Journal of Biosciences and Medicines, 2024, 12, 62-76
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Long Term Administration of *Lannea acida* Rich. (Anacardiaceae) Reverses the Imidacloprid-Induced Fertility Impairments in Adult Male Rat through Androgenic and Antioxidant Properties

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How to cite this paper: Tetsatsi, A.C.M., Djuidje, G.K., Nsamou, P.C.N., Zeugang, K.G., Petnga, Y.T., Munyali, D.A., Ngadjui, E., Nkeng-Effouet, P.A. and Watcho, P. (2024) Long Term Administration of *Lannea acida* Rich. (Anacardiaceae) Reverses the Imidacloprid-Induced Fertility Impairments in Adult Male Rat through Androgenic and Antioxidant Properties. *Journal of Biosciences and Medicines*, **12**, 62-76.
https://doi.org/10.4236/jbm.2024.123006

Received: January 24, 2024
Accepted: March 10, 2024
Published: March 13, 2024

Abstract

Aim: The harmful effects of pesticides have been largely documented in recent times. But effective therapeutic solutions to pesticide related male infertility are yet to be established. This study investigated the curative effects of *Lannea acida* on imidacloprid (IMI)-induced hypofertility in male Wistar rats. **Methods:** Rats of 150 – 200 g were administered IMI (22.5 mg/kg) for two weeks and partitioned into control (distilled water, vitamin E, clomiphene citrate) or test (aqueous (340 mg/kg), methanol (170 mg/kg) extract) groups for eight weeks treatment. Animals were sacrificed at the end of the treatment and samples were collected for sperm, antioxidant and hormonal analysis. Fertility tests were performed from treatment day 47 for fertility indices estimation. Results were expressed as mean ± SEM and one way ANOVA was applied using STATISTICA Software. **Results:** Exposition to IMI resulted in a significant decrease in sperm count, motility, viability and normality, testosterone and LH, coupled to an increase in oxidative stress markers. Moreover, IMI impaired male fertility evidenced by a significant drop in fertility index and litter size. Similar to clomiphene citrate and vitamin E, plant extracts sig-

nificantly improved the sperm parameters, sexual hormones and decreased the oxidative stress markers. More importantly, the fertility index and litter size were restored, especially with the aqueous extract. **Conclusion:** Present results indicate that *L. acida* possesses curative potentials against IMI-induced hypofertility through its androgenic and antioxidant properties. However, the effects the extract on spermatozoa DNA structure and the fertility of offsprings from exposed parents are yet to be studied to conclude on total recovery from IMI toxicity.

Keywords

Imidacloprid, *Lannea acida*, Hypofertility, Male Rat

1. Introduction

Endocrine disrupting chemicals (EDCs) among which pesticides, have gained much interest nowadays. They exert their toxic effects even at low doses through food consumption including fruits, vegetables or water [1] [2]. Pesticides (one of the most important EDCs) are used in agriculture or veterinary medicine to promote productivity by fighting against devastating insects [3] [4] [5]. Pesticides are toxic chemicals that interfere with synthesis, secretion or action of endogenous hormones and disrupt their function in target organisms [6]. Although useful in promoting food sufficiency to cope with the demographic burden especially in developing countries, they have been proven to be food and water pollutants. Over the last few decades, several studies have emphasized on the environmental pollution of pesticides and their health impact on non-target organisms including mammals [4] [7] [8]. The United States Department of Agriculture pesticide report of 2018 revealed a detection rate of 77.3% for neonicotinoids insecticides in fruit and vegetables. Imidacloprid (IMI), the commonest neonicotinoid insecticide [IMI, 1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine] was the most frequently observed neonicotinoid residue [9].

IMI is the first neonicotinoid insecticide registered for use by the United States Environmental Protection Agency. The World Health Organization (WHO) and United States Environmental Protection Agency classified this molecule as a "moderately toxic" Class II or III requiring a Warning or Caution labels on sold products [3]. Indeed, IMI causes neurotoxicity in target insects by blocking nicotinic acetylcholine receptors (nAChR) thereby preventing acetylcholine action and resulting in the insect's death. This effect is thought to be specific to insects as IMI has a selective affinity for insect's nAChRs over those of vertebrates [2]. However, many reports have documented toxic effects of neonicotinoids in humans including congenital heart defects, neural tube defects, autism spectrum disorders and reproductive toxicity [4]. The effects on humans arise after direct or indirect contact in the environment as IMI has a relatively long half-life [5]. Moreover, their high-water solubility contributes to its persistence and transport

in food and water products [10] [11]. Experimental studies have revealed the harmful impact of IMI in mammals and especially on the reproductive system. Testicular damage, sexual hormone imbalance, altered spermatogenesis and testicular DNA damage are the main impairments of IMI on the reproductive system and which may lead to infertility [12] [13] [14]. The mechanism underlining these effects remains unclear but, many reports suggest of a specific action through interaction with mammals nAChRs [15] [16] and a common nonspecific pathway through oxidative stress, resulting from the generation of reactive oxygen species [2] [6] [17].

