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Original Research Article

Effects of Tacrolimus (Prograf) on the Reproductive Efficiency of Mice

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ABSTRACT

Tacrolimus is an immunosuppressant used after organ transplantation to prevent graft rejection. It involves long-term usage which may be associated with detrimental effects. This research aims to investigate the effects of Tacrolimus intake on the reproductive efficiency of mice. Tacrolimus was administered to the test group in doses of 0.5, 1.0, 2.0, and 3.0 mg/kg, while the control groups (positive and negative) were administered 1 ml/kg of distilled water and 20 % methanol, respectively. Reproductive parameters were measured following standard procedure. Male mice in the test group experienced a significant decrease ($P<0.05$) in their testes, epididymis, and sperm count compared to the control group. The oestrogen levels and gestation index of female mice in the test groups were significantly decreased ($P<0.05$) in a dose-dependent manner, along with the litter size and weight, in relation to the control group. However, the life birth index of mice did not vary significantly ($P>0.05$). There was a significant decrease ($P<0.05$) in the mean values of male and female reproductive parameters. This study has showcases that Tacrolimus capsule intake could cause reproductive organ malfunction and impair potency and fertility.

Keywords: Reproductive Toxicology, Immunosuppression, Reproductive parameters, Tacrolimus.

Introduction

The primary purpose of deliberately induced immunosuppression is to prevent the human body from rejecting an organ transplant. However, it can also be used for treating diseases caused by graft versus-host infection after a bone marrow transplant, or for the treatment of immunological ailments like rheumatoid arthritis or Crohn's disease.[1] Tacrolimus is a commonly used immunosuppressant which come in different brand names like Hecoria, Prograf, FK-506, Astragraf XL, Envarsus XR, Hecoria, Prograf, and FK-506. Tacrolimus are known for preventing the body from rejecting a transplanted kidney or liver. It may also be used to treat rheumatoid arthritis when the usual therapies have failed to work.[2] Though the white blood cells transplanted organ such as liver or kidney can be fought or rejected by the immune system, the action of Tacrolimus is to lower the activity of white blood cells in the body and inhibit the action of T lymphocytes. It acts as an immunosuppressant by binding to FK506-binding protein, also known as FKBP, in T-lymphocytes. The complex formed binds to calcineurin, which impedes its phosphatase activities, resulting to nuclear factor of activated T cells (NFAT) from being dephosphorylated[2].

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This prevents the displacement of NFAT from cytoplasm to the T cell nucleus and subsequently inhibits the gene transcription factors that are vital for activation of early T cell. This results in the obstruction of T lymphocyte reaction, the formation of cytotoxic T cells, and B cell activation that is reliant on T cells. These actions collectively suppresses the immune system which prevents it from attacking the new organ as an invader.[3] Orally, Tacrolimus can be administered as capsules that contain 0.5 mg, 1.0 mg, 3.0 mg, and 5.0 mg of anhydrous Tacrolimus. Lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate are the ingredients that are not active in Tacrolimus.[2] Gelatin, titanium dioxide, and ferric oxide form the shells of the 0.5 mg and 5.0 mg capsules, while gelatin and titanium form the shell of the 1 mg capsule. Tacrolimus is also available in protopic form, for instance, in 0.3 and 0.1%. Tacrolimus can also be taken in combination with some other drugs like azathioprine and mmF /IL -2 receptor antagonist.[1,2]

It has also been noticed that Tacrolimus usage is not without side effects, which have been found to be based on the duration of treatment and type of immunosuppressant used.[2] Male kidney transplant recipients' fertility capacity was correlated with the time after transplantation and the amount of immunosuppressive agents utilized during fertilization.[4,5] Effects are developed about two years post transplantation, when immunosuppressive dosages are decreased.[3] A number of reports have also shown that apart from a change in macrophage polarization, Tacrolimus also affects monocyte function.[6,7] A burning or itching sensation on the first applications, less common symptoms like flu, headache, cough, and burning eyes are the most prevalent negative effects of using protopic form (ointment), particularly if applied over a large area.[8]

Generally, the quality of life of those who undergo kidney transplant is better than those on dialysis. Sexuality is an aspect of life that seems not to improve after transplant.[9] However, several reports have

revealed that nearly 50 % of those that underwent kidney transplant have sexual functioning problems.[9] However, not much has been recorded on the effect of Tacrolimus capsules on reproduction despite the increasing patronage due their efficient immunusuppression and reduced record of graft rejection in patients. Few of the recorded reports on Tacrolimus are one-sided, either on males or on females. Therefore, this research is focused the effects of Tacrolimus intake on the reproductive efficiency of reproducing mice. This is to ascertain the fate of organ-reproducing transplant patients, who are most often faced with the challenge of ingesting this drug for up to six months post-transplant.

