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# Artesunate-induced testicular injury: Oil from selected spices blend modulates redox homeostasis and exacerbates steroidogenesis in rat models

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SUMMARY

The therapeutic potential of oil from blends of selected culinary spices against artesunate-induced testicular injury in albino rats was investigated. Two groups of rats each were pretreated with the oil at 1.5 and 3.00 mL respectively for seven days and after which administered artesunate (100 mg/kg bw) for seven days; two other groups were administered artesunate for seven days and after which post treated with the oil at both doses respectively for another seven days; another groups were co-administered artesunate and the oil for seven days. A group was administered artesunate only for seven days, while another was fed chows only. After sacrifice, the testicular homogenates of the rats were analysed for GSH, Superoxide Dismutase (SOD), Catalase (CAT), Lipid peroxidation (LPO), 3β-HSD and 17β-HSD activities. LPO and GSH levels, SOD and CAT activities were significantly ($p < 0.05$) higher in rats administered artesunate only, these were significantly lowered in all treatment groups. Administration of artesunate significantly suppressed steroidogenesis, this was attenuated in all treatment groups. The antioxidant, anti-lipid peroxidative and

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steriodogenetic effects of the oil indicate its protective potential against artesunate-induced oxidative testicular damage.



## 1. Introduction

Malaria has been recognized as a major cause of morbidity and mortality in sub-Saharan Africa. Effective treatment has been a great concern over the years coupled to increasing resistance of the malaria parasite to standard antimalarial agents [1]. Two classes of drugs are currently available for the treatment of malaria: the cinchona alkaloids (quinine) and the artemisinin derivatives (artesunate, artemether and artemotil). Much attention has been shifted to artemisinin and its derivatives, with artesunate being the most common in developing countries [2].

A link has been drawn between the mechanism of action of artemisinins and the generation of reactive oxygen species (ROS) such as hydroxyl, alkoxyl, (protonated) superoxide or peroxyl radicals owing to the presence of a peroxide lactone group in their structure [3]. An imbalance between the generated ROS and the body's antioxidant system results to oxidative stress. Thus, portraying a detrimental effect of artesunate overdose especially during self-medications. Oxidative stress has been implicated in several physiological complications including male infertility. The anti-fertility effect of artesunate in males is well [4] and has been linked to the generated ROS. Peroxidative damage has been recognized as the major cause of impaired testicular function characterized by low sperm count and motility, and down regulation of β-hydroxysteroid dehydrogenase leading to suppressed steroidogenesis [5], with the testicular and spermatozoa lipids being the main substrate [6].

The medicinal properties of spices have been documented in several studies. They have been recognized for their potent antioxidant activities which are functions of their bioactive components [7]. They are major sources of essential oils and unsaturated fatty acids which constitute effective alternatives in nutritional, pharmaceutical, and agricultural fields owing to reported antimicrobial, antiviral, nematicidal, antifungal, insecticidal, and antioxidant properties [8,9]. These spices are often combined when used as major ingredients in food and herbal medicine to give a synergetic effect. Erukainure et al. [10] reported the free radical scavenging potentials and anti-peroxidative activity of essential oil from blend of selected spices.

This study aims at reporting the therapeutic effect of oil from blends of selected culinary spices against artesunate-induced oxidative injury in testicular tissues of albino rats (Wistar strain).

## 2. Materials and methods

### 2.1. Plant material

Selected culinary spices consisting of *Monodora myristica* (African nutmeg), *Myristica fragrans* (nutmeg), *Tetrapleura tetraptera* (Aridan), and *Aframomum sceptrum* (black amomum) were purchased from a local market in Benin City, Nigeria. They were dehulled and air-dried at room temperature. After which, they were blended to fine powder, using a laboratory mill. The powders were mixed in the ratio of 1:1:1:1 and stored in air-tight containers for laboratory analysis.

### 2.2. Oil extraction and analysis

The blended sample was subjected to hexane extraction using soxhlet apparatus. The hexane extract was concentrated with a rotary evaporator and the resulting oil stored in glass vials.

Physicochemical Properties of the oil were carried out according to the AOAC Official Methods of Analysis which covers for saponification, acid, peroxide, and iodine values as well as refractive index and density [11].

