Impact of Co-administered Lopinavir/Ritonavir and Sulfamethoxazole/Trimethoprim on Reproductive Indices of Male Albino Rats

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Abstract Antiretroviral drugs containing Lopinavir/ ritonavir (LPV/r) are usually co-administered with sulfamethoxazole / trimethoprim (SMX/TMP) in the management of HIV/AIDS and co infections. The concurrent use of these drugs may place more adverse burden on testicular function because reports have associated these drugs individually with decreased testicular function. This study therefore evaluated the effects of the co-administration of SMX/TMP + LPV/r on reproductive indices of male albino rats. Eighty (80) adult male rats which were divided into five (5) groups A-E were used in this study. Animals in group A which served as the control were treated with 1% ethanol while animals in groups B-E were treated orally with SMX/TMP, (22.4/4.6mg/kg), LPV/r (22.8/5.8mg/kg) and combined doses of SMX/TMP + LPV/r for 2-8 weeks respectively. Animals were sacrificed at the end of treatment, testes were collected and weighed. Sperm count, sperm motility, and morphology were evaluated. Testicular levels of malondialdehyde (MDA), super oxide dismutase (SOD) and histopathological changes were also analyzed. Single doses of LPV/r and SMX/TMP produced significant time dependent decrease in total sperm count and sperm motility, with increase in abnormal sperm morphology. Treatment with single doses of these agents time dependently increased testicular MDA, decreased SOD level and induced abnormal testicular histopathological changes. No significant synergistic effects were observed in all evaluated parameters when these agents were co-administered. **Conclusion**: Due to lack of significant synergistic effects on all evaluated parameters with the co-administration of these agents, the concurrent use of these agents in the management of HIV and co- infections may not have any deleterious effect on male reproductive function.

***Keywords**: testis, toxicity, sulfamethoxazole/trimethoprim, lopinavir/ritonavir, rats*

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1. Introduction

Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse [1]. Infertility is reported to be on the increase over the past decade due to the number of couples seeking consultation for infertility problems with about 50% attributed to male factors [2,3]. Infertility among males could be basically diagnosed through semen analysis which involves the evaluation of sperm count, sperm motility, and sperm morphology [4,5,6,7]. Quite a number of factors have been reported to be actively associated with infertility. Among them are pharmaceutical medications as well as recreational drugs which have been shown to affect fertility through decreased ejaculation, induction of erectile dysfunction and decreased libido by impacting seriously on testicular function and structure [8,9,10,11].

Lopinavir/Ritonavir and sulfamethoxazole/trimethoprim are antimicrobial agents used in the management of HIV/AIDS and co-infections. Lopinavir is usually boosted with ritonavir to increase its pharmacological profile. In the management of HIV/AIDS lopinavir/ritonavir is used as a combination therapy with other antiretroviral drugs [12,13,14,15]. Lopinavir/ritonavir mediates its antiviral activity by binding the protease enzyme, and preventing the cleavage of the gag and gag/pol polyproteins into structural functional proteins and enzymes thereby preventing the formation of new viral particles [16]. Sulfamethoxazole/trimethoprim mediates its action by causing sequential blockade of the various steps involved in microbial folate synthesis, which is necessary for the formation of purines and, ultimately, of DNA [17,18].

As a result of HIV/AIDS associated co-morbidity and co-infection the concurrent use of antiretroviral and antibacterial drugs is inevitable and in some cases antiretroviral drugs containig lopinavir/ritonavir are concurrently used with sulfamethoxazole/trimethoprim [19,20]. The concurrent use of these drugs may place more toxicological burden on male reproductive function because reports have

associated these drugs individually with decreased testicular function [21,22,23,24] but with no literature on the possible toxicological interaction of the co-administration of these agents on male reproductive function. This study therefore, evaluated the possible effects of co-administered lopinavir/ritonavir and sulfamethoxazole/trimethoprim on reproductive indices of male albino rats.

2. Materials and Methods

Drugs: Lopinavir/ritonavir (LPV/r) used in this work was manufactured by Myland Laboratories Limited India. Other ingredients in the LPV/r tablet include colloidal silicon dioxide, copovidone, sodium stearyl fumarate and sorbitan monolaurate. Sulfamethoxazole/Trimethoprim (SMX/TMP) used in this study was manufactured by CSPC Ouyi Pharmaceuticals China. Other ingredients in SMX/TMP tablets include magnesium stearate, maize starch, silica and sodium lauryl sulphate.