Materials and method

Ethical approval
The ethical permission for the management of experimental animals was approved by the Institutional Research and Ethics Committee of the University of Nigeria Nsukka (IREC UNN); IREC223UNN

Animal model
A total of 72 virgin mice between three and four months of age and weighing 29 and 35 g were used. They consist of 18 males and 54 females. The entire animal model was procured from the genetics and animal breeding unit of the Zoology and Environmental Biology Department, University of Nigeria, Nsukka. The rats were fed commercial feed (Grand Brand) with 30 % crude protein ad-libitum. The mice were given access to food and water and were acclimatized for two weeks in clean mouse cages.

Procurement and administration of drug
Different concentrations of prograf (Tacrolimus) capsules were obtained from Panacea Biotec Pharmacy. Doses of 0.5, 1.0, 2.0 and 3.0 mg/kg of Tacrolimus were administered to the mice. Each concentration used was dissolved in 20% methanol. Thereafter, the drug concentrations were measured out with 1mL syringes and administered orally to the mice according to their body weights using oral gavage.

LD_{50} Determination
The lethal dose (LD_{50}) of the extract was determined according to the method of.[10]

First phase
Three groups of three mice were used in this study and were administered 500, 1000 and 2000 µg/kg of Tacrolimus. The mice were observed for toxic effects as well as mortality for a period of 24 hours.

Second phase
Three mice were grouped into three groups, with one mouse assigned to each group. They were monitored for 24 hours after they were given 5, 15 and 30 mg of Tacrolimus per kilogram.
LD_{50} was determined using the formula:

$LD_{50} = \sqrt{(D_0 \times D_{100})}$ ……Equation 1

D_0 = Highest concentration that did not cause mortality, D_{100} = Lowest concentration that caused mortality.

Experimental design
A completely randomized experimental design was adopted. A total of 72 adult mice (54 females and 18 males) weighing between 29 to 35 g were used for the experiment. The mice were grouped into 12 before mating, which were further divided into two major groups (6 groups for males and 6 groups for females). Each major group comprises a positive control, a negative control and four treatments, consisting separately of three males and nine females. The positive and negative control group in either sex were administered 1 ml/kg of distilled water and 20% methanol, respectively, while 0.5, 1.0, 2.0 and 3.0 mg/kg of Tacrolimus (Trade name: Prograf) were administered to the treatment groups for 15 days. Thereafter, the mice were regrouped into six groups of 12 mice (three males and nine females) which consist of three replicates of one male and three female. At this point, males and females of the same treatment groups were kept together for mating for 84 days at the ratio of 1:3.

Determination of body weight of adult mice
The body weights of the adult test animals were determined individually in grammes (g) using a sensitive weighing balance (Mettler 2000, China). This was carried out before the commencement of the treatment, at five days intervals as treatment progressed and finally, at the end of the study.

Determination of testes weight of male mice
The weights of the dissected testes of male mice were determined at the end of the study using a sensitive weighing balance (Mettler 2000). The testes weights were determined in grammes (g).

Determination of litter size and weight
The litter size for each female was determined by counting the number of young ones (dead and alive) immediately after delivery. Similarly, the litter weights were determined by weighing immediately after birth. The whole young ones delivered by each mother were weighed using a sensitive weighing balance (Mettler 2000) in grammes (g).

Determination of Gestation Index
The gestation index was determined by multiplying the number of live litters born in a group by 100 and then dividing by the number of animals present in that group. These were summed up and calculated at the end of the study using the formula of.[11]

$$\text{Gestation Index} = \frac{\text{Number of litters} \times 100}{\text{Number of animals in a group}} \quad \text{Equation 2}$$

Determination of live birth index
The live birth index was determined by multiplying the total number of live offspring at Postnatal day 1 (PND1) by 100 and then dividing by the total number of offspring born. This will be summed up and calculated after the study according to.[12]

$$\text{Life Birth Index} = \frac{\text{Total no. of life offspring at PND1} \times 100}{\text{Total no. of offspring born in a group}} \quad \text{Equation 3}$$

Determination of sperm count in adult male mice
The sperm counts of the adult male mice were examined at the end of the experiment. Sperm solutions were prepared in accordance with the methodology of.[13] The epididymis were minced and filtered in 2 mls of physiological salt solution. The smears of sperm cells were prepared, viewed, and counted using Neubauer chamber as recommended by.[14]

Determination of oestrogen level in female mice
The oestrogen levels of the female mice were determined using procedure as described in DSI kit of oestrogen hormonal assay.