Fatty Acid Methyl Esters (FAME) was generated from the oil and subjected to GC–MS analysis as described by Zhao [12] with slight modifications.

### 2.3. Tablets

Artesunate (GVS Laboratories, Dombivli, India) was purchased from a local pharmacy at Ota, Nigeria.

### 2.4. Animals

Forty eight male albino rats of Wistar strain weighing 180–200 g were used for the present investigation. They were reared at the Animal House of Bells University of Technology, Ota, Nigeria. The rats were acclimatized for seven days on normal diet of pelletized mouse chow, and water given *ad libitum* at room temperature with a 12-h light and dark cycle before the commencement of the experiment. The animals used in the present study were maintained in accordance with the approval of the Animal Ethical Committee, Bells University of Technology, Ota, Nigeria. They were divided into eight groups, each consisting of 6 animals:

**Group 1**: Control only (water only)
**Group 2**: Artesunate only 100 mg/kg bw
**Group 3**: Pretreatment with 1.5 mL of oil then post treatment with artesunate 100 mg/kg bw
**Group 4**: Pretreatment with 3 mL of oil then post treatment with artesunate 100 mg/kg bw
**Group 5**: Treatment with artesunate 100 mg/kg bw then post treatment with 1.5 mL oil
**Group 6**: Treatment with artesunate 100 mg/kg bw then post treatment with 3 mL oil
**Group 7**: Co treatment with artesunate (100 mg/kg bw) and 1.5 mL oil
**Group 8**: Co treatment with artesunate (100 mg/kg bw) and 3.0 mL oil

All administrations were done on daily basis. Treatments lasted 7 and 14 days for groups 7–8 and groups 1–6 respectively. At the end of the treatment trials, the rats were fasted overnight and sacrificed by cervical dislocation.

### 2.5. Preparation of tissue homogenates

The testes were removed, washed off excess blood using ice-cold 1.15% KCl solution, blotted dry with filter paper and weighed. The organs were homogenized in phosphate buffer (0.01 mM) and centrifuged at 10,000*g* for 15 min. The supernatant was decanted and stored at −4 °C for subsequent analysis. Each time the supernatant was outside the freezer, it was kept in ice bags.

### 2.6. Determination of oxidative stress parameters

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formed by thiobarbituric acid reaction (TBAR) [13]. Catalase (CAT) activity was estimated by measuring the rate of decomposition of $H_2O_2$ [14]. Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich [15], while the method of Ellman [16] was adopted in estimating the level of reduced glutathione (GSH).

### 2.7. Testicular key androgenic enzyme activities

The testicular 3β-HSD and 17β-HSD activity was determined according to the method described by Talalay [17] with slight modifications.

### 2.8. Statistical analysis

To address the biological variability, each set of experiments was repeated at least three times (n = 3) for physicochemical analysis and six times for experimental rats (n = 6). Differences between the groups were analysed by one-way analysis of variance (ANOVA) with the aid of SPSS software (SPSS Inc., Chicago, IL, USA) standard version 17. The p values of <0.05 were considered statistically significant for differences in mean using the least of significance difference, and data were reported as mean ± standard deviation.

## 3. Results

Physicochemical properties of the oil showed higher values (176.72 ± 25.00 mg KOH/g and 107.87 ± 15.45 g/100 g) for saponification and iodine values respectively as depicted in Table 1. Lower values were observed peroxide value and refractive index, with acid value being the lowest.

GC–MS characterization of fatty acid methyl esters (FAMEs) revealed Dodecanoic acid, 1,2,3-propanetriyl ester to be the predominant constituent (24.00%) as shown in Fig. 1 and Table 2. This was followed by 9-Octadecenoic acid, methyl ester, (E)- (20.79%) and 8,11-Octadecadienoic acid, methyl ester (18.05%) respectively. Other non-fatty acid constituents present included 4,4,4-Trifluoro-2-butanone (0.16%), alpha.-Phellandrene (0.25%), .alpha.-Cubebene (0.35%) and (−)-.beta.-Bourbonene (1.74%) respectively.

