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Original Research Article

Ameliorative Potential of *Azadirachta indica* and *Gongronema latifolium* on *Plasmodium-induced* Gonadotoxicity in Swiss Mice

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ABSTRACT

Background: Polyherbal decoctions are frequently utilized by some Africans for anti-malaria treatment because they are readily accessible, and assumed low toxicity despite possible gonadotoxicities. We investigated ameliorative potential of anti-plasmodial plants *Azadirachta indica* (AI) and *Gongronema latifolium* (GL) on Plasmodium-induced gonadotoxities.

Materials and methods: Adult male Swiss mice (n = 50) were allotted into 10 groups of (n = 5) animals. All groups were parasitized with 1×10^6 *P. berghei inoculum* for three days except for groups 1 and 10; (normal control [NC]) and (non-parasitized mice administered *Arthemeter-Lumefantrine* [AL]). Group 2 was parasitized non-treated (PNT), groups 3 and 4 received low and high doses AI (86.60 and 259.81 mg), groups 5 and 6 received low and high doses GL (337.27 and 1011.80 mg), Groups 7, and 8 received low doses AI + GL (86.60 + 337.27 mg), and high doses AI + GL (259.81 + 1011.80 mg), while Group 9 received AL (8 mg), all odes via oral gavage per kg body weights, respectively. Rectal temperature, seminal/hormonal, testicular morphometry and histology assessments were evaluated using standard protocols.

Results: Concurrent administration of AI and GL alleviated pyrexia and parasitemia in test groups, compared to NC and PNT groups. Testosterone concentrations were reduced in parasitized groups compared to NC, while sperm parameters significantly decreased ($p < 0.05$) in PNT and high AI + GL extract groups, but improved in combined low dose with testicular histoarchitecture. Conclusions: AI and GL concurrently administered at low doses mitigate *P. berghei*-induced gonadotoxicity better than as singly.

KEYWORDS: Anti-Malaria, *Azadirachta Indica, Gongronema Latifolium*, Gonadotoxicity, Histology.

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INTRODUCTION

Malaria, a disease caused by protozoa parasite in the genus Plasmodium; is the most significant parasitic ailment that impacts both humans and animals. The malady is propagated by female Anopheles mosquitoes and prevails in region such as in Southern Asia, sub-Sahara Africa, and Northern Brazil [1].

P. falciparum, P. ovale, P. malariae and P. vivax although morphologically distinct, are the principal species that can infect humans [2].

P. vivax and P. falciparium are the most dominant species globally with the former causing the most serious and life-threatening condition to humans [3]. The World Health Organization currently estimates 241 million malaria cases globally and about 627 000 malaria-associated mortalities [1].

The emergence of drug-resistant malaria in new regions and its resurgence in previously controlled area pose significant challenges to eradicating the disease. [4]. However, very good anti-malarial activities on the various parasite strains have been achieved by the co-administration of plant-based concoctions when compared with orthodox drugs [5].

Approximately 80% of the global populations relies on herbal remedies as the principal source of medicinal treatments for various illnesses [6]. Notably, certain anti-malarial medications like *quinine and artemisinin,* have either been derived from plants or constructed based on their chemical compositions [7]. *Azadirachta indica* and *Gongronema latifolium* are two plants that have found usage in the management of malaria.

Azadirachta indica (Neem), locally called ''Dogonyaro'' in Nigeria, is a large evergreen tree with a wide trunk, which belongs to the family Meliaceae [8]. The leaves of "Neem" have been reported to completely cure experimental malaria at 600 mg in two days, which is quite a high dose [9].

The wonder plant, *Azadirachta indica*, possesses anti-malarial, anti-microbial, anti-bacterial [6,8], anti- oxidant, anti-inflammatory [10], antipyretic and anti-parasitic properties [11].

Nevertheless, Las et al. [12] had reported on the contraceptive effects of Neem oil which included a reduction in sperm motility. In addition, Awasthy [13] and Raji et al. [14] reported that the consumption of Neem reduces serum testosterone and luteinizing hormone levels (anti-fertility effects). Male mice fed with crushed fresh Neem leaves were reported to impregnate fewer mice and had smaller average litter sizes [15].

According to Santra and Manna [16], the process of spermatogenesis was adversely affected by the administration of Neem leaf extract.

Gongronema latifolium has both medicinal and nutritional values, is a climbing perennial shrub with broad and heart-shaped leaves of the family *Asclepiadaceae.* It is commonly called amaranth globe or bush buck [17].

Several reports have indicated that *G.latifolium* exhibits several herbal actions which include; anti-malarial, analgesic, anti-tumor, antimicrobial, antipyretic, antioxidant, anti-inflammatory, anti-ulcer, anti-asthmatic, mild expectorant, hypoglycemic, hypolipodemic, hepatoprotective, digestive tonic and laxative properties [18,19,20].

Phytochemical evaluation of the plant shows it to be rich in essential oils, saponins and pregnanes, alkaloids, tannins, phytates, flavoniods, and oligosaccharides [21, 22]. Akpaso et al. [23] reported that there were increases in sperm cell concentration in animals treated with G. latifolium. Dasofunjo et al. [24] reported also that the plant extract can be used in the management of erectile-related dysfunction since it enhances sexual health and libido in male Wistar rats.

Therefore, this study investigated whether *Azadirachta indica* which is an effective plasmocidal agent but with some reported gonadotoxicity, when co-administered with *Gongronema latifolia* a known sperm and libido-booster, can ameliorate a Plasmodium-induced male gonado-toxicity in an experimental animal model.

MATERIALS AND METHODS

Procurement and Preparation of *Azadirachta indica* and *Gongronema latifolium* Leaf Extracts: Fresh leaves of *A. indica* and *G. latifolium* were obtained from the medicinal plant farm of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria. The plants were identified and authenticated by the principal curator at the Pharmacognosy Herbarium of the Faculty of

Pharmacy. Specimen voucher numbers UUPH49 (a) and UUPH9 (a) were issued for *A. indica* and *G. latifolium* respectively. The leaves were washed to remove dirt and dried under a shade for three weeks. The dried leaves were macerated separately to obtain a fine powder. They were preserved in separate airtight containers. One hundred and fifty gram (150 g) of A. indica powered leaf was mixed with 1700 mL of 70 % ethanol, while 94 g of G. latifolium was mixed with 1050 mL of 70 % ethanol for 72hr at room temperature. The extracts were filtered with cheese cloth and later with filter paper. The filtrate was allowed to stand for 30 minutes, and thereafter evaporated to dryness using water bath at 40 æ%C. The extracts were then stored in a closed container and kept in the refrigerator until use.

Animal Care and Use: A total of 50 male Swiss mice weighing 18 – 31 g were acquired from the Animal House of Faculty of Basic Medical Sciences, University of Uyo. The animals were housed in clean well ventilated plastic cages and exposed to 12 hours light and dark cycles at room temperature. They were fed ad libitum with normal commercial pellet and clean water throughout the duration of the experiment. The animals were handled according to Guide for the Care and Use of Laboratory Animals [25]. They were allowed to acclimatize for 2 weeks before commencement of the experiment.

Ethical Approval: Ethical clearance was obtained from the State Health Research Ethics Committee of the Akwa Ibom State Ministry of Health (Nigeria), with reference number MH/PRS/99/VOL.V/625.