Animals: The animals used in this research work were obtained from the animal house of the Department of Pharmacology and Toxicology, Madonna University, Elele, Rivers State. The animals were allowed free access to food and water *ad libitum* and were allowed to acclimatize for 14 days. Animals were handled according to Helsinki declaration on the handling and use of animals.

Dose Selection: 22.4/4.6mg/kg of sulfamethoxazole/ trimethoprim and 22.8/5.8mg/kg of Lopinavir/ritonavir were used in this study [25,26].

2.1. Preparation of Drug

Lopinavir/ritonavir tablets were crushed and dissolved in 1% ethanol while sulfamethoxazole/ trimethoprim tablets were crushed and suspended in water [27].

2.2. Grouping of Animals

Eighty (80) healthy male albino rats of average weight 300g ±5 were used in this study. The rats were housed in a large mesh cage and divided into five groups A - E.

2.3. Drug Administration

Group A: This served as the control and contained twenty (20) animals which were treated with 1% ethanol orally throughout the duration of study.

Group B: This group contained 15 animals which were further divided into 3 subgroups (B1-B3). Animals in group B1 were treated with 22.4/4.6mg/kg of SMX/TMP. Animals in group B2 were treated with 22.8/5.8mg/kg of LPV/r. Animals in group B3 were treated with combined doses of SMX/TMP + LPV/r. All animals in this group were treated for 2 weeks

Group C: This group contained 15 animals which were further divided into 3 subgroups (C1-C3). Animals in group C1 were treated with 22.4/4.6mg/kg of SMX/TMP. Animals in group C2 were treated with 22.8/5.8mg/kg of LPV/r. Animals in group C3 were treated with combined doses of SMX/TMP + LPV/r. All animals in this group were treated for 4 weeks.

Group D: This group contained 15 animals which were further divided into 3 subgroups (D1-D3). Animals in group D1 were treated with 22.4/4.6mg/kg of SMX/TMP.

Animals in group D2 were treated with 22.8/5.8mg/kg of LPV/r. Animals in group D3 were treated with combined doses of SMX/TMP + LPV/r. All animals in this group were treated for 6 weeks.

Group E: This group contained 15 animals which were further divided into 3 subgroups (E1-E3). Animals in group E1 were treated with 22.4/4.6mg/kg of SMX/TMP. Animals in group E2 were treated with 22.8/5.8mg/kg of LPV/r. Animals in group E3 were treated with combined doses of SMX/TMP + LPV/r. All animals in this group were treated for 8 weeks.

2.4. Collection of Sample for Analysis

Animals were sacrificed using chloroform anesthesia at the end of 2, 4, 6 and 8 weeks of treatment respectively, the testes were harvested, weighed and analyzed for histopathological changes.

2.5. Preparation of Tissue Homogenate

Testicular tissues homogenate was prepared as reported by Nathiya and Nandhini, 2014 [28].

2.6. Biochemical Evaluations

2.6.1. Malondialdehyde and Superoxide Dismutase

Testes malondialdehyde (MDA) concentrations, a lipid per oxidation index, were determined according to Draper and Hadley, 1990. [29] Superoxide dismutase (SOD) activity was estimated according to Beauchamp and Fridovich [30]

2.7. Semen Analysis

2.7.1. Sperm Count and Sperm Motility

The epididymal sperm count and sperm motility were evaluated as reported by WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction [31].

2.7.2. Sperm Morphology

Sperm morphology was evaluated according to the method reported by Wyrobek and Bruce, 1975, [32] Narayana et al., 2002 [33].

2.8. Histopathological Analysis

Histopathological analysis of the testes was performed using standard laboratory technique [34].

2.9. Statistical Analysis

Results were expressed as mean ± S.E.M. Statistical analysis was done with the aid of SPSS for windows; SPSS Inc., Chicago, Standard version 14.0 to determine difference between mean using one way analysis of variance (ANOVA).

3. Results

3.1. Effects on Testicular Weight, Malondialdehyde and Superoxide Dismutase

Exposure to single doses of SMX/TMP, LPV/r, and combined doses of SMX/TMP + LPV/r did not produce any significant ($p>0.05$) change in testicular weight with respect to the control [Table 1]. Treatment with SMX/TMP produced a time dependent increase in MDA level which was significant ($p<0.05$) at 6 and 8 weeks with respect to the control. Significant ($p<0.05$) increase in testicular MDA which represents 92.2% was observed in animals exposed to LPV/r at week 8 when compared with the control. Co-administration of SMX/TMP + LPV/r produced a time dependent increase in testicular MDA with significance (71% and 139% $p<0.05$) at 6 and 8 weeks when compared with the control [Table 2].