Administration of artesunate led to significant ($p < 0.05$) increased level of MDA (group 2) as depicted in Fig. 2. Pretreatment with the oil led to significant ($p < 0.05$) reduction (groups 3 and 4). This was also observed in the post treated and co-treated groups respectively. In all treatment groups, the double dose-treated were observed to be the most effective.

There was an increase in GSH level and antioxidant enzymes on administration of artesunate (group 2), though not significant compared to group 1 as shown in Fig. 3. These were observed to be significantly reduced in all treatment (pre, post and co) groups, with the double dose being the most effective.

Treatment with artesunate led to downregulation of testicular 3β-HSD and 17β-HSD activities as depicted in Fig. 4. These were significantly ($p < 0.05$) revised in all treatment types, with the co-administrative treatment having the activities (groups 7 and 8). Except for the co-administrative treated groups, the double dose treated rats were the most effective.

## 4. Discussion

An imbalance in ROS and testicular antioxidant system has been implicated in most male testicular oxidative injury and has been linked to male infertility [18]. The mechanism of artemisinins has been linked to the generation of ROS. Being the main drug after chloroquine in the treatment of malaria, its overdose is of tremendous fertility health concern especially in most developing countries where self-prescription is rampant owing to unavailability and unaffordability of proper health care. The use of synthetic drug in ameliorating these effects may further complicate it, thus the need of a natural

**Table 1**
Physicochemical properties of extracted oil.

| Parameters | Values |
|---|---|
| Saponification value (mg KOH/g) | 176.72 ± 25.00 |
| Acid value (mg KOH/g) | 0.47 ± 0.02 |
| Peroxide value (meq) | 5.59 ± 0.10 |
| Iodine value (g/100 g) | 107.87 ± 15.45 |
| Refractive index | 1.472 ± 0.05 |
| Density (g/ml) | 0.896 ± 0.07 |

Values = mean ± SD; n = 3.

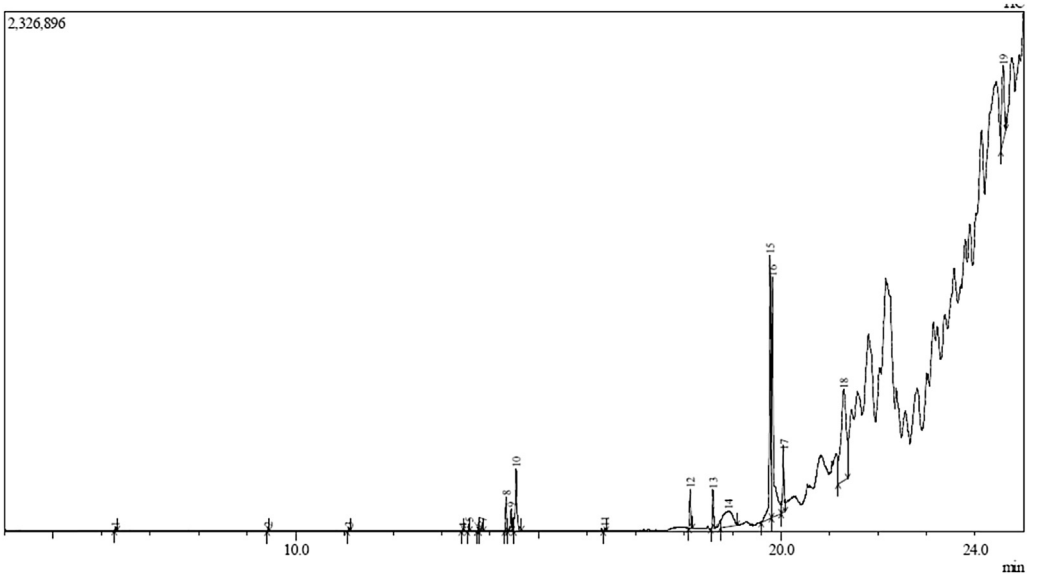


**Fig. 1.** GC–MS chromatogram of fatty acids constituents of oil from selected spice blend.

remedy with little or no side effects. In this study, the ameliorative effect of oil from blend of selected Nigerian indigenous spices on artesunate induced testicular oxidative injury was investigated.

