$) up-regulated in the artesunate treated rats' ovaries as compared to the control. However, (**Figure 3**) revealed that there was insignificant ($p > 0.05$) difference in SOD expression in the artesunate treated rats' ovaries when compared with the control. In addition, (**Figure 4**) suggested that aromatase expression was down-regulated significantly ($p < 0.05$) in the artesunate treated rats' ovaries relative to the control. (**Figure 5**) revealed that IL-1β expression was up-regulated significantly ($p < 0.05$) in the artesunate treated rats' ovaries when compared with the control.

From (**Figure 6**), it was reported that p53 expression was up-regulated significantly ($p < 0.05$) in the artesunate treated rats' uteri relative to the control. (**Figure 7**) reported that the Bcl-2 expression was significantly ($p < 0.05$) up-regulated in the artesunate treated rats' uteri as compared to the control. (**Figure 8**) revealed that the SOD expression was up-regulated significantly ($p < 0.05$) in the artesunate treated rats' uteri relative to the control. In addition, (**Figure 9**) reported that aromatase expression was down-regulated significantly ($p < 0.05$) in the artesunate treated rats' uteri relative to the control. The result presented in (**Figure 10**) revealed that IL-1β expression was up-regulated significantly ($p < 0.05$) in the artesunate treated rats' uteri relative to the control.
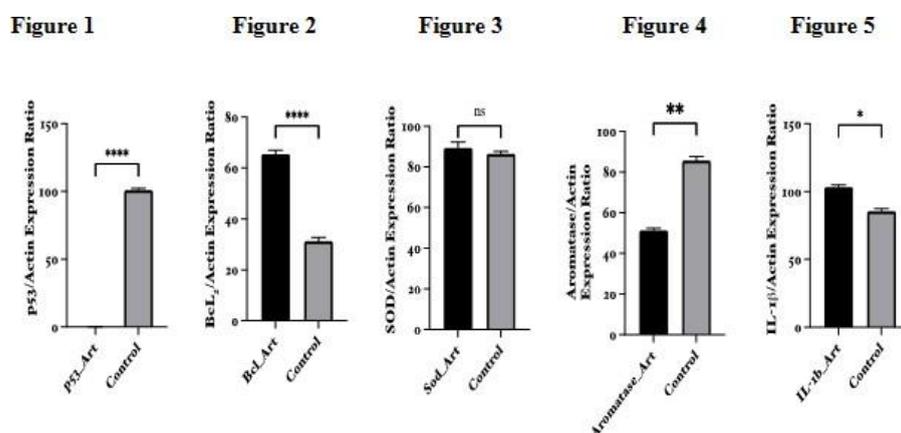
Figure 1: Comparative expression of p53 in the ovaries of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph

(n=5, p<0.05). **Figure 2:** Comparative expression of Bcl-2 in the ovaries of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05). **Figure 3:** Comparative expression of SOD in the ovaries of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05). **Figure 4:** Comparative expression of aromatase in the ovaries of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05). **Figure 5:** Comparative expression of IL-1β in the ovaries of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05).
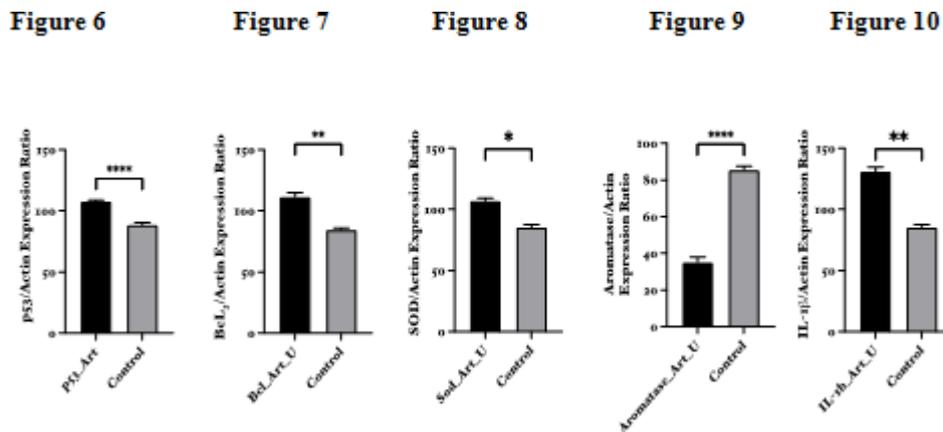

Figure 6: Comparative expression of p53 in the uteri of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05). **Figure 7:** Comparative expression of Bcl-2 in the uteri of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05). **Figure 8:** Comparative expression of SOD in the uteri of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05). **Figure 9:** Comparative expression of aromatase in the uteri of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05). **Figure 10:** Comparative expression of IL-1β in the uteri of rats treated with artesunate (Art). The band density (image J) was plotted as a bar graph (n=5, p<0.05).