Statistical analysis
The Generated data were statistically analyzed using SPSS version 17. Data were presented as Mean ± Standard Error of Mean. One-way analysis of variance was used to evaluate the effect of treatment, while Duncan's Multiple Range Test (DMRT) was used to test the significant differences among treatment groups. Linear regression was used to ascertain the relationships between variables. between Significance levels were set at $P < 0.05$.

Results and Discussion

The toxicity test result of the adult mice (29 – 35 g body weight) treated with Tacrolimus was shown in figure 1. No mortality was recorded in 0.5, 1.0, 2.0 and 5.0 mg/kg of Tacrolimus administered mice, though there were observable side behaviours such as panting and restlessness in those mice administered 5 mg/kg. There was a

record of mortality in the animal group administered 15 and 30 mg/kg within 24 hours. However, the lethal dose of Tacrolimus was found to be 8.66 mg/kg. This is an indication that higher dose of Tacrolimus could be harmful if precautionary measures are not taken. Table 1 displays the effects of tacrolimus on the average weight of male mice, both 15 days into the treatment and 84 days after. When male mice treated with 2.0 and 3.0 mg/kg of Tacrolimus were compared to the control group, their mean weight considerably dropped ($P < 0.05$). From day one to day five of treatment, there was a characteristic loss in weight and appetite and a record of diarrhoea, which dropped significantly ($P < 0.05$) with time in each of the treatment groups. The reduction in weight was more noticeable from day 10 of the treatment in all the treatment groups. The results of the effect of Tacrolimus on the body weight of female mice are shown in table 3. There was an observable overall fluctuation in the weight of female mice in the entire treatment groups across the duration, with the exception of the animals given 0.5 and 1.0 mg/kg. The body weight of female mice decreased significantly ($P < 0.05$) throughout a period of time from day 5 to day 15 in comparison to the control group. The mice given 3.0 mg/kg exhibited diarrhoea, loss of appetite, and weight loss with a general weakness at the first five days of administration. When compared to the control group, the mice given 0.5 mg/kg and 1.0 mg/kg did not exhibit a significant change in body weight at day 5, while significant increase ($P < 0.05$) was found in the weight of the mice given 2.0 and 3.0 mg/kg. In contrast, mice given 2.0 and 3.0 mg/kg showed no significant change in body weight at day 10 ($P >0.05$), whereas mice given 0.5 and 1.0 mg/kg showed substantial declines in body weight ($P <0.05$) in comparison to the control group. Additionally, at day 15, the body weight of female mice given 0.5, 1.0, and 2.0, 3.0 mg/kg, respectively, showed substantial increases and decreases ($P < 0.05$).

Table 1: Effect of Tacrolimus on mean body weight of adult male mice during treatment

Treatment (mg/kg)	**Initial weight**	**Duration of treatment**			**84 days post treatment**
		5 days	10 days	15 days	
0.5	35.68 ± 8.87^{a2}	36.06 ± 8.15^{ab2}	33.50 ± 8.80^{b1}	33.36 ± 8.67^{bc1}	37.19 ± 0.40^{ab}
1.0	39.62 ± 7.09^{a2}	40.79 ± 6.16^{b2}	37.01 ± 0.00^{bc1}	38.73 ± 5.52^{bcd1}	37.02 ± 0.26^{ab}
2.0	30.46 ± 3.25^{a3}	26.56 ± 0.93^{a2}	24.19 ± 0.84^{a1}	23.94 ± 0.77^{a1}	33.33 ± 2.29^{b}
3.0	33.01 ± 10.06^{a3}	30.29 ± 7.51^{a2}	29.45 ± 6.44^{ab2}	24.84 ± 1.24^{a1}	39.62 ± 4.79^{a}
Control (-ve)	34.81 ± 3.39^{a2}	33.50 ± 4.48^{ab2}	32.96 ± 5.19^{ab2}	31.31 ± 5.08^{abc1}	36.08 ± 3.23^{ab}
Control (+ve)	40.03 ± 1.04^{a1}	41.88 ± 0.79^{b1}	43.42 ± 0.66^{c2}	44.78 ± 0.52^{d2}	45.21 ± 0.24^{c}