Determination of Median Lethal Dose: The median lethal dose (LD_{50}) of leaf extracts of *Azadirahta indica* and *Gongronema latifolium* was assessed in the Department of Pharmacology, Faculty of Pharmacy, University of Uyo. The dosage of the extracts to be administered to the mice was determined using the method of Lorke [33]. Fifteen (15) animals were divided into five groups of three animals each which were administered leaf extracts 1000, 1500, 2000, 2,500 and 3000 mg/kg body weights, respectively. The animals were closely monitored for 24 hours to assess their behavior and potential mortality. Where there was no mortality, animals were administered 3500 and 4000 mg/kg body weights, respectively with 24 hours observation for behavior changes and mortality.

Phytochemical Screening of Leaf Extracts: Standard qualitative methods, in accordance with the protocols outlined by Trease and Evans [18] and Sofowora [16], were employed to conduct phytochemical screening for major constituents. The ethanol extracts of *A. indica and G. latifolium* leaves underwent a thorough phytochemical screening to ascertain the presence of alkaloids, cardiac glucosides, flavonoids, phenols, saponins, tannins, terpenes, and steroids. The screening process adhered to established phytochemical methodologies.

Procurement of Malaria Parasite and Inoculum Preparation: Plasmodium berghei blood-stage samples were procured from a laboratory-maintained stock through successive blood passages between mice at the Faculty of Pharmacy, University of Uyo. The procedure for preparing the inoculum followed the approach outlined by Ubulum et al. [26]. A blood sample was extracted from a donor mouse afflicted with parasitic infection using cardiac puncture, and the collected sample was stored in a sterile heparinized container. The inoculum was formulated by diluting 2 mL of parasitized blood with 10 mL of sterile normal saline, culminating in a final inoculum volume of 0.2 mL (1.0×10^6 parasites), which represents the established standard for infecting a single mouse.

Infection of Mice: The infection process of the recipient mice commenced by transferring the prepared parasite inoculum from the donor to healthy test animals using a needle, administered through the intraperitoneal route, following the method outlined by Udoh and Peter [5]. The infection's confirmation was established through a daily assessment of parasitemia, achieved by examining Giemsa-stained thin blood smears extracted from the tail blood of the mice.

Determination of Parasitaemia and Calculation of Parasite Density: The percentage of parasitaemia was determined using the

method of WHO [27]. Thin smears were prepared on glass slide by collecting blood from cut-tip of the tail of the mice, before drug administration and after drug administration. Three percent (3%) stain was prepared from a Giemsa stock solution as outlined by WHO [27] and used to stain the slides. The slides were viewed under the light microscope under oil immersion at × 100 magnification. The parasite density was calculated using the formula:

% parasitaemia =

$$\frac{\text{Number of parasitized RBC}}{\text{Total number of RBC}} \times 100$$

where RBC = red blood cells

Also, the percentage inhibition of the parasite for each group was calculated by the formula:

$$\frac{\text{mean \% parasitemia of untreated group} - \text{mean \% parasitaemia of treated group}}{\text{mean \% parasitaemia of untreated group}} \times 100$$

Treatment began for the experimental groups when the parasitaemia reached 5 % of the initial inoculation. Parasitaemia was recorded after every other day for each mouse.

Experimental Design: Ten experimental groups of five Swiss male mice were used for the experiment. After acclimatization for two weeks, all the groups were inoculated with *Plasmodium berghei* except normal control and standard anti-malarial groups. All animals were provided with normal feed and clean water daily. The administration of treatment was done orally using hypodermic syringe attached to an oral cannula for three days according the standard drug regimen for malaria treatment in humans. The animal grouping and treatment schedules are shown below:

Serial No.	Group	Description	Treatment per kg body weight	Duration (days)
1	NC	Normal control	10 mL distilled H_2O	3
2	PNT	Parasitized & not treated with drug / extract	10 mL dist. H_2O	3
3	PAI_{LD}	Parasitized animals treated with low dose *A. indica* leaf extract	86.6 mg	3
4	PAI_{HD}	Parasitized animals treated with high dose *A. indica* extract	259.8 mg	3
5	PGL_{LD}	Parasitized animals treated with low dose *G. latifolium* leaf extract	337.3 mg	3
6	PGL_{HD}	Parasitized animals treated with high dose *G. latifolium* leaf extract	1011.9 mg	3
7	$PAIGL_{LD}$	Parasitized animals treated with combined low doses *A. indica* + *G. latifolium* leaf extract	86.6 + 337.3 mg, respectively	3
8	$PAIGL_{HD}$	Parasitized animals treated with combined high doses *A. indica* + *G. latifolium* leaf extract	259.8 + 1011 mg, respectively	3
9	PAL	Parasitized animals treated with Arthemeter-Lumefantrine (AL)	8 mg	3
10	AL	Non-parasitized animals treated with Arthemeter-Lumefantrine (AL)	8 mg	3

Legend:
NC - Normal control;
PNT - Parasitized none treated;
PAI_{LD} - Parasitized mice treated with 86.6 mg of *A. indica*;
PAI_{HD} - Parasitized mice treated with 259.8mg of *A. indica;*
PGL_{LD} – Parasited mice treated with 337.3 mg of *G. latifolium*;
PGL_{HD} – Parasitized mice treated with 1011.9 mg of *G. latifolium*;
$PAIGL_{LD}$ – Parasitized mice treated with 86.6 mg of *A. indica* and 337.3 mg of *G. latifolium;* $PAIGL_{HD}$ - Parasitized mice treated with 259.8 mg of *A. indica* and 1011.9 mg of *G. latifolium*;
PAL – Parasitized mice treated with 8mg Arthemeter-Lumefantrine
AL – Mice administered 8 mg Arthemeter-Lumefantrine per kg body weights, respectively.

Determination of Weight, Rectal Temperature and Physical Activity:

The weights of the mice were recorded daily using an electronic weighing balance (Zeiss, West Germany (Pty) Ltd; 0.000 g). Rectal temperature were measured by inserting an electronic rectal thermometer 2 cm deep gently through the anal orifice at specific intervals throughout the period of drug administration. Physical activities were monitored and clinical experimental cerebral malaria (ECM) scores determined, as defined by the following signs: ruffled fur, hunching, wobbly gait, limb paralysis, convulsion and coma. Each sign was ascribed a score of 1, and scores was determined daily. Animals with severe ECM (cumulative score of 4) were sacrificed [5].

Sacrifice of Experimental Animals: At the expiration of the treatment, 24 hours after the last dosage, the animals were anaesthetized using 50 mg ketamine hydrochloride (Rotex Medica, Germany) per kg body weights intraperitoneally, respectively. Thoraco-abdominal walls of each mouse were dissected to assess the heart, and blood was aspirated from the left ventricle of the heart. The heparinized blood was collected immediately and centrifuged 3000 rpm for 10 minutes. Obtained plasma were refrigerated at 4 ^{o}C and subsequently assayed for sera testosterone and FSH concentrations. The testes were excised and fixed in Bouin's fluid for 6 hours before transfer to alcohol for 24 hours then processed for light microscopy.

Gross Morphometry

Testicular weight: Testes were excised from the animal, and blood connective tissue and blood were cleaned off. The cleaned testis was placed on a weighing balance and measured. Weight of both testes was recorded as average weights. The relative testis weight was obtained by using the formula: (organ weight)/(final body weight) x 100.