Several treatments strategies are used against infertility, among which medicinal plants. Some natural products and medicinal plants have been proven to possess beneficial effects against various models of pesticides induced-reproductive toxicity. [17] reported the preventive effects of *Origanum majorana* against sexual behavior and testicular oxidative damages induced by IMI. Moreover, [18] highlighted the protective effects of quercetin, a plant-derived natural product against IMI-induced biochemical imbalance and DNA damage in adult rats. In our previous study, we demonstrated the alleviating effects of *Lannea acida* extracts, a local medicinal plant, on the reproductive impairments of IMI through the improvement of sex organs weight, hormones and spermatozoa quality after a short-term period treatment [19]. These effects were mediated through the regulation of testis oxidative stress markers. In the present study, we focused on the long-term alleviating effects of *Lannea acida* on spermatogenesis and fertility potentials of IMI-induced hypofertility adult male rats.

2. Methodology

2.1. Ethical Approval

The project was presented and validated by the scientific committee of the Department of Animal Biology, University of Dschang, which follows the internationally accepted standard ethical guidelines for laboratory animal use and care.

2.2. Chemicals

Commercial IMI (Colibri®: 30% IMI and 70% inactive ingredients) was purchased from the Dschang market (Sun Valley Hall Limited-Hong Kong; Bach N˚ SVH161104) and, the working solution prepared in distilled water. Methanol and Anhydrous sodium sulfate were purchased from Sigma Chemical Co., Germany. Assay kits for testosterone, LH and FSH (Accubind, Monobind. Lake Forest, USA) were used according to the manufacturer's instructions. All other chemicals and reagents were of analytical grade and purchased from local suppliers.

2.3. *Lannea acida* Harvesting and Extract Preparation

The stem barks of *L. acida* were received from the Alango Foundation (Dschang, Cameroon) and shade-dried. The dried material was reduced into powder for extract's preparation. Aqueous and methanol extracts were prepared as pre-

viously described [19]. Briefly, the aqueous extract was prepared by decoction of 250 g of plant powder in 1.5 L of boiled distilled water for 10 minutes. The solution was then filtered at room temperature and the filtrate was oven-dried to obtain a brown powder. The methanol extract was prepared by maceration of 250 g of the powder of *L. acida* in 1 L of methanol for 72 h at room temperature. The product was then filtered and the filtrate was evaporated under reduced pressure and oven-dried to obtain a brown powder representing the methanol extract.

2.4. Doses Selection and Preparation

In our previous study, the aqueous and methanol extracts of *L. acida* at 340 mg/kg and 170 mg/kg, respectively, were the most active plant doses in alleviating sperm, hormonal, oxidative and testis histological impairments induced by IMI and were chosen for this study [19]. Moreover, the same doses of IMI (1/20 of the LD_{50}: 22.5 mg/kg bw), clomiphene citrate and vitamin E were used as described in our previous study. The working solution of IMI was freshly prepared by mixing 1.5 mL of the commercial IMI to 18.5 mL of distilled water to obtain 20 mL of IMI solution at 2.25 mg/mL. For aqueous extract solution, 680 mg of the extract powder were dissolved in 14 mL of distilled water. The volume of the homogenized mixture was then adjusted to 20 mL to obtain a concentration of 34 mg/mL. The preparation of the methanol solution followed the same procedure using 340 mg of methanol extract powder. All working solutions were freshly prepared and administered at a volume of 10 mL/kg.

2.5. Animals and IMI Exposure

Adult male (30) and female (60) Wistar rats weighing 150 - 200 g were obtained from the animal house of the Department of Animal Biology, Faculty of Science of University of Dschang, Cameroon. They were acclimated in plastic cages (4/cage) at room temperature with a natural light/dark cycle for 2 weeks before experiments. Animals had free access to standard rat diet and tap water throughout the experiment.