The testis is recognized as potent target of lipid peroxidation owing to the high concentration of polyunsaturated fatty acids [6]. The generated toxic peroxides have been documented to cause impairments of the sperm cells leading to infertility [19]. In the present study, administration of artesunate led to enhanced lipid peroxidation in the testicular tissue (Fig. 2). The observed elevation indicates an occurrence of oxidative stress. The reduced level observed in all the treatment groups indicates an anti-lipid peroxidative effect of the oil against artesunate induced testicular lipid peroxidation. The pretreated groups indicate the chemopreventive potentials of the oil; that of the post-treated, the curative effect of the oil; while the co-treated groups indicate a protective effect of the oil.

GSH is well documented to be the most abundant non-thiol protein in mammalian cells and its deficiency has been implicated in defective motility [20]. Its reduced cellular level is a marker of oxidative stress [6]. However, in this study administration of artesunate led to an increased level. This indicates an alteration in the redox system which can be attributed to the adaptation period and/or excessive release of this non-enzymatic antioxidant to scavenge the increased free radicals induced by artesunate [21]. The observed chemopreventive and curative effect of the oil at single dose indicates a potent restoration of the testicular GSH level.

Increased SOD and CAT activities due to oxidative stress have been reported [21]. Thus, their observed increase in the testes of rats administered only artesunate (group 2) may also be attributed to excessive release to scavenge the increased free radicals and lipid peroxides induced on ingestion of artesunate. The protective role of SOD against superoxide toxicity and lipid peroxidation in spermatozoa has been reported [22]. CAT breaks down $H_2O_2$ into oxygen and water molecules [23]. It also activates nitrous oxide (NO)-induced sperm capacitation [24]. Their significant reduction in all treatments groups corroborates the anti-oxidative stress protective effect of the oil against artesunate-induced oxidative injury in testicular tissues.

3β-HSD and 17β-HSD belong to the short-chain dehydrogenase reductase (SDR) protein superfamily and involved in the biosynthetic pathway of testosterone [25,26]. They also have regulatory function in steroidogenesis. The activities of these enzymes were significantly lower in the testicular tissues of rats administered artesunate only (Fig. 4), indicating an impairment of steroidogenesis [27,28]. This also

**Table 2**
Identified fatty acids constituents of oil from selected spice blend.

| Peak no. | Retention time (min) | %Area | Compounds |
|---|---|---|---|
| 1 | 6.28 | 0.25 | alpha.-Phellandrene |
| 2 | 9.44 | 0.15 | 2-(Phenylacetamido) propionic acid, (dl)- |
| 3 | 11.090 | 0.16 | 4,4,4-Trifluoro-2-butanone |
| 4 | 13.44 | 0.08 | L-Alanine, N-(4-butylbenzoyl)-, hexyl ester |
| 6 | 13.76 | 0.15 | Propanamide, 2-(4-isobutylphenyl)-N-(3,5-dinitrophenyl)- |
| 7 | 13.84 | 0.35 | .alpha.-Cubebene |
| 8 | 14.33 | 1.74 | (−)-.beta.-Bourbonene |
| 9 | 14.54 | 5.16 | 1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene |
| 10 | 16.37 | 0.16 | 3-Acetoxy-2-trifluoromethylbut-3-enoic acid, ethyl ester |
| 11 | 18.13 | 2.15 | Hexadecanoic acid, methyl ester |
| 12 | 18.59 | 2.42 | Pyridine, 5-ethyl-2-phenyl- |
| 13 | 19.78 | 18.05 | 8,11-Octadecadienoic acid, methyl ester |
| 14 | 19.83 | 20.79 | 9-Octadecenoic acid, methyl ester, (E)- |
| 15 | 20.05 | 4.75 | Heptadecanoic acid, 10-methyl-, methyl ester |
| 16 | 21.29 | 24.00 | Dodecanoic acid, 1,2,3-propanetriyl ester |
| 17 | 24.58 | 9.85 | Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester |

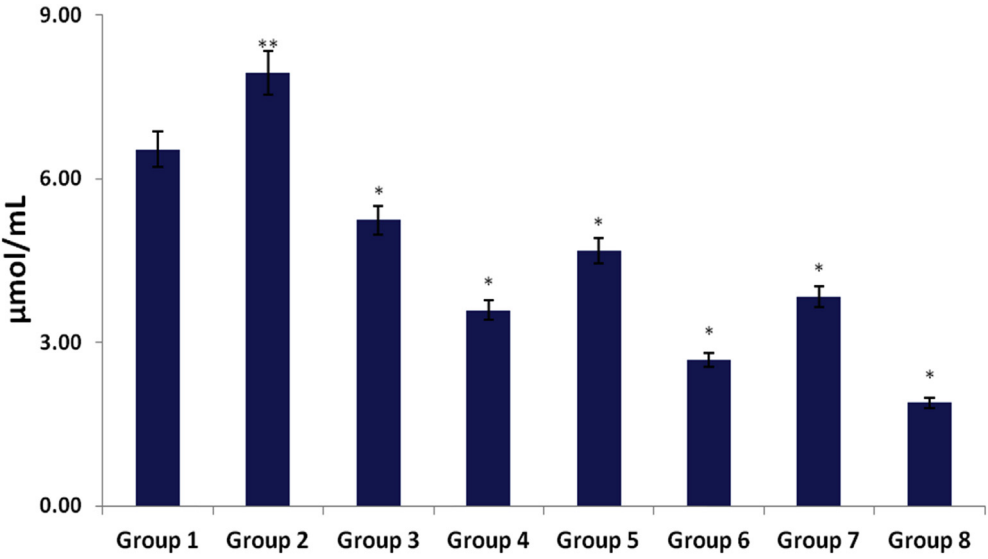


**Fig. 2.** MDA values of experimental groups. Values = mean ± SD; n = 6. *Statistically significant ($p < 0.05$) as compared to group 2; **statistically significant ($p < 0.05$) as compared to group 1.

reflects a decreased androgen production which in turn leads to disruption of spermatogenesis [26]. Oxidative stress in testicular tissues has been implicated in impaired steroidogenesis [5,27,28]. The reduced level of GSH as well as concomitant increase in lipid peroxidation by artesunate, reflects the observed suppressed steroidogenesis. Thus, implying a disruption of spermatogenesis by artesunate-induced oxidative stress. Chemopreventive and curative treatment with oil at double dose significantly increased the activities of 3β-HSD and 17β-HSD. These activities were more pronounced on co-treatment with the oil. The increased activities indicate a reversion of the suppressed steroidogenesis. Thus implying an exacerbative effect on spermatogenesis. This is further corroborated by the observed anti-oxidative effect of the treatments (Figs. 2 and 3).

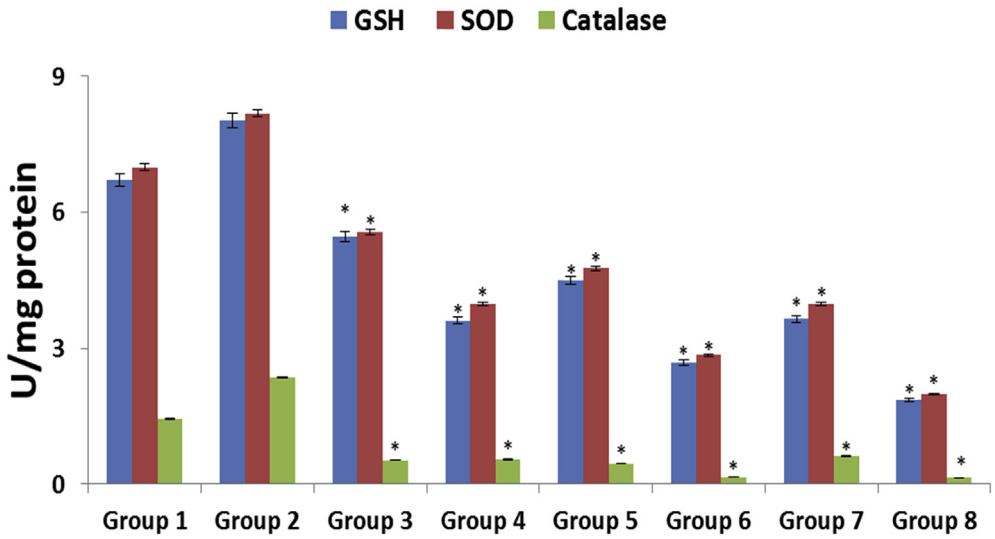


**Fig. 3.** Antioxidant activities of experimental groups. Values = mean + SD; n = 6. *Statistically significant ($p < 0.05$) as compared to group 2.