Testicular length: The cleaned testis was placed in a Petri dish. Jaws of the digital Vernier caliper were extended to touch both pools of each testis (right and left) at the equatorial pool. Values were observed and recorded.

Testicular width: Cleaned testis was placed in a Petri dish. Jaws of the Digital Vernier caliper were extended to touch the circumference of each testis (right and left) at the equatorial pool. Values were observed and recorded.

Sperm Morphometry Procedures

Estimation of Sperm motility: Cauda (tail region) of epididymis was incised to expose fluid. 5 µL of the epididymal fluid was collected with a micro pipette**.** Fluid was delivered onto a glass slide and covered with a 22 x 22 mm cover slip. Microscope glass slide was taken to light microscope with magnification of × 400. Motility estimation was carried out at room temperature (24 - 28 ºC). Microscopic field was scanned systematically, and each spermatozoon encountered was assessed. Motility was recorded in percentage and classified as progressively motile, non - progressively motile and non-motile. Procedure was repeated and averages taken.

Estimation of Sperm Cell Count and Concentration: The extracted epididymis was carefully sectioned using anatomical scissors and then finely minced within a Petri dish. A volume of 50 µL of epididymal spermatozoa was diluted with 950 µl of physiological saline. The well-mixed solution was pipetted into both chambers of a hemocytometer. The hemocytometer was placed onto the microscope's stage, and the microscope's objective was adjusted to a magnification of x40. The hemocytometer was observed, and the process of counting was carried out. The recorded counts were obtained after repetition in each chamber, and an average count was computed. The average number of cells, cell density, dilution factor, and cell concentration were determined by applying the formula:

Average number of cells

$$= \frac{\text{sum of cells in each square}}{\text{Number of squares}}$$

$$\text{Cell density} = \frac{\text{Average number of cells} \times \text{Dilution factor}}{\text{Volume of a square}}$$

Volume of a square = 10^{-4}mL (0.0001mL)

$$\text{Dilution factor} = \frac{\text{Final volume}}{\text{Volume of cell}}$$

Concentration (viable cells/mL) = Average number of cell/square x Dilution Factor x 10^4

Sperm Morphology Procedure: A thin layer of semen was applied to a microscope glass slide to create sections depicting the head, midpiece, and tail of the sperm. The slide was fixed using 75% alcohol and left to air dry for a minute. Subsequently, the slide was rinsed with slow-running tap water and allowed to air dry. The staining process was carried out by flooding the slide with a 1:20 dilution of carbol fuchsin for approximately 3 minutes. Afterward, the slide was again rinsed under slow-running tap water and subjected to counter-staining using methylene blue for a duration of five minutes. Following counter-staining, the slide was once more rinsed under slow-running tap water and left to air dry. Under a light microscope (employing oil immersion), the slide was observed to detect abnormalities in sperm morphology. These abnormalities encompassed head and mid-piece defects (e.g., microcephalus, detached head, flattened head, doubled head, and bent neck) as well as tail defects (e.g., coiled tail, bent tail, and doubled tail).

Histopathological Assessments: Fixed testicular specimens were dehydrated through upgraded ethanol series, embedded in paraffin wax, sectioned at thickness of 5 ìm, placed on slides and stained with hematoxylin and eosin (H&E) [28]. Demonstrable observations were made, and photomicrographs were taken with the light microscope (Olympus - CX31, Japan). Images were captured with Amscope digital camera (MU 1000, China) attached to the light microscope, and were assessed by three independent histopathologists unassociated with the study to avoid bias.

Determination of concentration of testosterone in serum: The serum testosterone concentrations were measured using Monobind Inc (California, USA) based assay kits with ELISA method of Tietz [29]. Values were expressed in ng/mL.

Determination of concentration of Follicle Stimulating Hormone: The serum FSH concentrations were measured using Monobind Inc (California, USA) based assay kits with ELISA method of Saxana et al. [30]. Values were expressed in ng/mL.

Statistical Analysis: Data obtained from this study were expressed using GraphPad prism 6.0 statistical software, Results were expressed as Mean ± SEM. Significant difference among groups were determined using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test, with the significance level of $p < 0.05$.

RESULTS

Median Lethal Dose of Azadirachta indica and Gongronema latifolium Ethanol Leaf Extracts: The results for the median lethal doses of the ethanol leaf extracts of 2 experimental plants used in this study are presented in Table 1.

With *A. indica*, no sign of toxicity or mortality was observed with increasing doses up to 750 mg/kg body weight (bw). The median lethal dose of the ethanol leaf extracts of *A. indica* was calculated to be over 866 mg/kg bw. No death was recorded with increasing doses of *G. latifolium* leaf extract up to 3250 mg/kg bw. Also, no adverse effect on the animal behavioural and physical appearance was recorded with the above dose. The median lethal dose of the ethanol leaf extract of *G. latifolium* was calculated to be over 3373 mg/kg bw.

Phytochemical Analysis of *A. indica* and *G. latifolium* Ethanol Leaf Extracts: Phytochemial screening of the leaf extracts of both plants showed they were rich in alkaloids, tannins, saponins, carbohydrates, flavonoids, polyphenols, balsams, glycosides, phytosteriods, cardiac glycosides, and terpeniods. Steroids were absent in *G. latifolium* as shown in Table 2.

Effect of *Azadirachta indica* and *Gongronema latifolium* Leaf Extracts on Total Body Weight, Testicular Weight and Morphometry: There were body weight gains (g) for animals in normal control NC (0.36), parasitized untreated PNT (0.12), Artemether-Lumefantrine AL-administered and (non-parasitized AL/ parasitized PAL) (0.22 /1.12 respectively). Low dose single therapy extract-treated groups (PAI_{LD} and PGL_{LD}) had 0.56 and 0.16, respectively. However, the following single therapy high dos, and combined extract-treated (low and high dose) groups had weight losses (g): PAI_{HD} (-1.22), PGL_{HD} (-2.26), $PAIGL_{LD}$ and $PAIGL_{HD}$ (-1.96 and -1.36, respectively).

Table 1: Median Lethal Doses of Azadirachta indica and Gongronema latifolium Ethanol Leaf Extracts.

Group (n = 3)	Experimental Plant			
	Azadirachta indica		*Gongronema latifolium*	
	Dose of leaf extract mg/kg body weight (bw)	Mice mortality	Dose of leaf extract (mg/kg bw)	Mice mortality
1	250	None	1000	None
2	500	None	2000	None
3	750	None	2500	None
4	1000	1	3000	None
5	-	-	3250	None

*LD_{50} is over 750 mg/kg for A. indica; while for G. latifolium, LD_{50} is over 3250 mg/kg bw.

Table 2: Phytochemical screening for ethanol leaf extracts of Azadirachta indica and Gongronema latifolium

Phytochemical constituents	Plant Leaf Extract	
	Azadirachta indica	*Gongronema latifolium*
Alkaloids:		
(a) Dragendoff Reagent	+++	++
(b) Maeyer's Reagent	+++	++
Tannins	+++	+
Saponins	++	+++
Carbohydrates	++	++
Flavonoids	+++	++
Polyphenols	+++	+++
Anthraquinones	-	-
Balsams	+++	+
Glycosides	++	+
Cardiac glycosides	++	++
Phytosteroids	+++	+++
Steroids	+	-
Terpenoids	+++	+++

Legend: + = Mildly Present, ++ = Moderately Present, +++ = Abundantly present; - = Absent

Table 3: Effect of Azadirachta indica and Gongronema latifolium Leaf Extracts on Total Body Weight, Testicular Weight and Morphometry.