The induction of the reproductive toxicity using IMI was done as previously described [19]. 25 male rats were daily administered 22.5 mg/kg of IMI for two consecutive weeks. The weight of animals was monitored every 3 days and IMI solution volume was adjusted accordingly. 5 other rats constituted the control group and received distilled water (10 mL/kg) throughout the experiment.

2.6. Animal Grouping and Treatment

After two weeks of repeated oral exposure to IMI, 25 sexually experienced male rats were randomly divided into 5 groups of 5 animals each. Group 1 was the negative control animals and received distilled water (IMI + DW). Groups 2 and 3 were the positive control animals and were treated with clomiphene citrate (IMI + CC) and vitamin E (IMI + Vit E), respectively. Groups 4 and 5

were respectively treated with aqueous (IMI + AE) and methanol (IMI + ME) extract of *L. acida*. Control animals without IMI exposure received distilled water (Group 6) throughout the experiment. All treatments were orally given for 8 consecutive weeks, the time necessary for spermatogenesis achievement in rats.

2.7. Fertility Test

The fertility test was conducted as described by [3] with minor adjustments. The test was performed during the last 10 days of treatments (days 47 to 56). For this purpose, each male was paired with 2 adult females of proven fertility. Vaginal smears were daily examined under a light microscope (OLYMPUS, ×400) for the presence of spermatozoa. Sperm positive females were considered inseminated, caged separately and followed-up till delivery. Quantic gestation [(number of sperm positive females/number of pregnant rats) × 100], fertility index [(number of pregnant rats/number of females mated) × 100] and mean litter size were determined for each treatment group

2.8. Animal Sacrifice and Sample Collection

At the end of the treatment (day 57), male rats were sacrificed under diazepam/ketamine anesthesia. Blood sample was collected from the abdominal artery and centrifuged for 15 min at 3000 rpm. The supernatant plasma was pipetted and kept in sealed tubes at −20˚C for the measurement of sexual hormone levels. Testes, vas deferens, epididymis, seminal vesicles and prostate were excised, freed from surrounding tissues and weighted using a sensitive laboratory scale. The left cauda epididymis served for sperm parameters analysis. Testes were kept at −20˚C for the measurement of oxidative stress markers.

2.9. Sexual Hormone Assay

The plasma testosterone, FSH and LH were measured through ELISA technic according to the standard guidelines instructed by the kit supplier (Accubind, Monobind. Lake Forest, USA).

2.10. Sperm Parameters Analysis

After sacrifice, the fresh left cauda epididymis of each rat was crushed in 10ml of saline solution (0.9%) at 34˚C to evaluate sperm motility, count, viability and morphology (normality). The procedure followed the steps described by [20] and [3]. 10 µl of a diluted solution were inserted in A (Ratio: 1/1) and B (Ratio: 1/2) chambers of the Mallassez hemocytometer. Motile and non-motile spermatozoa were then counted in 10 random squares (5 in either chamber) using a light microscope (OLYMPUS, 40×). This permitted to determine sperm motility as the as the percentage of motile spermatozoa over the total number of spermatozoa counted.

The epididymis solution was kept for 24 h to allow sperm cells migration from the tissue to the physiological solution before sperm count estimation. After di-

lution (1/11 ratio), 10 µl of the solution were placed in each chamber of the Mallassez hemocytometer. The density was expressed as the total number of spermatozoa counted in 20 random squares using light microscope (OLYMPUS, 40×).

For the measurement of sperm viability and morphology, sperm smear was prepared using a drop of the solution obtained from the cauda epididymis and eosin-nigrosine staining. The preparation was dried at room temperature and a minimum of 200 sperm cells were examined in 20 randomly selected fields using light microscope. Sperms with stained cytoplasm were considered nonviable and the total viability was calculated as the percentage of viable sperm cells over the total number. In the same fields, sperms with morphological abnormalities were counted and the total normality was estimated as the percentage of total cells without morphological abnormalities over the total number of sperm cells observed.

2.11. Assessment of Oxidative Stress Markers

Testis homogenate was prepared at 10% in ice-cold 10 mM Tris buffer (pH 7.4) and centrifuged for 10min at 3000 rpm at 4˚C. The supernatant was collected separately and stored at −20˚C for oxidative stress markers estimation. Total proteins were quantified using bovine serum albumin as standard and lipid peroxidation (LPO) was measured in terms of malondialdehyde (MDA) production using the thiobarbituric acid method [21]. Super oxide dismutase (SOD) and catalase (CAT) activities were measured following the procedure described by [22]. Potassium iodate method was employed for total peroxidase activities measurement [23].