Mean values with different alphabets are significantly different ($P < 0.05$) within the column and mean values with different numeric superscript are significantly different ($P < 0.05$) across the row. Control (+ve) =animals fed with water only. Control (-ve) = animals fed with only 20% methanol

Table 2: Effect of Tacrolimus on reproductive parameters of male mice, 84 days post treatment

Treatment groups (mg/kg)	**Testes weight (g)**	**Epididymal weight (g)**	**Sperm count (10^6/ml)**
0.1	0.115 ± 0.000^{b}	0.113 ± 0.004^{b}	$262.5\pm10.6b^{c}$
1.0	0.100 ± 0.007^{bd}	0.103 ± 0.004^{ab}	$233.5\pm16.3b^{c}$
2.0	0.083 ± 0.003^{a}	0.081 ± 0.001^{ab}	208.67 ± 9.81^{b}
3.0	0.075 ± 0.005^{a}	0.062 ± 0.0109^{a}	126.3 ± 69.88^{a}
Control (-ve)	0.132 ± 0.007^{bc}	0.180 ± 0.047^{c}	312.67 ± 65.4^{c}
Control (+ve)	0.213 ± 0.015^{d}	0.313 ± 0.012^{d}	561.67 ± 56.2^{d}

Mean values with different alphabets are significantly different ($P<0.05$) within the column
Control (+ve) =animals fed with water only. Control (-ve) = animals fed with only 20 % methanol

The immune system's ability to be suppressed by tacrolimus has enhanced the rate of graft survival in the field of transplant medicine and life support in general.[1] Tacrolimus capsule intake as a remedy for graft rejection is accompanied with some side effects in patients, of which the reproductive effect is one of them.[2] However, the dose-dependent weight loss seen in the immunosuppressed mice may be attributed to the recurrent diarrhea and loss of appetite observed in those receiving treatment.[15] This results corroborates a similar works done with Tacrolimus ointment [16]. The effect of Tacrolimus on the mean sperm count, testes, and epididymal weight of male mice after 84 days post-treatment was shown in table 2. All other groups were considerably ($P < 0.05$) lower than the control group, with the exception of the testes weight of the group provided at 1 mg/kg, which did not differ from the positive control group. Furthermore, compared to the control group, there was a dose-dependent significant decrease ($P < 0.05$) in the mean number of sperm and weight of the male mice's epididymis in the treatment group. Significant decline observed in sperm count sperm count could be attributed to reduced epididymal and testicular content.[17] The testicular abnormalities linked to the use of two other widely used immunosuppressants, sirolimus and cyclosporine, are relatively mild when compared to the severe effects observed with tacrolimus.[18] Prograf (Tacrolimus) has been shown to reduce sperm counts and motility in a dose-dependent manner, although it had no effect on the amount of testosterone in mice's serum. [17] However, report showed that reduction in weight of testes and epidydimis with the use of immunossupressant Bisphenol was noticed at levels as low as 20 µm/kg (parts per million) while, with FK-506, sperm are sparse often with aberrant morphology, and they have reduced fertilizing capacity.[19] FK-506 may alter the morphology of the sperm head, resulting in decreased fertility, loss of the embryo, and heritable alterations in male germ cells.[18] It was discovered that extended FK-506 treatment caused abnormalities in the seminiferous tubules, which led to spermatogenic damage and sperm cell degeneration in the epididymis.[18] Table 4 shows the result of the effect of Tacrolimus on the weight of reproducing females, litter size and weight during the 84 days treatment. When compared to the control

group, the weight of the Tacrolimus-treated reproducing female mice did not significantly ($P < 0.05$) decrease. Furthermore, when comparing the mice given 3.0 mg/kg, 2.0 mg/kg, and 1.0 mg/kg of Tacrolimus to the control group, the litter size and litter weight were significantly reduced ($P < 0.05$), with the exception of the animals given 0.5 mg/kg.