Time dependant decrease in testicular superoxide dismutase level was noted in animals exposed to SMX/TMP which was significant at week 8 with respect to the control [Table 3]. Treatment with LPV/r produced time dependent decreases in SOD to 9.83 ± 2.07, 9.52 ± 1.06, 6.23 ± 1.05 and 5.00 ± 1.17 at 2-8 weeks respectively. These decreases were observed to be significant ($p<0.05$) at 6 and 8 weeks with respect to the control.[Table 3] Treatment with combined doses of SMX/TMP + LPV/r produced a time dependent decrease in testicular SOD with significance at 6 and 8 weeks when compared with the control [Table 3].

3.2. Effects on Sperm Count

Results showed that treatment with SMX/TMP produced a time dependent decrease in sperm count with significance (47% and 54 % $p<0.05$) at week 6 and 8 while treatment with LPV/r produced time dependent decrease in sperm count with significance (42 % $p<0.05$) at week 8 with respect to the control. Treatment with combined doses of SMX/TMP + LPV/r produced a significant ($p<0.05$) time dependent decrease in sperm count at weeks 4, 6 and 8 respectively when compared with control [Table 4].

3.3. Effects on Sperm Motility

Treatment with SMX/TMP produced a time dependent decrease in sperm motility which was observed to be statistically significant ($p<0.05$) at week 6 and 8 while treatment with LPV/r decreased sperm motility time dependently with significance (49%, 56 % $p<0.05$) at week 6, and 8 with respect to the control. Results showed that treatment with co- administered SMX/TMP + LPV/r, time dependently and significantly ($p<0.05$) decreased sperm motility at weeks 6 and 8 respectively, when compared with the control [Table 5].

3.4. Effects on Sperm Morphology

Time dependent increase in abnormal sperm morphology which becomes significant ($p<0.05$) at week 6 and 8 with respect to the control was noted in animals treated with LPV/r while treatment with SMX/TMP produced significant increase ($p<0.05$) in abnormal sperm morphology at week 8 with respect to the control. Combined doses of these agents produced time dependant increase in abnormal sperm morphology with significance at week 6 and 8 when compared with the control [Table 6].

## 3.5. Effects on Histopathology of the Testes

The control testes of rats treated with 1% ethanol showed normal seminiferous tubules and maturation stages of spermatocytes [Photomicrograph A]. Testes of rats treated with 22.4/4.6mg/kg of SMX/TMP for 8 weeks showed normal seminiferous tubules but with mildly widened interstitium by edema fluid [Photomicrograph B]. Testes of rats treated with 22.8/5.8mg/kg of LPV/r for 8 weeks revealed thick basement membrane of the seminiferous tubules with loss of maturation stages of spermatocytes and focal cytolysis [Photomicrograph C]. The testes of rats treated with combined doses of SMX/TMP + LPV/r for 8 weeks showed thick basement membrane of the seminiferous tubules with loss of maturation stages of spermatocytes, focal cytolysis and edematous interstitium infiltrated by mononuclear inflammatory cells [Photomicrograph D].
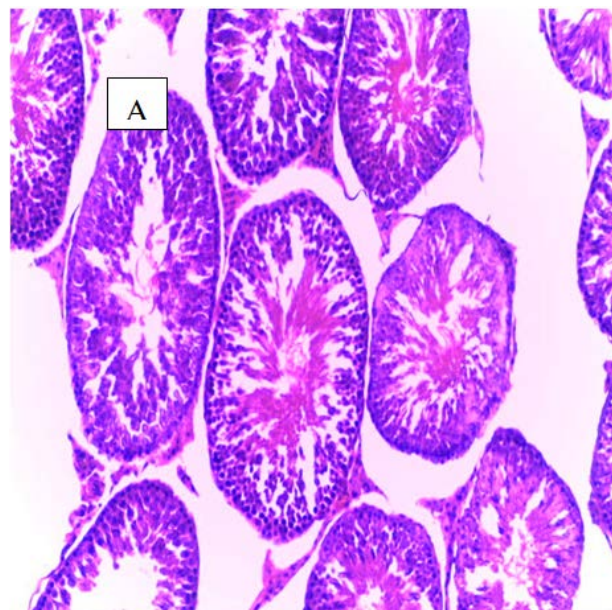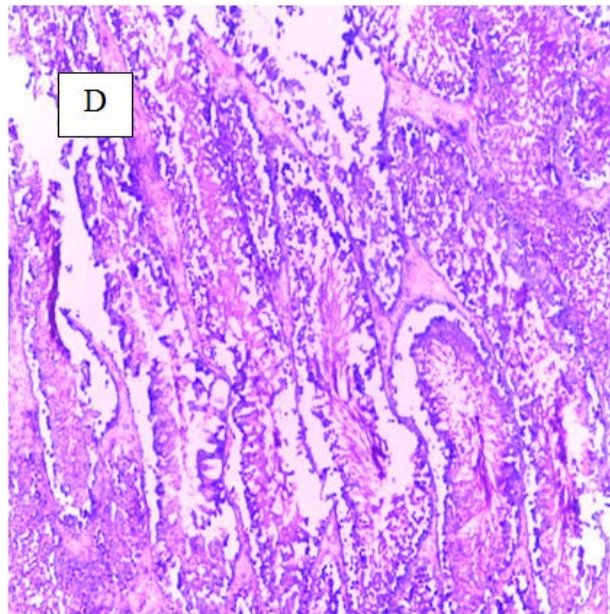