2.12. Data Analysis

Results are presented as mean ± SEM. After testing conformity to normality of data, comparisons were analyzed by a one-way analysis of variance (ANOVA) followed by a Tukey-HSD post-hoc test to determine statistical difference. Values were considered statistically significant if $p < 0.05$. STATISTICA/PC program (Version 8.0.) was used for data analysis.

3. Results

3.1. Body Weight Changes throughout the Experiments

After two weeks of repeated oral exposure to IMI, a significant decrease ($p \leq 0.0001$) in body weight was recorded in all exposed rats compared to the control (**Table 1**). During the treatment phase, there was a marked recovery in body weight. The increase was balanced in all treated groups except for control which recorded the lowest increase (**Table 1**).

3.2. Effect of Treatments on Sexual Glands

The effects of treatments on testis, seminal vesicles and prostate weights are

Table 1. Effects of treatments on body weight changes.

Treatments	Induction period			Treatment period		
	Initial weight (g)	Final weight (g)	Variation (%)	Initial weight (g)	Final weight (g)	Variation (%)
Control	172.80 ± 1.16	187.60 ± 2.58	8.56 ± 2.35	187.60 ± 2.58	215.43 ± 12.80	14.83 ± 2.35
IMI + DW	177.00 ± 6.87	157.40 ± 11.46	−11.36 ± 3.75###	157.40 ± 11.46	233.80 ± 16.86	48.54 ± 4.57
IMI + CC	183.00 ± 4.51	161.00 ± 4.17	−12.01 ± 1.03###	161.00 ± 4.17	236.00 ± 8.17	45.10 ± 4.53
IMI + Vit E	173.60 ± 3.67	155.80 ± 4.73	−10.08 ± 3.35###	155.80 ± 4.73	213.60 ± 11.75	34.79 ± 4.28
IMI + AE	174.40 ± 4.20	154.80 ± 6.74	−11.13 ± 3.82###	154.80 ± 6.74	247.60 ± 6.76	54.99 ± 8.02
IMI + ME	177.20 ± 7.39	158.80 ± 10.36	−10.60 ± 2.99###	158.80 ± 10.36	227.60 ± 7.30	40.23 ± 6.40

Values are presented as mean ± Standard Error of the Mean. Number of animals per group = 5. Control group received only distilled water throughout the experiment. IMI + DW = Imidacloprid + Distilled Water (10 ml/kg). IMI + CC = Imidacloprid + Clomiphene Citrate (0.25 mg/kg). IMI + Vit E = Imidacloprid + Vitamin E (200 mg/kg). IMI + AE = Imidacloprid + Aqueous Extract at 340 mg/kg. IMI + ME = Imidacloprid + Methanol Extract at 170 mg/kg. ###; $P < 0.001$: Significantly different compared to Control group.

presented in Table 2. After 8 weeks of continuous gavage, no major difference was observed between the absolute and relative weights of IMI + DW and Control groups. However, a significant increase ($p < 0.05$) in the relative weight of testis was noted in animals treated with the methanol extract at 170 mg/kg.

3.3. Effects of Treatments on Rat Epididymis and vas Deferens Weights

Similar to effects on sexual glands, no noticeable difference was observed between treatment groups (Table 3).

3.4. Effects of Sexual Hormones

The administration of IMI induced a significant decrease ($P < 0.05$ - 0.001) in testosterone, LH and FSH as compared with the untreated control group. Similar to clomiphene citrate and vitamin E, aqueous and methanol extract of *L. acida* significantly increased testosterone ($P < 0.05$ - 0.001), and LH ($P < 0.001$) levels compared with the untreated control group. Moreover, a significant ($P < 0.05$) improvement of FSH concentration was noticed in animals receiving the aqueous extract. Comparatively, the aqueous extract of *L. acida* appeared to be more active than the methanol extract (Table 4).