Table 3: Effect of Tacrolimus on mean body weight of adult female mice during treatment

Treatment groups (mg/kg)	Initial weight	5 days	10 days	15 days
0.1	30.14±1.65^{a2}	27.14±2.21^{a2}	25.29±2.69^{a1}	25.34±2.87^{a1}
1.0	29.96±1.10^{a3}	26.29±3.89^{a2}	25.79±3.14^{a2}	25.29±2.77^{a2}
2.0	30.15±2.07^{a3}	32.29±2.95^{b2}	37.98±3.19^{b3}	29.16±1.22^{c1}
3.0	30.11±2.71^{a1}	35.48±3.37^{b2}	30.43±1.93^{bc1}	41.89±1.61^{d3}
Control (-ve)	31.68±2.78^{a2}	25.76±2.39^{a1}	25.91±2.14^{a1}	26.41±2.88^{ab2}
Control (+ve)	31.53±1.79^{a1}	28.76±3.69^{a1}	29.02±3.67^{b1}	28.17±2.58^{b1}

Mean values with different figures are significantly different ($P < 0.05$) across the row while, mean values with different numeric superscript are significantly different within the same column. Control (+ve) =animals fed with water only. Control (-ve) = animals fed with only 20% methanol

Table 4: Effect of Tacrolimus on reproductive parameters of female mice 84 days post treatment

Treatment (mg/kg)	Weight of reproducing female (g)	Litter size	Sperm count (10^6/ml)	Litter weight (g)
0.5	34.81±4.14^{a}	8.96±3.53^{b}	262.5±10.6b^{c}	16.06±5.58^{b}
1	35.66±4.03^{a}	5.06±3.02^{a}	233.5±16.3b^{c}	9.51±5.73^{a}
2	36.19±3.31^{a}	4.56±3.03^{a}	208.67±9.81^{b}	7.82±5.63^{a}
3	35.57±3.36^{a}	4.62± 3.56^{a}	126.3±69.88^{a}	7.79±6.11^{a}
Control (-ve)	35.75±4.80^{a}	8.57±3.82^{b}	312.67±65.4^{c}	16.29±6.59^{b}
Control (+ve)	42.73±1.42^{b}	10.00±2.48^{b}	561.67±56.2^{d}	20.32±3.36^{c}

Mean values with different alphabets are significantly different ($P < 0.05$) across the row
Control (+ve) =animals fed with water only. Control (-ve) = animals fed with only 20% methanol

This decline may have contributed to the foetal deaths of some mice given tacrolimus at doses of 3.0 mg/kg, 2.0 mg/kg, and 1.0 mg/kg. Records of foetal death in the early stage of the study, though minimal could probably due to the duration of treatment. Malformations and death of fetuses at birth, as recorded in this study in the 3mg/kg treatment groups, could be attributed to FK506-binding protein being enriched in the foetal brain. Stimulation of such site by FK506 (Tacrolimus) through its intake in pregnant and lactating mothers causes changes in the foetal brain.[20] This could be as a result of poor implantation, as was noted by [21] at high doses of FK506 compared with control. These results corroborates the meta-analysis findings, which confirmed this through obvious risks of congenital malformations, low birth weight, or preterm delivery from cyclosporine immunosuppressant treatment during pregnancy.[20,22]

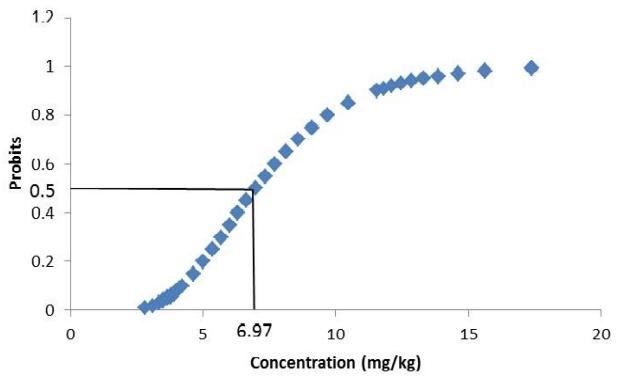

Figure 1. Probit-concentration plot showing LD_{50} of Tacrolimus

Figure 2 shows a linear representation of the correlation between the testes and body weight of male mice. The model equation is TW = 0.0069BW - 0.1398, where TW represents the testes weight and BW, the body weight. The testes weight of adult male mice showed a positive correlation ($r = 0.632$, $P < 0.01$, $n = 15$) with the body weight. The weight of male mice explains 40.68% variation in the testes weight of male mice ($R^2 = 0.4068$). This observation showed that the testes weight of adult male mice is dependent on their body mass. In other words, larger male mice tend to possess larger testes, which ultimately enhance the reproductive activity of such individuals. The linear representation of the relationship is shown in figure 3. The model equation is EW = 0.0119BWM – 0.3079, where EW is epididymal weight and BWM is body weight of male mice.