Photomicrograph A. The control testis of rat treated with 1% ethanol showing normal seminiferous tubules and maturation stages of spermatocytes (H&E x400)
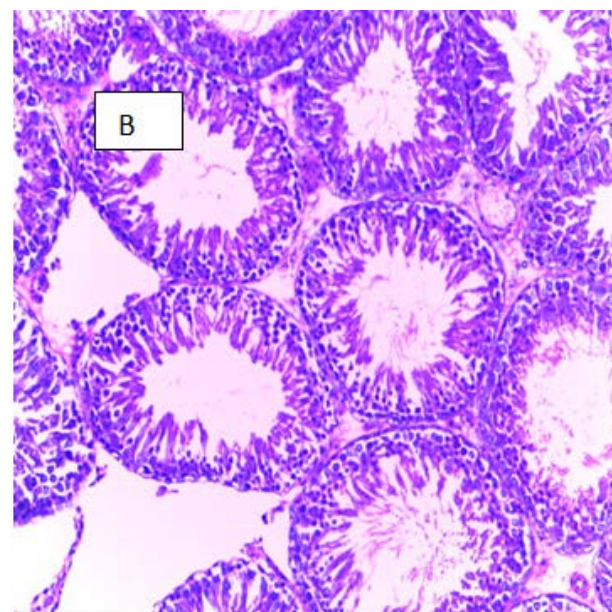
Photomicrograph B. The testis of rat treated with 22.4/4.6mg/kg of SMX/TMP for 8 weeks showing normal seminiferous tubules but with mildly widened interstitium by edema fluid. (H&E x 400)
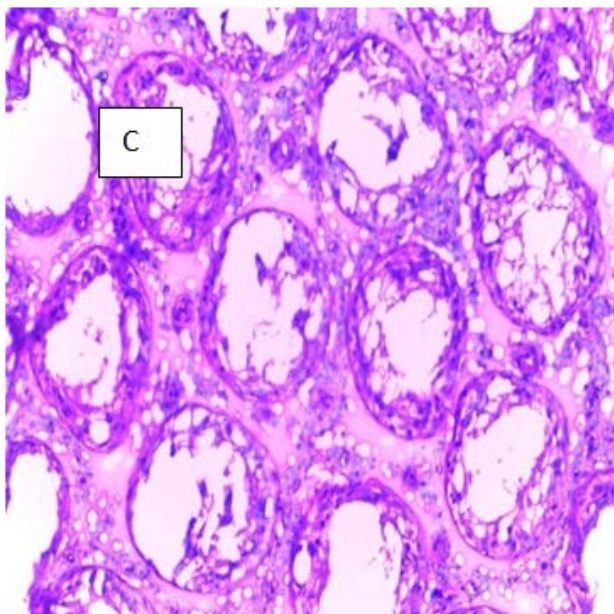
Photomicrograph C. The testis of rat treated with 22.8/5.8mg/kg of LPV/r for 8 weeks showing thick basement membrane of the seminiferous tubules with loss of maturation stages of spermatocytes and focal cytolysis (H& Ex 400)