Group	Total Body Weight (g)				Testicular Weight (g)	Testicular Width and & Length (mm)	
	Initial	Final	Weight change	Absolute	Relative g/100g body weight	Width	Length
NC	29.02±2.03	29.38±2.86	0.36±0.30	0.38±0.17	1.29±0.66	4.40±0.15	7.32±0.20
PNT	25.10±0.52	25.22±0.73	0.12±0.24	0.50±0.17	1.98±0.72	$4.72±0.09^{fg}$	7.43±0.22
PAI_{LD}	28.68±0.78	29.24±0.73	0.56±1.02	0.41±0.18	1.40±0.64	4.56±0.09	6.92±0.20
PAI_{HD}	26.10±1.98	24.88±2.03	-1.22±0.67	0.65±0.14	2.61±0.62	$4.80±0.09^{fg}$	7.00±0.33
PGL_{LD}	22.58 ± 0.95	22.74 ± 1.17	0.16±0.69	0.42 ± 0.20	1.85 ± 1.02	4.42 ± 0.11	6.74 ± 0.12
PGL_{HD}	23.58 ± 2.14	21.58 ± 0.36	$-2.26 ± 0.30^{ac}$	0.52 ± 0.17	2.41 ± 0.83	4.26 ± 0.06	6.72 ± 0.12
$PAIGL_{LD}$	23.64 ± 0.49	21.68 ± 0.67	$-1.96 ± 0.66^{c}$	0.64 ± 0.14	2.95 ± 0.67	4.28 ± 0.15	6.92 ± 0.29
$PAIGL_{HD}$	27.30 ± 1.81	25.94 ± 1.16	-1.36 ± 0.46	0.52 ± 0.17	2.01 ± 0.73	4.58 ± 0.06	6.92 ± 0.29
PAL	26.70 ± 1.08	25.58 ± 0.95	-1.12 ± 0.46	0.42 ± 0.19	1.64 ± 0.77	$4.74 ± 0.07^{fg}$	7.44 ± 0.14
AL	26.04 ± 1.30	26.26 ± 1.39	0.22 ± 0.20	0.11 ± 0.01	0.42 ± 0.04	4.42 ± 0.07	7.38 ± 0.07
P value	0.075	0.012	0.002	0.548	0.372	0.002	0.152

Data are presented in Mean ± Standard Error of Mean. Minus sign (-) indicates weight loss.
a = p < 0.05 relative to NC; c = p < 0.05 relative to PAI_{LD}; f = p < 0.05 relative to $PGL_{HD;}$ g = P < 0.05 relative to $PAIGL_{LD.}$

Legend:
NC = Normal control administered distilled water 10 mL/kg
PNT = Parasitized mice administered distilled water 10 mL/kg
PAI_{LD} = Parasitized mice treated with low dose *A. indica* extract (86.60 mg kg^{-1}bw)
PAI_{HD} = Parasitized mice treated with high dose *A. indica* extract (259.81 mg kg^{-1}bw)
PGL_{LD} = Parasitized mice treated with low dose *G. latifolium* extract (337.27mg kg^{-1}bw)
PGL_{HD} = Parasitized mice treated with high dose *G. latifolium* extract (1011.80 mg kg^{-1}bw)
$PAIGL_{LD}$ = Parasitized mice given combined low dose *A. indica + G. latifolium* extracts $PAIGL_{HD}$ = Parasitized mice given combined high dose *A. indica + G. latifolium* extracts
PAL = Parasitized mice treated with Artemether-Lumefantrine (8 mg kg^{-1}bw)
AL = Non-parasitized mice administered Artemether-Lumefantrine (8 mg kg^{-1}bw)

There were significant changes ($p < 0.002$) in body weights of $PAIGL_{HD}$, $PAIGL_{LD}$, PGL_{HD} and PAI_{HD} when compared with NC group, and a significant decrease in the body weights (g) of PGL_{HD} (-4.02) when compared with PAI_{HD} (-1.22), $PAIGL_{LD}$ (-1.96) and $PAIGL_{HD}$ groups (-1.36).

There were no significant differences ($p > 0.05$) in the testicular parameters assessed (relative/ absolute weights, lengths and widths) between the controls and other experimental groups as shown in Table 3.

Effect of *A. indica* and *G. latifolium* Leaf Extracts on Rectal Temperature (°C) and Parasitemia (%): Rectal temperature of animals prior to experiment commencement was 36.56 - 36.58 °C. Animals rectal temperatures in groups NC and AL remained normal (35. 74 – 36.24 °C) throughout the course of the experiment.

There was a progressive increase in temperature (37.04 – 37.58°C) in the parasitizedgroups; PNT, PAI, PGL, PAIGL and PAL, respectively following the inoculation of *P. berghei* as measured on day 3 of post-infection. There was abridgment of temperature after administration of the extracts and drugs, with the temperature normalizing (36.12 - 37.16 °C) by day 5 post-infection. Normalcy of rectal temperatures were observed in all drug-treated groups by day 7 post-infection (36.08 - 36.46 °C). The temperature of animals in the PNT group which received no treatment (37.8 °C), remained high till the expiration of the experiment as shown in Table 4.

Table 4: Effect of Azadirachta indica and Gongronema latifolium leaf extracts on rectal temperature (°C) and Parasitemia (%).

Experimental Group	Rectal Temperature (°C)				Parasitemia (%)	
	Post Infection Days				Initial	Final
	0 (Baseline)	3	5	7		
NC	36.10 ± 0.14	36.00 ± 0.20	35.74 ± 0.42	36.16 ± 0.25^{b}	0.00 ± 0.00	0.00 ± 0.00^{f}
PNT	36.18 ± 0.20	37.04 ± 0.16^{ac}	37.74 ± 0.13	37.80 ± 0.25	47.00 ± 1.22	63.60 ± 2.23^{x}
PAI_{LD}	35.86 ± 0.19	37.34 ± 0.14^{ac}	36.88 ± 0.28	36.08 ± 1.01^{b}	47.60 ± 1.12	34.60 ± 1.63^{d}
PAI_{HD}	36.58 ± 0.21	37.50 ± 0.30^{ac}	36.58 ± 0.46	36.26 ± 0.51^{b}	46.20 ± 1.24	33.40 ± 1.66^{d}
PGL_{LD}	36.46 ± 0.18	37.58 ± 0.20^{ac}	37.14 ± 0.32	36.46 ± 0.50^{b}	47.20 ± 1.39	35.00 ± 2.05^{d}
PGL_{HD}	36.34 ± 0.05	37.52 ± 0.30^{ac}	37.16 ± 0.38	36.32 ± 0.46^{b}	48.20 ± 0.92	31.00 ± 0.49^{d}
$PAIGL_{LD}$	35.56 ± 0.37	36.94 ± 0.22^{ac}	36.12 ± 0.34	36.08 ± 0.51^{b}	46.60 ± 1.03	27.40 ± 1.29^{d}
$PAIGL_{HD}$	35.90 ± 0.09	37.16 ± 0.42^{ac}	36.52 ± 0.54	36.42 ± 0.23^{b}	47.20 ± 0.97	25.60 ± 1.12^{d}
PAL	36.26 ± 0.11	37.26 ± 0.20^{ac}	37.02 ± 0.20	36.18 ± 0.32^{b}	47.60 ± 1.12	7.60 ± 1.12^{e}
AL	36.24 ± 0.22	36.02 ± 0.33	36.12 ± 0.40	36.06 ± 0.24^{b}	0.00 ± 0.00	0.00 ± 0.00^{f}
P value	0.026	0.0001	0.038	0.022	0.948	0.0001