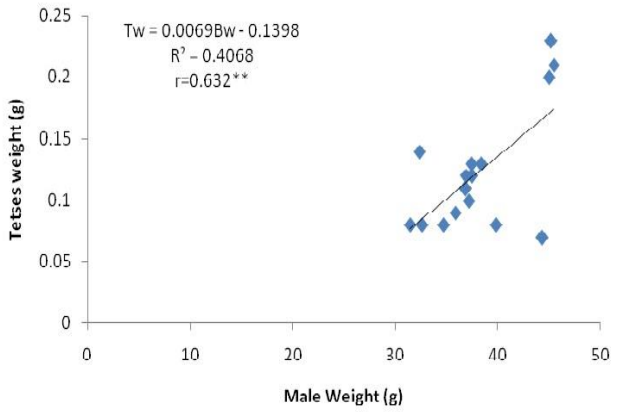

Figure 2: Linear representation of the Correlation between testes weight and body weight of male mice.** significant at P < 0.01

Male mice's body weight and epididymal weight showed a positive relationship (r = 0.551, P < 0.05, n = 15). The Weight of the male mice explained 30.4% variations in epididymal weight. This is an indication that the epididymal weight is dependent on the body weight of the adult male mice. That is, larger males are likely to have heavier epididymis which ultimately enhances their reproductive efficiency. The relationship between the sperm count and the body weight of male mice is shown in figure 4. The model equation as shown in figure 5 is SC = 16.232BWM – 329.88, where: SC is sperm count and BWM is the body weight of adult male mice. There was a positive relationship (r = 0.493, P > 0.05, n = 15) between the weight and sperm count of mature male mice. There was no discernible difference (P > 0.05) in the link between the male mice's weight and sperm count. The body mass of male mice had a low prediction (24.3%) of sperm count. In other words, sperm count of male mice is not dependent on the body weight.

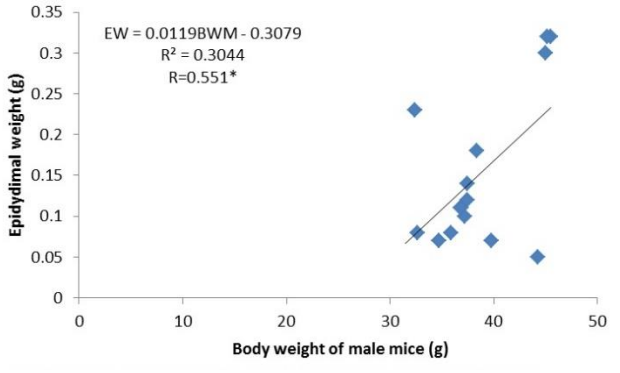

Figure 3: Linear representation of the Correlation between epididymal weight and the body weight of adult male mice. * significant at P < 0.05

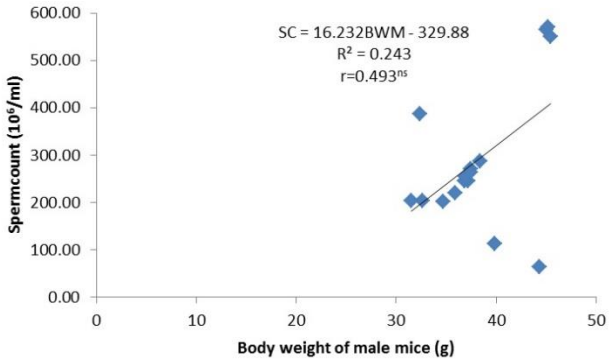

Figure 4: Linear representation of the Correlation between sperm count and Body weight of adult male mice. ns = not significant

Figure 5 shows the relationship between the sperm count and the body weight of male mice. The model equation is as shown in Figure 6, where: SC is sperm count and TWM is the testes weight of male mice. There was a positive correlation (r = 0.967, P > 0.05, n = 15) between the weight and sperm count of mature male mice. The weight and sperm count of male mice, however, showed a significant difference (P < 0.05) in their relationship. The body weight of male mice had a high prediction (93.47%) of sperm count. In other words, sperm count of male mice is dependent on the testes weight. Figure 6 has a succinct explanation of the effect of Tacrolimus on the oestrogen level of mice after 15 days of treatment. Comparing to the treatment groups, there was a substantial overall rise (P < 0.05) in the oestrogen level of the control. A dose dependent effect of Tacrolimus on the female oestrogen levels was noticed in the drastic fall in the oestrogen of those mice administered doses above 0.5 mg/kg after 15 days of treatment. The correlation between the mature female mice's weight and oestrogen levels is represented linearly in Figure 7.The model equation is as follows: OL = 0.4519BWF – 9.7566, where OL is the oestrogen level and BWF is the body weight of adult female mice. The relationship between oestrogen level and weight of female mice was highly significant (P < 0.01, n = 45). The weight of female mice had a strong positive correlation (r = 0.861) with the oestrogen level. The percentage prediction of oestrogen levels from the weight of female mice was 74.05%. This observation shows that the oestrogen level is dependent on the body weight of adult female mice. In other words, the higher the weight of female mice, the higher the oestrogen level.