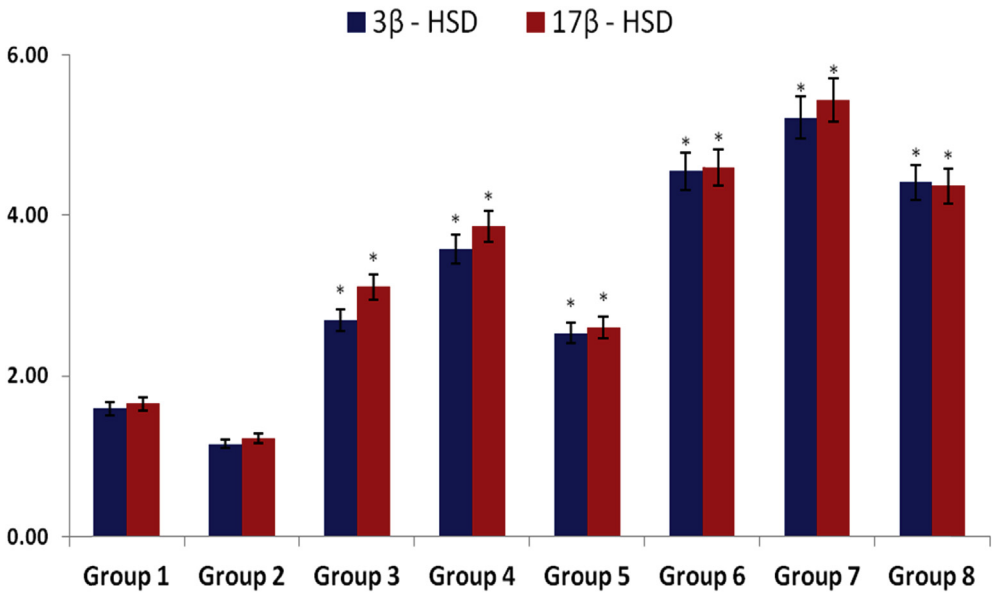


**Fig. 4.** 3β-HSD and 17β-HSD levels of experimental groups. Values = mean ± SD; n = 6. *Statistically significant ($p < 0.05$) as compared to group 2.

The observed antioxidant, anti-lipid peroxidative and steriodogenetic properties of the oil can be attributed to the synergistic effect of the observed high concentration of polyunsaturated fatty acids (PUFA) and trace concentrations of non-fatty acid constituents (Fig. 1 and Table 2). The antioxidant properties of PUFA have been documented in several studies [29,30]. The stimulatory effect of PUFA on steroidogenesis has also been reported [31]. Dietary fatty acid especially PUFA has been reported to

influence steroidogenesis [32]. This corresponds to [33] earlier report of higher activity of testicular 17β-dehydrogenase and plasma androgen concentration in rats fed diets rich in unsaturated fatty acids. Hughes et al. [31] further reported the stimulatory effect of PUFA on steroidogenesis. The affinity of steroid hormone receptors to fatty acids is well documented [33,34]. The non-fatty acids identified in the oil, are major bioactive compounds found in most essential oils. Erukainure et al. [10] reported similar compounds for essential oil extracted from same selected spices blend. The antioxidant protective effects of these compounds have also been documented [10].

In conclusion, the antioxidant, anti-lipid peroxidative and steriodogenetic effects of the oil indicate its protective potential against artesunate-induced oxidative testicular damage. These activities can be attributed to the synergistic effect of the identified PUFAs which were predominant and other non-fatty acid compounds.

## Conflict of interest

None.

## Acknowledgement

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