Data are presented as Mean ± Standard Error of Mean

a = $p < 0.05$ relative to NC; b = $p < 0.05$ relative to PNT; c = $p < 0.05$ relative to AL

d = Significantly different from Groups PNT, NC, PAL and AL at $p < 0.01$

x = Significantly different from Groups NC, AL, PAL, PAI PGL and PAIGL at $p < 0.01$

NC = Normal control administered distilled water 10 mL/kg

PNT = Parasitized mice administered distilled water 10 mL/kg

PAI_{LD} = Parasitized mice treated with low dose *A. indica* extract (86.60 mg kg^{-1}bw)

PAI_{HD} = Parasitized mice treated with high dose *A. indica* extract (259.81 mg kg^{-1}bw)

PGL_{LD} = Parasitized mice treated with low dose *G. latifolium* extract (337.27mg kg^{-1}bw)

PGL_{HD} = Parasitized mice treated with high dose *G. latifolium* extract (1011.80 mg kg^{-1}bw)

$PAIGL_{LD}$ = Parasitized mice administered combined low dose *A. indica + G. latifolium* extracts $PAIGL_{HD}$ = Parasitized mice administered high dose *A. indica + G. latifolium* extracts

PAL = Parasitized mice treated with Artemether-Lumefantrine (8 mg kg^{-1}bw)

AL = Non-parasitized mice administered Artemether-Lumefantrine (8 mg kg^{-1}bw)

All treatment groups had significant reduction in percentage parasitemia level ($p < 0.01$), when compared with the PNT group. There was also a significant decrease in percentage parasitemia in PAL group compared to the extracts administered groups, as presented in Table 4.

Effect of *Azadirachta indica* and *Gongronema latifolium* Ethanolic Leaf Extracts on Sperm Count, Motility and Morphology: The effects of the plant extracts on sperm motility and morphology are presented as shown in Table 5. The sperm counts ($\times 10^6$/L) of the PNT group (10.4) was decreased when compared to NC (42.20) and other experimental groups (14.80 -31.40). There was a statistically significant decrease in the sperm count of PNT groupcompared to NC, PAI_{HD}, $PAIGL_{LD}$, and AL. There was also a significant decrease ($p < 0.05$) in the sperm count ($\times 10^6$/L) of $PGL_{LD,}$ $PAIGL_{HD}$ and PAL groups (14.80 – 17.60) compared to NC group**.**

There were no significant variations ($p > 0.05$) between the motile sperms (%) of NC, $PGL_{LD,}$ $PAIGL_{LD}$ and $PAIGL_{HD}$ groups (55 – 60 %). There was lower sperm motility of PAL, AL and PNT groups (17.00 – 32.00 %) when compared with the NC group, which had 58.00 %. Also, there the sperms of the PNT group (17 % motile) exhibited significantly lower motility ($p < 0.05$) than the NC, PAI_{HD} (60.00 %), PGL_{LD} (46.00 %) $PAIGL_{LD}$ (60.00 %) and $PAIGL_{HD}$ groups (58.00 %) as presented in Table 5.

Table 5: Effects of Azadirachta indica and Gongronema latifolium ethanolic leaf extracts on sperm count, motility and morphology.

Group	Sperm concentration (x 106/mL)	Motility (%)				Morphology	
		Motile	Immotile	Progressive	Non- progressive	% Normal	% Abnormal
NC	42.20 ± 10.93	58.00 ± 1.22[b]	42.00 ± 1.22[b]	75.00 ± 14.05	25.00 ±14.05[i]	90.00 ± 00.00	10.00 ± 00.00
PNT	10.40± 2.27[a]	17.00 ± 5.61	83.00 ± 5.61	43.00 ± 11.58	37.00 ± 10.20[i]	50.00 ± 04.47[c]	50.00 ± 04.47
PAI_{LD}	15.00 ± 2.43[a]	46.00 ± 11.34	8.00 ± 11.02	43.00 ± 16.91	56.00 ± 16.91	92.60 ± 01.12	07.04 ± 01.12
PAI_{HD}	21.20 ± 2.63[a]	60.00 ± 4.47[b]	49.00 ± 12.08	27.00 ± 8.60	73.00 ± 8.60	54.00 ± 09.80	46.00 ± 09.80
PGL_{LD}	15.00 ±2.72[a]	55.00 ± 9.22[b]	52.00 ± 10.07	34.00 ± 15.12	66.00 ± 15.12	56.00 ± 10.89	40.00 ± 10.12
PGL_{HD}	19.20 ± 3.65[a]	44.00 ± 5.34	56.00 ± 5.34	42.00 ± 3.74	58.00 ± 3.74	54.00 ± 09.80	50.00 ± 06.32
$PAIGL_{LD}$	31.40 ± 4.72	60.00 ± 5.24[b]	40.00 ± 5.24[b]	60.00 ± 5.48	40.00 ± 5.48[i]	53.00 ± 10.71	47.00 ± 10.79
$PAIGL_{HD}$	14.80± 3.01[a]	58.00 ± 13.19[b]	42.00 ± 13.19[b]	43.00 ± 15.30	57.00 ± 15.30	63.00 ± 12.31	37.00 ± 12.31
PAL	17.60 ± 2.58[a]	24.00 ± 5.10	75.00 ± 5.00	7.00 ± 1.22[a]	93.00 ± 1.22	41.00 ± 06.00	59.00 ± 06.78
AL	20.60 ± 4.45[a]	32.00 ± 10.20	66.00 ± 10.30	30.00 ± 13.04	70.00 ± 13.04	63.00 ± 12.31	44.00 ± 11.22
P-value	0.0001	0.0001	0.009	0.024	0.009	0.002	0

Data are presented in Mean ± Standard Error of Mean
a = $p < 0.05$ relative to NC; b = $p < 0.05$ relative to PNT; i = $p < 0.05$ relative to PAL
NC = Normal control administered distilled water 10 mL/kg
PNT = Parasitized mice administered distilled water 10 mL/kg
PAI_{LD} = Parasitized mice treated with low dose *A. indica* extract (86.60 mg kg^{-1}bw)
PAI_{HD} = Parasitized mice treated with high dose *A. indica* extract (259.81 mg kg^{-1}bw)
PGL_{LD} = Parasitized mice treated with low dose *G. latifolium* extract (337.27mg kg^{-1}bw)
PGL_{HD} = Parasitized mice treated with high dose *G. latifolium* extract (1011.80 mg kg^{-1}bw)
$PAIGL_{LD}$ = Parasitized mice administered combined low dose *A. indica + G. latifolium* extracts $PAIGL_{HD}$ = Parasitized mice administered high dose *A. indica + G. latifolium* extracts
PAL = Parasitized mice treated with Artemether-Lumefantrine (8 mg kg^{-1}bw)
AL = Non-parasitized mice administered Artemether-Lumefantrine (8 mg kg^{-1}bw)

The percentages of sluggish sperms in AL, PAL and PNT groups (66.00, 75.00 and 83.00 % respectively) were comparatively higher than those of the NC (42.00 %) and other experimental groups (40.00 - 56.00 %). The AL, PAL and PNT groups had fewer motile sperms (17.00 – 32.00 %) than extract-treated animals (44.00 – 60.00 %).