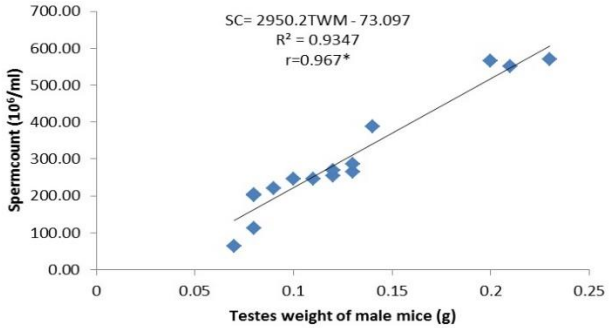

Figure 5: Linear representation of the Correlation between sperm count and testes weight of adult male mice. * significant at 0.05

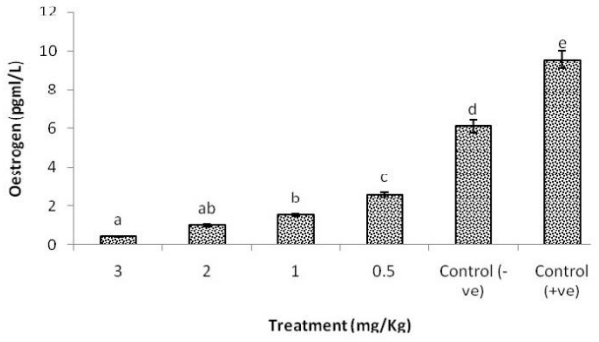

Figure 6: Effect of Tacrolimus on the oestrogen level of adult females mice after 15 days of treatment
Mean value with different alphabets are significantly different ($p<0.05$).

The effect of Tacrolimus on the gestation and live birth index of adult female mice after 84 days post-treatment is shown in figure 8. Between the live birth indexes of the treatment groups and the controls, there was no overall significant difference ($P > 0.05$). As opposed to the positive control, treatment groups' gestation indices decreased significantly ($P < 0.05$). Nonetheless, compared to treatment groups given 0.5 mg/kg, animals receiving large doses (3.0 mg/kg, 2.0 mg/kg, and 1.0 mg/kg) had significantly lower gestation indices ($P < 0.05$). Immunossuppressants and steroids can lead to irregular menstrual cycle and infertility.[23] The pituitary gland could be blocked by these drugs from secreting sufficient female sex hormones (FSH and LH) for regular ovulation to take place.[23] Malformations of foetuses and death at birth were recorded in this study at the 3mg/kg treatment groups.

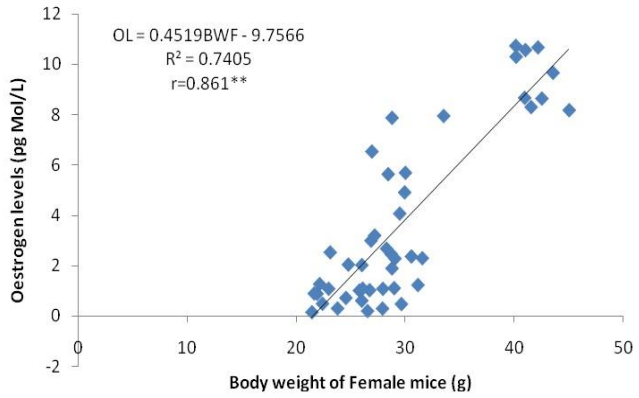

Figure 7: Linear representation of the Correlation between Oestrogen level and body weight of adult female mice. ** significant at 0.01