Histopathological effect (3.2)

(**Figure 12**) revealed that treating rats with artesunate presented with medullary regions that were severely congested, contrary observation was made in the control rats (**Figure 11**). (**Figure 14**) revealed that treating rats with artesunate presented with endometrial glands with expanded lumens, contrary observation was made in the control rats (**Figure 13).**
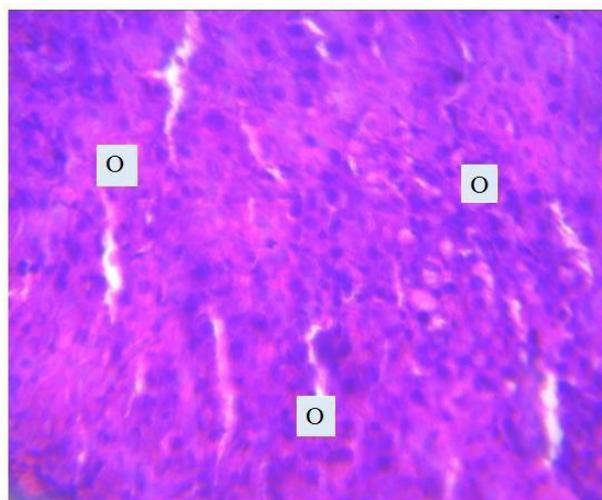

Figure 11: Effect of distilled water (control) (0.5 ml/100 g) on the ovary at x100. Photomicrograph revealing a normal ovary (O) without pathological lesions.
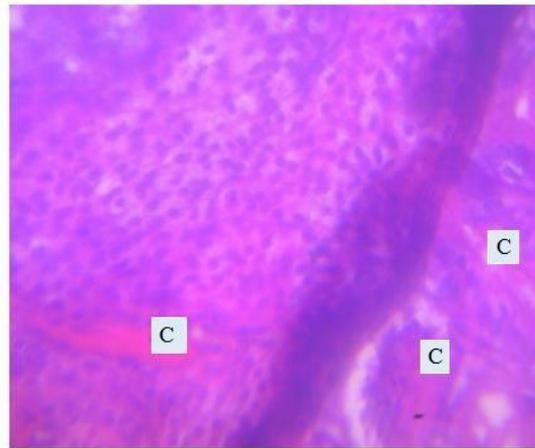
Figure 12: Effect of artesunate (1.43 mg/kg) on the ovary at x40. Photomicrograph revealing severely congested (C) medullary regions.
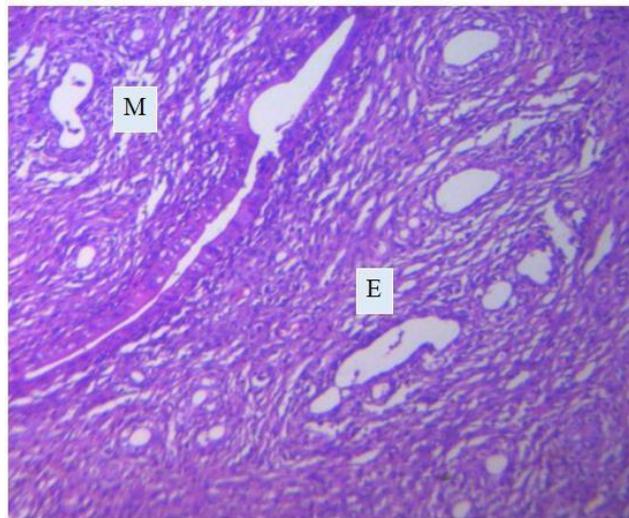
Figure 13: Effect of distilled water (control) (0.5 ml/100 g) on the uterus at x100. Photomicrograph revealing normal myometrium (M) and endometria (E) without visible lesions.
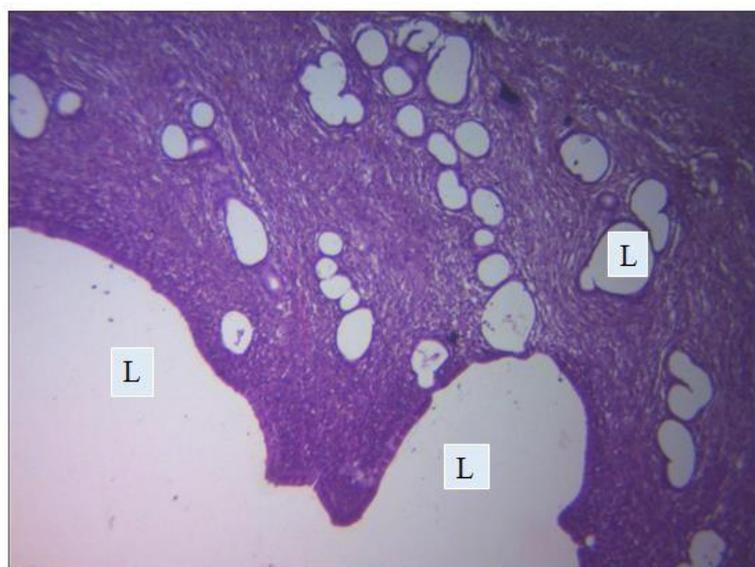
Figure 14: Effect of artesunate (1.43 mg/kg) on the uterus at x100.Photomicrograph revealing endometrial gland with expanded lumens (L).