Sperm morphology of PAI_{LD} group (92.60 %) compared favorably with that of the normal

control (90.00 %). The PAL and PNT groups exhibited lower percentage of normal sperms (47.00 and 50.00 % respectively) than other animals used in the study. Plant extracts, especially singly at low levels and high dose combined extracts produced more normal sperms (56.00 – 92.00 %) than low dose combined extracts (53.00 %).

Effect of Azadirachta indica and Gongronema latifolium ethanol leaf extracts on testicular hormones: There was no significant trend ($p > 0.05$) between the serum testosterone concentrations of NC, PGL_{LD}, PAI_{HD} and AL groups (2.36 – 2.82 ng/ mL) as shown in Fig. 1.

The NC (2.82) group had statistically increased testosterone concentration (ng/mL) compared to PNT (1.56), PAI_{LD} (2.01), PGL_{HD} (1.88), $PAIGL_{HD}$ (1.43) and PAL groups (2.15). Also, there was a significant increase of serum testosterone of $PAIGL_{LD}$ group compared to PNT, PAL, PGL_{HD}, and $PAIGL_{HD}$ groups. There were no significant differences in follicle hormone (FSH) concentrations of the experimental animals (1.36 – 1.75 mIU/mL).

The mean differences of the concentrations of follicle stimulating hormone were not statistically significant as presented in Fig. 2.

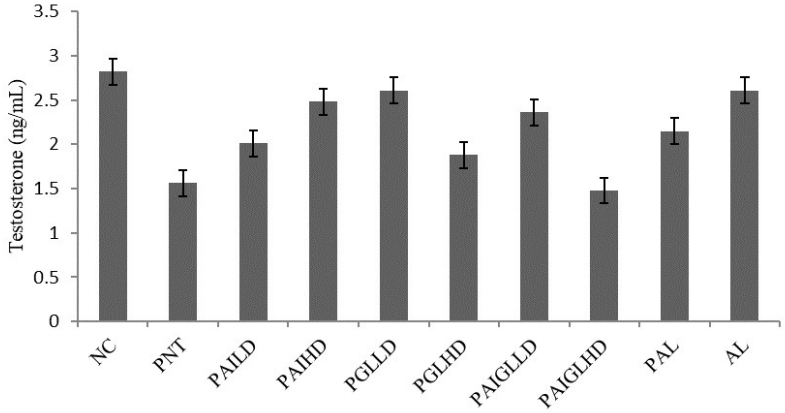

Fig. 1: Effect of Azadirachta indica and Gongronema latifolium ethanol leaf extracts on testicular hormone concentration in parasitized Swiss mice

Legend: a = $p < 0.05$ relative to NC; b = $p < 0.05$ relative to PAI_{HD}, PGL_{LD} and AL
c = $p < 0.05$ relative to $PAIGL_{LD}$; d = $p < 0.05$ relative to PAI_{LD}, PGL_{HD} and PAL

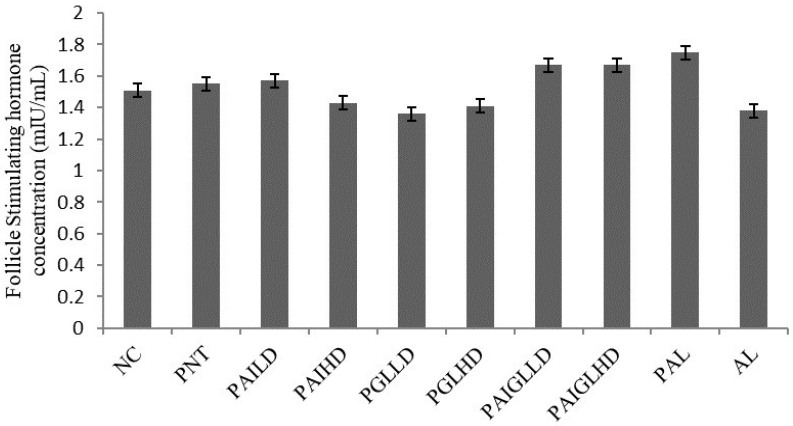

Fig. 2: Effect of Azadirachta indica and Gongronema latifolium ethanol leaf extracts on follicle stimulating hormone concentration in parasitized Swiss mice

Legend: NC = Normal control administered distilled water 10 mL/kg
PNT = Parasitized mice administered distilled water 10 mL/kg
PAI_{LD} = Parasitized mice treated with low dose *A. indica* extract (86.60 mg kg^{-1}bw)
PAI_{HD} = Parasitized mice treated with high dose *A. indica* extract (259.81 mg kg^{-1}bw)
PGL_{LD} = Parasitized mice treated with low dose *G. latifolium* extract (337.27mg kg^{-1}bw)
PGL_{HD} = Parasitized mice treated with high dose *G. latifolium* extract (1011.80 mg kg^{-1}bw)
$PAIGL_{LD}$ = Parasitized mice administered combined low dose *A. indica + G. latifolium* extracts $PAIGL_{HD}$ = Parasitized mice administered high dose *A. indica + G. latifolium* extracts
PAL = Parasitized mice treated with Artemether-Lumefantrine (8 mg kg^{-1}bw)
AL = Non-parasitized mice administered Artemether-Lumefantrine (8 mg kg^{-1}bw)

Effect of Azadirachta indica and Gongronema latifolium ethanol leaf extracts on testicular histoarchitecture: Photomicrographs indicating the effects of both leaf extracts of A. indica and G. latifolium on testicular architecture are demonstrated in Figure 3.