3.5. Effects of Treatments on Testis Oxidative Stress Markers

As described in Table 5 there was a moderate change in testis total proteins, but no significant difference was recorded across the groups. In contrast, IMI exposition led to a significant increase in testis MDA content ($P \leq 0.001$), SOD ($P \leq 0.05$) coupled with a significant increase in Catalase ($P \leq 0.01$) and total peroxidases ($P \leq 0.001$) activities. Similar to vitamin E, treatment with plant extracts, especially the aqueous extract, significantly decreased ($P \leq 0.05$ - 0.001) these oxidative stress markers compared to IMI + DW group.

Table 2. Effects of treatments on sexual gland weights.

Treatments	Testis		Seminal Vesicles		Prostate	
	Abs weight (g)	Rel weight (g/100g bw)	Abs weight (g)	Rel weight (g/100g bw)	Abs weight (g)	Rel weight (g/100g bw)
Control	2.37 ± 0.10	1100.13 ± 35.69	1.25 ± 0.10	580.23 ± 13.37	0.28 ± 0.01	129.97 ± 53.21
IMI + DW	2.41 ± 0.16	1030.80 ± 28.46	1.24 ± 0.70	530.37 ± 19.33	0.28 ± 0.07	119.76 ± 40.23
IMI + CC	2.62 ± 0.14	1110.17 ± 43.75	1.24 ± 0.13	525.42 ± 17.31	0.36 ± 0.05	152.54 ± 35.35
IMI + Vit E	2.37 ± 0.10	1109.55 ± 59.90	1.25 ± 0.10	585.21 ± 5.36	0.28 ± 0.01	131.09 ± 47.56
IMI + AE	2.68 ± 0.11	1082.39 ± 32.57	1.23 ± 0.11	496.77 ± 4.23	0.32 ± 0.02	129.24 ± 44.15
IMI + ME	2.62 ± 0.14	1151.14 ± 64.25*	1.24 ± 0.13	544.82 ± 14.05	0.36 ± 0.05	158.17 ± 52.37

Values are presented as mean ± Standard Error of the Mean. Number of animals per group = 5. Abs weight = Absolute weight. Rel weight = relative weight. bw= Body Weight. Control group received only distilled water throughout the experiment. IMI + DW = Imidacloprid + Distilled Water (10 ml/kg). IMI + CC= Imidacloprid + Clomiphene Citrate (0.25 mg/kg). IMI + Vit E = Imidacloprid + Vitamin E (200 mg/kg). IMI + AE = Imidacloprid + Aqueous Extract at 340 mg/kg. IMI + ME = Imidacloprid + Methanol Extract at 170 mg/kg. *: $P < 0.05$ = Significantly different compared to IMI + DW group

Table 3. Effects of treatments on the epididymis and vas deferens weight.

Treatments	Epididymis		Vas deferens	
	Abs weight (g)	Rel weight (g/100g bw)	Abs weight (g)	Rel weight (g/100g bw)
Control	0.77 ± 0.04	357.42 ± 30.44	0.15 ± 0.02	69.63 ± 2.78
IMI + DW	0.76 ± 0.10	325.06 ± 20.09	0.13 ± 0.02	55.60 ± 3.36
IMI + CC	0.88 ± 0.05	372.88 ± 20.71	0.18 ± 0.01	76.27 ± 6.88
IMI + Vit E	0.77 ± 0.04	360.49 ± 15.36	0.15 ± 0.02	70.22 ± 5.97
IMI + AE	0.96 ± 0.04	387.72 ± 21.72	0.17 ± 0.01	68.66 ± 3.99
IMI + ME	0.88 ± 0.05	386.64 ± 19.64	0.18 ± 0.01	79.09 ± 7.49

Values are presented as mean ± Standard Error of the Mean. Number of animals per group = 5. Abs weight = Absolute weight. Rel weight = relative weight. bw = Body Weight. Control group received only distilled water throughout the experiment. IMI + DW = Imidacloprid + Distilled Water (10 ml/kg). IMI + CC = Imidacloprid + Clomiphene Citrate (0.25 mg/kg). IMI + Vit E = Imidacloprid + Vitamin E (200 mg/kg). IMI + AE = Imidacloprid + Aqueous Extract at 340 mg/kg. IMI + ME = Imidacloprid + Methanol Extract at 170 mg/kg.

Table 4. Effects of treatments on sexual hormones.