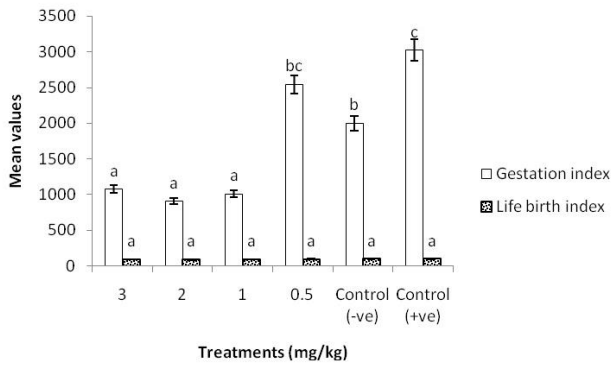

Figure 8: Effects of Tacrolimus on the gestation and live birth index of adult female mice 84 days post treatment**.** Mean values with different alphabets are significantly different ($P < 0.05$)

The fluctuations in the gestation index of female mice in the groups administered 3.0 mg/kg of Tacrolimus and their inability to give birth even when males were introduced at the same time with others could be due to frequency of menstrual alteration, which was above other groups exposed to the immunossupressants.[7] The non-significant difference in the life birth index of all the treatment groups, very low record of foetal mortality and still births could be associated with implantation loss.[20]

The linear representation of the relationship between the litter size and body weight of female mice is shown in figure 9. The model equation is as follows: LS = 0.1461BWF + 1.8505, where LS is the litter size and BWF is the body weight of adult female mice ($P > 0.05$, $r = 0.171$, $n = 45$). However, the weight of female mice can only predict 2.93% variation in the size of litter. This shows that the litter size of mice is independent of the weight of adult female mice.

Figure 10 shows the linear representation of the correlation between the litter weight and female weight. There was a noteworthy positive correlation ($P < 0.01$, $r = 0.243$, $n = 45$) between the weight of adult female mice and the weight of the litter. The equation of the model is as follows: LW = 0.3851BWF – 0.6634, where LW is the litter weight and BWF is the body weight of female mice. The percentage prediction of litter weight from weight of adult female mice was low (5.89%). This shows that litter weight is not dependent of body weight of adult female mice.

This result is in line with the already established normal oestrogen level (9.3 - 28.9 pgml/L) for non-breeding females.[24] High oestrogen level above 9.3 was recorded in the positive control and less in the higher dose (above 0.5mg/kg) treatment groups. This could be as a result of the interaction between Tacrolimus and oestrogen which was discovered to hamper the 2- hydroxylation or 17-oxidation of Estrogen in the liver.[25] This could act as a trigger that can lead to a hike in the estradiol secretion, which may result to gynecomastia.

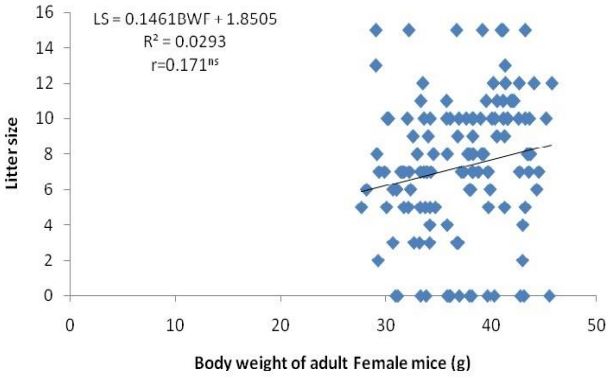

Figure 9: Linear representation of the Correlation between litter size and Body weight of adult female mice. ns = not significant

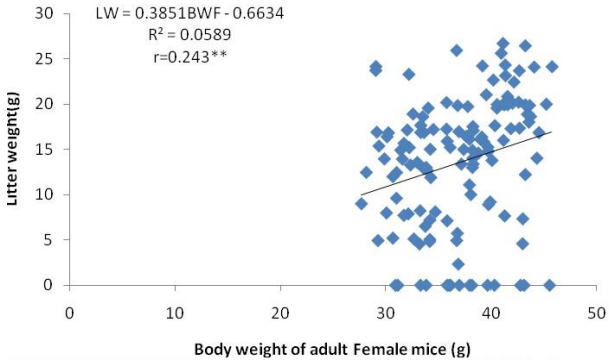

Figure 10: Linear representation of the Correlation between litter weight and body weight of adult female mice. ** significant at 0.01

Conclusion

Duration of treatment and doses were analyzed to determine the effects of Tacrolimus on the reproductive efficiency of mice. The significant changes in the reproductive parameters indicated that the intake of Tacrolimus had notable effects on the reproducing mice. Future research is underway to thoroughly assess the genetic responses of mice treated with Tacrolimus constituents which may provide answers to unclear conditions recorded after its use and to validate its intake comprehensively

Conflict of Interest

The authors declare that there is no conflict of interest.

Declaration of Authors

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by authors.

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