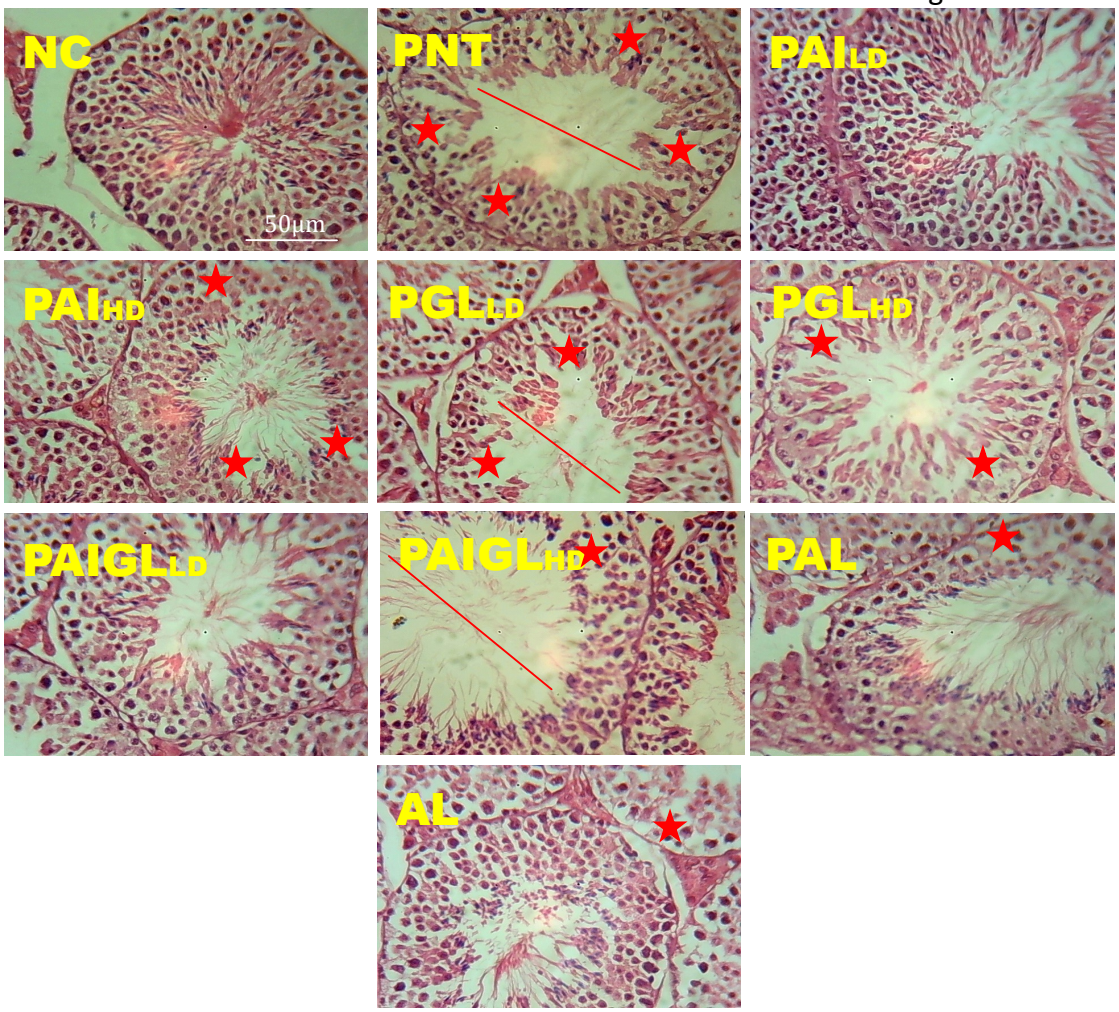

Fig. 3: Photomicrographs of transverse sections of the testes (H&E) stained x 400. Red asterisk = Degenerated spermatogenic lineage cells; Red line = Widened tubular lumen of seminiferous tubules.

Legend:

NC = Normal control, PNT = Parasitized animals not treated with drug or extract,
PAI_{LD} = Parasitized mice treated with low dose A. indica extract (86.60 mg kg^{-1}bw)
PAI_{HD} = Parasitized mice treated with high dose A. indica extract (259.81 mg kg^{-1}bw)
PGL_{LD} = Parasitized mice treated with low dose G. latifolium extract (337.27mg kg^{-1}bw)
PGL_{HD} = Parasitized mice treated with high dose G. latifolium extract (1011.80 mg kg^{-1}bw)
$PAIGL_{LD}$ = Parasitized mice administered combined low dose A. indica + G. latifolium extracts $PAIGL_{HD}$ = Parasitized mice administered high dose A. indica + G. latifolium extracts
PAL = Parasitized mice administered with Artemether-Lumefantrine (8 mg kg^{-1}bw)
AL = Non-parasitized mice administered Artemether-Lumefantrine (8 mg kg^{-1}bw)

DISCUSSION

Malaria continues to be a significant public health concern in Nigeria with about 97% of the population being exposed to this infectious disease [1]. This study was designed to explore the potential effects of co-administering Azadirachta indica and Gongronema latifolium against Plasmodium berghei parasites and their effect on the testes and hypothalamic micro-structure behaviour of Swiss male mice.

The result of the acute toxicity study on the ethanol leaf extracts of A.indica showed that the median lethal dose (LD_{50}) of the extract is over 866 mg/kg body weight (bw). This value was similar to 1000 mg/kg bw obtained by Oseni and Akwetey, [31] and was different from 5000 mg/kg bw reported by Tepongning et al. [32]. The LD_{50} of the ethanol leaf extract of G. latifolium was over 3373 mg/kg bw. This value was similar to those obtained by Nwanjo et al. [18] and 1500 mg/kg obtained

by Sylvester et al. [33].

This shows that G. latifolium has lower acute toxicity than A. indica. According to the toxicity scale of Hodge and Sterner [34], both plants are classified as slightly toxic, because the values obtained were between 500 - 5000 mg/kg.

Phytochemical screening of the extracts showed that both extracts were rich in alkaloids, tannins, saponins, carbohydrates, flavonoids, polyphenols, balsams, glycosides, phytosteroids, cardiac glycosides, and terpenoids. These chemicals may account for the biological activities associated with these extracts. Several of these phytochemicals have been shown to have antiplasmodial effects. One of the important phytochemicals found in both extracts is flavonoids. Flavonoids are capable of impairing Plasmodium nucleic acid base pairing [32, 35]. Terpenoids and steroid saponins possess effects detrimental to infective protozoans including Plasmodium species [36] and alkaloids block parasitic protein synthesis [5, 37]. Saponins have cytotoxic and anti-tumor potentials [38]. Plants containing saponins have been used for medicinal purposes for decades [39]. Saponins, tannins and phenols also have some antiplasmodial activity. Phytochemical screening also indicated the extracts of A. indica to have more steroids, glycosides, tannins and alkaloids than G. latifolium. This may suggest increased biological activity, including a potential for toxicity in the A. indica extract.

It was observed that parasitized mice administered high doses of single extracts (viz. PAI_{HD} and PGL_{HD}) and those administered Artemether-Lumefantrine (PAL) had significant weight changes (-1.12 to -4.02g) when compared with normal control (NC) and parasitized mice on low doses of single extracts (viz. PAI_{LD} and PGL_{LD}), which had weight changes of +0.16 to +0.56 g. Animals that were given combined extracts ($PAIGL_{LD}$ and $PAIGL_{HD}$) also had significant weight loss (-1.36 to -1.96 g) when compared with the NC, PAI_{LD} and AL groups ($p < 0.05$). However, the group administered high dose G. latifolium (single) extract (PGL_{HD}) had the highest weight loss (-4.02 g).

Jayakumal et al. [40] and Ekong et al. [41] reported significant weight reduction in relation to use of G. latifolium. The extract at the dose of administration may have interfered with factors that affect availability, intake of food and growth rate [42, 43].

There was increased temperature with all the animals parasitized with P. berghei. When compared to the healthy (non-parasitized) animals in the NC and AL groups, (in which the temperature remained normal throughout the duration of experiment), it could be seen that P. berghei is a potent pyrogen. In P. falciparum infection, when merozoites rupture red blood cells, they release specific elements and toxins, including red cell membrane protein lipid and glycerol phosphophatidylinositol, which can directly trigger the release of cytokines like tumor necrosis factor (TNF) and interleukin-1 from macrophages. This process leads to symptoms such as chills and high-grade fever [39]. It is possible that P. berghei induces fever in mice through similar mechanism. This observation is corroborated by other studies and P. berghei induced pyrexia has been used as an experimental model for the study of pyrexia and its effects [44]. Both A. indica [30] and G. latifolium [45] have been reported to possess anti-pyretic effect against various pyrogens. In this study, all doses of extracts, both- singly and in combination showed significant anti-pyretic effect against P. berghei induced pyrexia; this is similar to the report by Udo and Peter, [5]. The extracts may have reduced the fever by inhibiting further prostaglandin synthesis and thus reducing the hypothalamic temperature set point, since pyrogens induce fever by increasing the secretion of prostaglandins in the hypothalamus [46]

It was also observed that A. indica had better antiplasmodial effect. The low dose (PAI_{LD}) had parasitemia decreased from 47.60 % before treatment to 34.60 % after treatment, while the high dose (PAI_{HD}) group had parasitemia reduction from 46.20 % to 33.40 % in similar period. The phytochemical screening of the extracts showed that both extracts are rich in polyphenol and saponin, while A. indica had more tannins than G. latifolium. Phenols,

saponins and tannins have been shown to possess the ability to subdue cellular immunity [47].