Photomicrograph D. the testis of rat treated with combined doses of SMX/TMP +LPV/r for 8 weeks showing thick basement membrane of the seminiferous tubules with loss of maturation stages of spermatocytes, focal cytolysis and edematous interstitium infiltrated by mononuclear inflammatory cells. (H&E x400)

Table 1. Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and their combination on testicular weight (gram) in male albino rats

DOSE	WK2	WK4	WK6	WK8
CONTROL	2.12±1.17	2.16±2.04	2.15±1.14	2.18±2.34
SMX/TMP (22.4/4.6mg/kg)	2.00±0.07	2.01±2.17	2.11±0.13	2.09±2.13
LPV/r (22.8/5.8mg/kg)	2.06±1.03	2.00±2.24	2.06±1.18	2.12±1.33
SMX/TMP +LPV/r	2.00±0.26	2.03±0.15	2.04±1.08	2.04±0.25

Table 2. Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and their combination on kidney malondialdehyde (n mole/gram tissue) in male albino rats

DOSE	WK2	WK4	WK6	WK8
CONTROL	6.49±3.13	6.53±2.27	6.51±3.12	6.50±0.23
SMX/TMP (22.4/4.6mg/kg)	6.82±1.I5	7.23±2.14	10.5±2.07*	13.26±2.26*
LPV/r (22.8/5.8mg/kg)	7.14±1.18	8.02±0.13	8.54±3.14	12.54±2.16*
SMX/TMP +LPV/r	7.51±1.24	8.1±2.06	11.10±0.06*	15.5±3.27*

Results are expressed as mean± SEM, n=5, the superscript (*) means significant difference with respect to the control at $p<0.05$(ANOVA).

Table 3. Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and their combination on testis superoxide dismutase (units/gram protein) in male albino rats

DOSE	WK2	WK4	WK6	WK8
CONTROL	10.1±2.40	10.4±1.23	10.1±3.12	10.2±3.31
SMX/TMP (22.4/4.6mg/kg)	10.3±1.15	8.50±2.06	6.01±2.11*	5.53±0.08*
LPV/r (22.8/5.8mg/kg)	9.83±2.07	9.52±4.06	6.23±3.05*	5.00±1.17*
SMX/TMP +LPV/r	9.01±2.02	8.53±1.01	5.91±0.86*	4.02±3.07*

Results are expressed as mean± SEM, the superscript (*) means significant difference with respect to the control at $p<0.05$(ANOVA).

Table 4. Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and their combination on sperm count (million/mil) in male albino rats

DOSE	WK2	WK4	WK6	WK8
CONTROL	61.3±2.12	65.1±4.38	63.5±3.14	65.5±1.27
SMX/TMP (22.4/4.6mg/kg)	63.4±3.16	58.3±2.11	35.1±2.20*	30.3±1.29*
LPV/r (22.8/5.8mg/kg)	60.4.00±1.21	56.2±3.18	53.7±4.21	38.2±2.11*
SMX/TMP +LPV/r	59.4±3.14	40.3±1.01*	30.5±2.21*	27.5±1.00*

Results are expressed as mean± S.E.M, n=5, the superscript (*) means significant difference with respect to the control at $p<0.05$(ANOVA).

Table 5. Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and their combination on sperm motility (%) in rats

DOSE	WK2	WK4	WK6	WK8
CONTROL	66.1±2.14	68.0±0.23	68.2±0.07	68.3±3.16
SMX/TMP (22.4/4.6mg/kg)	65.2±2.08	55.4±2.16	37.2±1.14*	31.6±0.08*
LPV/r (22.8/5.8mg/kg)	64.2±1.13	58.3±0.16	35.2±0.21*	30.1±1.09*
SMX/TMP +LPV/r	59.2±2.04	54.3±0.06	34.6±1.11*	28.7±1.06*

Results are expressed as mean± S.E.M, n=5, the superscript (*) means significant difference with respect to the control at *p*<0.05(ANOVA).