Treatments	Testosterone (ng/ml)	LH (mUI/ml)	FSH (mUI/ml)
Control	2.76 ± 0.24	2.40 ± 0.20	0.92 ± 0.08
IMI + DW	1.07 ± 0.15$^{###}$	0.57 ± 0.13$^{###}$	0.67 ± 0.06
IMI + CC	3.63 ± 0.29***	2.18 ± 0.30***	0.91 ± 0.11
IMI + Vit E	2.73 ± 0.09***	1.17 ± 0.16	0.78 ± 0.06
IMI + AE	2.73 ± 0.17***	2.17 ± 0.22***	1.04 ± 0.09*
IMI + ME	1.99 ± 0.16*	3.36 ± 0.14***	0.86 ± 0.11

Values are presented as mean ± Standard Error of the Mean. Number of animals per group = 5. LH = Luteinizing Hormone. FSH = Follicle Stimulating Hormone. Control group received only distilled water throughout the experiment. IMI + DW = Imidacloprid + Distilled Water (10 ml/kg). IMI + CC = Imidacloprid + Clomiphene Citrate (0.25 mg/kg). IMI + Vit E = Imidacloprid + Vitamin E (200 mg/kg). IMI + AE = Imidacloprid + Aqueous Extract at 340 mg/kg. IMI + ME = Imidacloprid + Methanol Extract at 170 mg/kg. ###; $P < 0.001$: Significantly different compared to Control group. *: $P < 0.05$; ***: $P < 0.001$: Significantly different compared to IMI + DW group.

Table 5. Effects of treatments on oxidative stress markers.

Treatments	Total Proteins	MDA	SOD	Catalase	Total Peroxidases
Control	217.61 ± 4.99	102.42 ± 2.37	52.05 ± 1.61	10.33 ± 0.58	13.55 ± 0.51
IMI + DW	200.71 ± 4.11	133.79 ± 3.43###	60.65 ± 2.44#	14.07 ± 0.61##	15.61 ± 0.61###
IMI + CC	233.28 ± 9.96	112.26 ± 2.47***	47.50 ± 1.65***	11.71 ± 0.37	9.95 ± 0.73***
IMI + Vit E	230.35 ± 11.24	106.60 ± 2.48***	42.76 ± 0.90***	13.15 ± 0.37	11.83 ± 1.33*
IMI + AE	218.97 ± 7.97	109.23 ± 1.52***	48.38 ± 1.67***	11.34 ± 0.41*	10.28 ± 0.72***
IMI + ME	202.63 ± 9.24	127.44 ± 0.90	56.28 ± 1.08	12.74 ± 0.84	10.50 ± 0.38**

Values are presented as mean ± Standard Error of the Mean. Number of animals per group = 5. MDA = Malondialdehyde. SOD = Superoxide Dismutase. Control group received only distilled water throughout the experiment. IMI + DW = Imidacloprid + Distilled Water (10 ml/kg). IMI + CC = Imidacloprid + Clomiphene Citrate (0.25 mg/kg). IMI + Vit E = Imidacloprid + Vitamin E (200 mg/kg). IMI + AE = Imidacloprid + Aqueous Extract at 340 mg/kg. IMI + ME = Imidacloprid + Methanol Extract at 170 mg/kg. ##; $P \leq 0.01$; ###; $P \leq 0.001$: Significantly different compared to Control group. *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$: Significantly different compared to IMI + DW group.

3.6. Effects of Treatments on Sperm Parameters

The effects of *L. acida* on sperm parameters are summarized in Table 6. Animals exposed to IMI and treated with distilled water for 8 weeks showed a significant drop in sperm count ($P \leq 0.01$), sperm motility ($P \leq 0.01$), sperm viability ($P \leq 0.001$) and sperm normality ($P \leq 0.001$) compared with control group. Plant extracts significantly improved ($P \leq 0.05$ - $P \leq 0.001$) the sperm quality and quantity as compared with IMI + DW. The aqueous extract was more active than the methanol extract.

3.7. Effects of Treatments on Fertility

Results of the fertility test performed in two weeks IMI-exposed male rats followed by 8 weeks of treatment are presented in Table 7. A drop by 40% and 60% in quantic gestation and fertility index, respectively, was recorded in females paired with males of IMI-DW group in comparison to control. Moreover, IMI caused a significant decrease ($P \leq 0.01$) in litter size in the females of this group. Interestingly, plant extract treatments reversed these fertility impairments. IMI + CC, IMI + Vit E and aqueous extract groups significantly ($P < 0.01$) improved the litter size.