Combining the extracts significantly improved the anti-plasmodial effect much more than that obtained in single extract administration. The low dosed combination of AI and GL ($PAIGL_{LD}$) decreased the parasitemia percentage from 46.60 to 27.40% and the high dose ($PAIGL_{HD}$) parasitemia percentage reduced from 47.20 to 25.60%. It is possible that the increased anti-plasmodial effect may be due to the extracts having a different mechanism of action against the parasite, and thus a synergistic effect when both are combined. It may also be a result of chemical interactions between the phytochemical constituents of the extracts producing a secondary metabolite that may be more active than single extracts against the parasite.

Sperm parameters showed that there was significant decrease in the sperm count and motility of PNT group compared to NC and other experimental groups. This gives credence to the fact that P. berghei may alter spermatogenic activities, thus reducing their count and motility. This agrees with the reports by Raji et al. [48] and Ekhoye and Nwangwa, [49] which demonstrated that P. berghei induced reduction in sperm count and motility. The significant decrease observed in PNT group may be as a result of oxidative stress induced by P. berghei infection on the spermatogenic cells [50]. There was a significant reduction in sperm cell concentration between the groups treated with A. indica (PAI_{LD} and PAI_{HD}). This report correlates with observations of Khan et al. [51] and Ofoego et al. [52].

These workers reported reduction in sperm cell concentration, in contrast to Irais et al. [53] who reported no reduction in sperm cell concentration following treatment with A. indica. Also, there was a decrease in sperm cell concentration of groups treated with G. latifolium (PGL_{LD} and PGL_{HD}), which is in contrast with reports by Akpaso et al. [23] of increases in sperm cell concentration in animals treated with G. latifolium. There was no significant reduction in sperm cell concentration and motility in the group treated with low dose of combined extract ($PAIGL_{LD}$) when compared with the NC group. This implies that the co administration of both extracts in reduced dosage has no negative impact on sperm cell concentration and motility.

There was no significant difference in the percentage ($p > 0.05$) of normal sperm morphometry (normal head, normal neck, long tail) between the NC group and the treatment groups, for except the PAL group. There was a significant decrease ($p < 0.05$) in the percentage of abnormal sperm morphology (headless, curved/slender neck, tails) between the NC and all treatment groups, except for the PAI_{LD} group This implied that A. indica at lower doses does not impact negatively on sperm morphometry; similar to what was reported by Irais et al. [53].

Testosterone was observed to be significantly decreased in the P. berghei infected untreated mice (PNT group) compared to NC and other experimental groups, except $PAIGL_{HD.}$ Testosterone is a male hormone with significant impact on spermatogenesis [54]. Therefore, the decrease in testosterone level of PNT group negatively affected sperm cell concentration, rendering it low in comparison with that of NC. This suggests suppression (by malaria parasite) of testosterone production. The finding agrees with that of Ojezele et al. [55], who reported significant reduction in testosterone concentrations in parasitized mice. The significant reduction in testosterone following treatment with co-administration of high dose of both extracts observed in this study suggests that co-administration of A. indica and G. latifolium could cause reproductive toxicity depending on their doses, since both extracts are rich in polyphenols. Phenolic compounds have been reported to possess contraceptive activity [53]. There was no statistically significant difference in FSH between the experimental groups. Although it was observed that PGL_{LD} and AL groups, each of which had high serum testosterone values of 2.61 ng/mL each and lowest follicle stimulating hormone (FSH) values of 1.36 - 1.38 mIU/mL in comparison with other treatment groups, this may be due to inhibition of FSH secretion via testosterone- mediated negative

feedback regulation of pituitary gonadotropins [54].

The photomicrographs in this study showed varying amount of testicular alterations in all groups, except NC. The PNT group showed testiculo-degeneration effects, by extensive areas of eroded spermatogenic cells and widened tubular lumen with absence of germinal cells (spermatozoa). The observations are in tandem with reports of malaria parasite-induced testicular toxicity effects by the following workers [14,49,56].

The gonadotoxic effects of P. berghei to the testes are also similar to the germinal epithelium degenerations using alcohol [57,58]. Malaria parasite may have influenced the tissue by introducing free radicals, which consequently influenced metabolism in vivo. The above-mentioned alterations were also observed in the groups treated with extracts, both singly and in combination. There were mild damages in the testicular histoarchitecture of groups administered with A. indica extracts (PAI_{LD} and PAI_{HD}). The findings of mild damages in testicular architecture of PAI groups disagree with those of Uzozie et al. [59] following treatment of rats with 500 mg /kg bw of methanol leaf extract of A. indica. These authors documented severe damage of seminiferous tubules including intraepithelial vacuolation, loosening of germinal epithelium, appearance of giant cells, mixing of various germ cell types during spermatogenesis, and degeneration of germ cells. These effects negatively impacted both the morphology and quantity of spermatozoa in the cauda epididymidis. This suggests that A. indica in a relatively low dose exhibits mild adverse effects on testes and therefore may be non-toxic to testicular tissues. There was mild to moderate damages in the testicular microstructure of the groups administered with G. latifolium extracts (PGL_{LD} and PGL_{HD}). This is in contrast with report by Azu et al. [60], of absence of testicular damage in animals treated with G. latifolium. There was also varying testicular toxicities in extract combination groups ($PAIGL_{LD}$ and $PAIGL_{HD}$) and the PAL group, showing degenerated appearance of germ cells, scanty Sertoli cells and severe arrest of spermatogenesis. This suggests that the leaf extract combination may be able to induce morphologic apoptotic changes such as: shrinkage, membrane leakage, cytoplasmic fragmentation (before germ cell degeneration), inhibition of testosterone production (by suppression of Leydig cell activity) and subsequent slowing down of spermatogenesis.

CONCLUSION

To sum up, this study illustrated that co-administration of the ethanol extracts of Azadirachta indica and Gongronema latifolium reverses P. berghei-induced pyrexia and significantly increased the inhibition of P. berghei. The co-administration of A. indica and G. latifolium at lose dose improved the microstructure of the testes, boosted sperm cell and serum testosterone concentrations better than standard anti-malarial, but had negative impact at high doses. Therefore, the co-administration of these extracts should be taken with caution, as they tend to impair reproductive functions in Swiss mice.

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Conflicts of Interests: The authors declare that there are no competing interests.

Author Contributions

IAE, AIP, JAU and DOE conceptualized and designed and supervised the study, AJP, BEK, IEA and MAA performed the experiments, analyzed the data, and co-drafted the manuscript with IAE, AIP, JAU and DOE.

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