DISCUSSION

The p53 expression was significantly down-regulated in the artesunate treated rats' ovaries which suggest that the drug inhibited apoptosis [21]. It also probably indicates that the drug inhibited cell cycle arrest [22]. Similar report was given by [23] in phenobarbital treated rats.

The expression of Bcl-2 was up-regulated significantly in the artesunate treated rats' ovaries which probably indicate that the drug inhibited apoptosis in ovarian tissue [24]. Contrary result was given by [25] in *Olea europaea* treated rats.

There was insignificant difference in the expression of SOD in the artesunate treated rats' ovaries which suggests that the drug had no effect on superoxide radicals (oxidative stress) [26]. Contrary result was given by [27] in umbelliferone treated rats.

The aromatase expression was significantly down-regulated in the artesunate treated rats' ovaries which suggest that the drug decreased estrogen level (steriodogenesis). Similar result was given by [28] in *Ginkgo biloba* extract flavonoids effects on estrogen biosynthesis.

The IL-1β expression was significantly up-regulated in the artesunate treated rats' ovaries which probably indicate that the drug induced inflammation and carcinogenesis [29]. Similar result was given by [30] in their study on human gametes expression of Interleukin-1 system genes.

The p53 expression was significantly up-regulated in the artesunate treated rats' uteri which suggest that the drug induced apoptosis [21]. Contrary outcome was reported by [23] phenobarbital treated rats.

The Bcl-2 expression was significantly up-regulated in the artesunate treated rats' uteri which probably indicate that the drug inhibited apoptosis in uterine tissues [24]. Contrary outcome was reported by [25] in *Olea europaea* treated rats.

The SOD expression was significantly up-regulated in the artesunate treated rats' uteri which suggest that the drug inhibited superoxide radicals (oxidative stress) [26]. Similar result was given by [27] in umbelliferone treated rats.

The aromatase expression was significantly down-regulated in the artesunate treated rats' uteri which suggest that the drug decreased estrogen level (steriodogenesis). Similar result was given by [28] in *Ginkgo biloba* extract flavonoids effects on estrogen biosynthesis.

The IL-1β expression was significantly up-regulated in the artesunate treated rats' uteri which probably indicate that the drug stimulated or induced inflammation and carcinogenesis [29]. Similar result was given by [30] in their study on human gametes expression of Interleukin-1 system genes.

The ovarian photomicrographs of the artesunate treated rats' revealed severe congested (hemorrhagic) ovarian medulla which could be as a result of venous thrombosis. Similar result was reported by [31] in diclofenac treated rats.

The photomicrographs of uteri of the artesunate treated rats presented with expanded lumens of the endometrial glands which could be due to degeneration of cells adjacent to the endometrial lumens.

CONCLUSION

Conclusively, this study has suggested that artesunate inhibited apoptosis and steroidogenesis, but induced inflammation and carcinogenesis in rats' ovaries. In addition, it probably inhibited apoptosis, superoxide radicals (oxidative stress) and steroidogenesis; but also induced apoptosis, inflammation and carcinogenesis in rats' uteri. Furthermore, it is also probably toxic to the ovaries and uteri at histological level.

COMPETING INTEREST

There is absence of conflicting interests in this research work.

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