4. Discussion

This study investigated the ameliorative effects of *Lannea acida* on the fertility of IMI-exposed male rats. Exposition to IMI led to body weight decrease in all rats. This result is consistent with our previous study [19] and could reflect the effect of IMI on both the intestinal and liver functions. It has been reported that IMI disrupts the intestinal epithelium thus, affecting nutrients absorption [24]. Moreover, the histological damaging effects of IMI have been largely documented [25], [26]. After the treatment period, no difference was observed between plant treated IMI-exposed rats and non-treated exposed rats. Cessation to

Table 6. Effects of treatments on sperm parameters.

Treatments	Sperm parameters			
	Count (million × 10^6/ml)	Motility (%)	Viability (%)	Normality (%)
Control	262.25 ± 17.47	89.82 ± 2.70	81.99 ± 2.61	79.58 ± 2.30
IMI + DW	125.50 ± 15.68##	65.66 ± 5.93##	57.64 ± 3.84###	52.23 ± 3.20###
IMI + CC	246.75 ± 35.23**	76.52 ± 5.20	70.15 ± 4.27	63.60 ± 2.18
IMI + Vit E	192.25 ± 11.39	78.22 ± 2.09	79.36 ± 1.23***	72.01 ± 1.80***
IMI + AE	262.50 ± 17.23**	82.52 ± 1.61*	81.01 ± 0.74***	76.10 ± 1.68***
IMI + ME	256.88 ± 20.19**	85.14 ± 2.43*	74.92 ± 4.33**	66.92 ± 4.19**

Values are presented as mean ± Standard Error of the Mean. Number of animals per group = 5. Control group received only distilled water throughout the experiment. IMI + DW = Imidacloprid + Distilled Water (10 ml/kg). IMI + CC = Imidacloprid + Clomiphene Citrate (0.25 mg/kg). IMI + Vit E = Imidacloprid + Vitamin E (200 mg/kg). IMI + AE = Imidacloprid + Aqueous Extract at 340 mg/kg. IMI + ME = Imidacloprid + Methanol Extract at 170 mg/kg. ##; $P \leq 0.01$; ###; $P \leq 0.001$: Significantly different compared to Control group. *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$: Significantly different compared to IMI + DW group.

Table 7. Effects *L. acida* on the fertility parameters of imidacloprid-exposed rats.

Treatments	Female studied	Positive vaginal smear	Pregnant females	Quantic gestation (%)	Fertility Index (%)	Litter size
Control	10	9	9	100.00	90.00	7.56 ± 0.34
IMI + DW	10	5	3	60.00	30.00	5.00 ± 0.58##
IMI + CC	10	8	6	75.00	60.00	7.00 ± 0.37*
IMI + Vit E	10	7	6	85.72	60.00	7.33 ± 0.21*
IMI + AE	10	8	7	87.50	70.00	7.71 ± 0.29**
IMI + ME	10	6	4	66.67	40.00	6.25 ± 0.63

Values are presented as mean ± Standard Error of the Mean. Number of male rats per group = 5. Control group received only distilled water throughout the experiment. IMI + DW = Imidacloprid + Distilled Water (10 ml/kg). IMI + CC = Imidacloprid + Clomiphene Citrate (0.25 mg/kg). IMI + Vit E = Imidacloprid + Vitamin E (200 mg/kg). IMI + AE = Imidacloprid + Aqueous Extract at 340 mg/kg. IMI + ME = Imidacloprid + Methanol Extract at 170 mg/kg. ##; $P < 0.01$: Significantly different compared to Control group. *: $P < 0.05$; **: $P < 0.01$: Significantly different compared to IMI + DW group.

IMI-exposition is accompanied by a time-dependent decrease of its metabolites in the system that could lead to a decrease or reversibility of the associated damaging effects [27].

The effects of IMI on sexual organs are used as predictors of the damaging effects as this molecule has been shown to affect these organs both structurally and functionally (Hassan *et al.*, 2019; Mikolić & Karačonji, 2018; Najafi *et al.*, 2010; Saber *et al.*, 2021). Contrary to our previous study, no significant difference was noted among the groups, probably due to the 8 weeks elapsed between exposition stop and the measurement. Although the testis weight did not vary in IMI-exposed group, its functions were markedly impacted as shown by the significant decreased in testosterone and LH concentrations, 8 weeks after the cessation of IMI. IMI is an endocrine disruptor and is known to interfere with cho-

linergic neurons in the hypothalamus and disrupt LH and FSH synthesis. At local stage, this trouble affects the steroidogenesis and thus testosterone release (Abdel-Razik *et al*., 2021; Al-Awar, 2021; Najafi *et al*., 2010). Similar to Clomiphene citrate, plant extract significantly improved sexual hormone level compared to untreated group. With the better activity recorded with the aqueous extract. Clomiphene citrate is a classical molecule with proven capacity to modulate sexual hormones in male and female [28] [29]. The effects recorded with the plant extract are in accordance with its androgenic properties previously reported [30].