Table 6. Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and their combination on sperm morphology (%) in rats

DOSE	WK2	WK4	WK6	WK8
CONTROL	14.7±3.15	16.4±2.33	15.5±3.45	15.3±2.24
SMX/TMP (22.4/4.6mg/kg)	17.3±2.08	19.5±2.16	22.2±1.14*	35.6±0.08*
LPV/r (22.8/5.8mg/kg)	15.3±1.13	16.2±0.16	18.5±0.21	30.5±1.09*
SMX/TMP +LPV/r	18.1±2.04	18.7±0.06	21.2±1.11*	37.6±1.06*

Results are expressed as mean± S.E.M, n=5, the superscript (*) means significant difference with respect to the control at *p*<0.05(ANOVA).

4. Discussion

Spermatogonia are sensitive to chemical agents interfering with DNA replication because these cells go through several mitotic divisions. Considering the pivotal involvement of spermatozoan functions in fertilization, measuring multiple sperm parameters, evaluation of testicular weight and oxidative stress markers could attest to drug induced testicular damage [35,36]. This study, therefore evaluates the effects of co-administered SMX/TMP+ LPV/r on semen parameters, testicular oxidative stress markers and architecture. Researchers have shown that change in organ weight induced by a chemical agent is a maker of its toxicity [37]. In this study it was noted that treatment with single and combined doses of these agents didn't produce any significant change in testicular weight. Malondialdehyde is the major oxidative product of the peroxidation of polyunsaturated fatty acids. This makes an increase in MDA level observed in this study, in animals treated with single doses of these agents a sign of lipid peroxidation induced by these agents [38,39]. Concurrent use of these agents may not be deleterious to testicular function due to lack of synergistic increase in testicular MDA level when they were co-administered as observed in this study. Testis contains antioxidant enzymes which include SOD to protect itself from the hazardous effects of oxidative attack. In this study decrease in SOD level was observed when single doses of these agents were administered which is in agreement with some reported observations [40,41]. This could be attributed to increased testicular oxidative stress via the generation of ROS [42,43]. Co- administration of these agents may not be toxic to testicular function due to lack of significant synergistic decrease in SOD level when these agents were co-administered as observed in our study. Documented evidence showed that decrease in sperm count is an important indicator of male infertility [44]. Decrease in sperm count associated with exposure to LPV/r agrees with some reported observations [45,46], also SMX/TMP induced decrease in sperm count observed in this study has been reported by some authors [47]. In the absence of significant synergistic decrease in sperm count when these agents were co-administered the concurrent use of these agents may be safe on testicular function. Decrease in sperm count by these agents may be attributed to damage to leydig cells and seminiferous tubules responsible for testosterone and sperm production respectively [48].

Decrease in sperm motility often indicates chemical-induced testicular toxicity, and in men; defect in sperm motility causes untreatable infertility or subinfertility [49,50]. Decrease in sperm motility observed with LPV/r treatment in this work is in agreement with reports from some quarters [51,52] while decrease in sperm motility observed in SMX/TMP treated animals is also supported by some observations [53]. Observation in this study shows that the co-administration of these agents may not have deleterious effect on sperm motility. Decrease in sperm motility noted with these agents could be attributed to their interference with energy production required for sperm motility [54] and damage to mitochondria which are component of spermatozoa required for energy production to sustain sperm motility [55,56,57].

Semen morphology as measured according to strict criteria appears to be the most informative semen measurement for discriminating between fertile and infertile men [58]. Increase in abnormal sperm morphology observed with single doses of LPV/r and SMX/TMP is in agreement with reports from some quarters [59,60,61]. Concurrent use of these agents may be safe on sperm morphology due to insignificant synergistic increase in abnormal sperm morphology when they were co-administered. Observed decrease in sperm morphology in this study could be attributed to inhibition of meiosis of primary spermatocytes to matured sperm cells or direct destruction of sperm cell [62]. Observed abnormal testicular histopathological changes in LPV/r treated animals is consistent with some reported observations [63]. Similar abnormal testicular histopathological changes were noted in SMX/TMP treated animals with more pronounced effects when co-administered with LPV/r. Histopathological changes observed in this study may be associated with testicular oxidative stress leading to damage of testicular micro molecules [64,65]. This could be correlated with observed elevated MDA level and decreased SOD level in this study.

5. Conclusion

In this study, observed decrease in semen quality, testicular superoxide dismutase and increase in

malondialdehyde level correlates with observed testicular histopathological changes. The concurrent use of these agents may be safe on male reproductive function due to lack of synergistic effects on all evaluated parameters when these agents were co- administered.

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