The curative effects of *L. acida* could also be due to its antioxidant properties [31]. The main mechanism underlying the toxic effects of IMI is the excessive generation of free radicals and then oxidative stress [32] [33]. As largely documented, the effect of IMI was evidenced in the present study by a significant increase of MDA coupled with decreased activity of SOD, CAT and peroxidases. The increase of MDA in exposed rats reflects tissue lipid peroxidation while depletion of SOD, CAT and peroxidases activities indicates testis antioxidant balance disruption. Treatment with vitamin E and plant extracts significantly decreased the oxidative stress markers in IMI-exposed animals compared with distilled water-treated animals. Vitamin E is one of the powerful antioxidant agents and is recommended in many therapeutic protocols. More specifically, vitamin E and vitamin C possess significant effects in managing IMI-induced oxidative stress in various systems [34] [35] [36] [37].

Results on sperm parameters were in accordance with that of the sexual hormones and oxidative stress makers. Indeed, as predicted, IMI impaired spermatogenesis, marked by a significant decrease in sperm count, motility and normality. Many authors documented these effects and suggested that they originated from the testicular oxidative stress and from the decreased sexual hormones [2] [13] [19]. Spermatogenic process involves rapid cell division and high energy demand. The relative low vascularization of the testis compared to other organs [38] and the high concentration of unsaturated fatty acids make spermatogenesis particularly sensitive to oxidative stress [39].

Treatment with plant extract significantly increased sperm parameters with the highest effect produced by the aqueous extract. These results are in accordance with the already proven properties of *L. acida* on the reproductive system [19] [30] [31]. These effects are similar to those obtained with Curcumin, Thymol and *Saccharomyces boulardiion* on testis functions of rats exposed to IMI [6] [40] [41]. Although many studies have evaluated the harmful impact of pesticides including IMI on the reproductive system and therapeutic potentials of medicinal plants, none of them, to the best of our knowledge, has investigated the effect on fertility. From the fertility test performed in the present study, we recorded a significant damaging impact of IMI on male rats. IMI decreased quantic gestation and fertility index by 40% and 60%, respectively in female mated to IMI-exposed rats and treated with distilled water. Consequently, the decrease in

the mean litter size was also statistically significant. These results are in line with the effects on sexual hormones, sperm parameters and positive stress. They further strengthen the opinion that harmful effects of IMI are not light as previously envisaged. Thanks to the profertility and antioxidant properties of *L. acida [30]*, plant treated groups recorded a significant improvement compared with control group. Clomiphene citrate, vitamin E and the aqueous extract of *L. acida* significantly increased the litter size compared to distilled water-treated group. Results of plant extracts confirmed the effects recorded on sexual hormones, sperm parameters and oxidative stress, not only in this study but also in previous reports which investigated their therapeutic potentials [19] [30] [31]. Some authors have suggested that IMI exert a multigenerational toxicity in experimental models through DNA damages [42] [43]; thus it could be insightful to proceed to a comparative assessment of the DNA structure and fertility status of pups from different groups to determine the depth of the plant effect.

5. Conclusion

This study highlighted the damaging effects of pesticides and specifically IMI on the fertilizing potential of male rats and the alleviating effects of *L. acida*. A significant impairment on sexual hormones and sperm parameters which led to decreased fertility was recorded 8 weeks after IMI exposure, indicating the lasting reproductive toxicity of IMI in mammals. *L. acida* extracts showed significant improvement of sperm characteristics and fertility status through regulation of sex hormones production and oxidative stress regulation. The aqueous extract showed the highest effects and was considered the most active. However, the effects of these extracts on spermatozoa DNA structure and the fertility of offsprings from exposed parents are yet to be studied to conclude on total recovery from IMI effects.

Author Contribution Statement

ACMT, GKD, PCNN and PW conceived the study, participated in data collection, analysis and manuscript drafting; KGZ, YTPDAM, EN and PAN participated in data collection, analysis and manuscript reviewing. All the authors approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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