Effects of Ethanolic Seed Extract of *Dacryodes Edulis* on the of Paraquat Induced on Testicular Toxicity in Male Adult Wistar Rats

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ABSTRACT

Dacryodes edulis, a multipurpose plant contains high concentrations of antioxidants, anti-inflammatory agents that protect against tissue damage. The study was aimed at determining the ethanolic seed extract of *dacryodes edulis* on the of paraquat induced on testicular toxicity in male adult wister rat. Fifty-four adult male albino wister rat weighing between 150-180g were used for the study. LD 50 was determined for both dacryodesedulis and paraquat. The rats used for this experiment were distributed into eight groups. Group A (control group) while B C D E F G H were the treated group. (group B paraquat 0.1ml only for 4 weeks, group C paraquat only for 2 weeks, group D paraquat + 500mg/kg of dacryodes edulis, group E paraquat only for 2 weeks and discontinued, Group F paraquat +1000mg/kg of dacryodes edulis for 4 weeks’ group G and H received 500mg/kg and 1000mg/kg of dacryodes edulis for 4 weeks. At the end of the experiment animals were anesthetized and samples were collected for assessment. The result from this study shows that paraquat produces destructive effects on testes evaluations. There was significant increase in LH, FSH testosterone level, was high in group C E F. There was also a significant decrease in the body weight and relative organ weight throughout the period of administration. Histopathological finding reveals distortion of the testicular tissues with mild toxicity in group B. The treated group showed sign of recovery and reversal effect of paraquat. In conclusion the ethanolic seed extract of dacryodes edulis possess ability to improve sperm morphology in paraquat toxicity.

KEYWORDS: Testes, Toxicity, Dacryodes Edulis, Ethanol.

INTRODUCTION

*Dacryodes edulis* (African pear tree) is a tropical oleiferous fruit tree that possesses enormous potential in Africa (Kengué, 1990). It is commonly known as Ube by the Igbos, Mzembe by the Tivs of Nigeria (Burkill, 1985). Various parts of the plantare used in traditional medicine to treat several diseases in different areas (Okafor, 1983; Duru et al., 2012). The fruits are edible, and the bark, leaves, stem, and roots are employed for a variety of purposes (Neuwinger 2000; Jirovetz et al., 2003,and Warhiu et al., 2004). The bark resin is used in Nigeria to treat parasitic skin disease and jiggers (Hutchinson, 1963). Seeds of *Dacryodes edulis* are chewed by the Tiv people of Nigeria as a remedy for stomach problems like diarrhoea, dysentery etc (Ajibesin, 2008), the wood serves for firewood and carpentry (Ndoye et al., 1997), while the entire tree is used in agroforestry systems for soil conservation, fertility, shade and apiculture (Ndangang, 1989). *Dacryodes edulis* fruit or safou is popular in the diets of many Africans. It can be eaten raw, roasted or boiled in hot water, and is eaten alone or used in garnishing cooked or roasted maize. It could also be used as spread to eat bread (Duru et al., 2012). *Dacryodes edulis* has a potential to improve nutrition and food security (Ayuku et al., 2000). Paraquat (1, 1’-dimethyl-4, 4’-bipyridilium dichloride - PQ), is one of the most widely used herbicides and holds a large share of the global herbicide market till today, it is a non-
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selective quaternary nitrogen herbicide, commonly used as a desiccant and defoliant in a variety of crops all around the world (Dasta, 1978; Bismuth et al., 1982, Bismuth et al., 1990; Raghu et al., 2013). Paraquat is also known as methyl viologen because of its dark blue–green colour (Dinis-Oliveira et al., 2008). It has been considered as a toxic compound over the past 60 years, which is why it is classified as a moderately hazardous herbicide and placed in class II poison for acute toxicity (WHO, 2009). Paraquat was found to be highly toxic towards animals and humans with fatalities being reported by Kelly et al., 1978 and Florkowski et al., 1992. The main risks are due to deliberate dose dependent ingestion resulting in multiple organ failure and death (Florkowski et al., 1992). Other routes of toxic exposure are inhalation, ocular and skin contacts (Bataller et al., 2000; Baharuddin et al., 2011). Toxicity resulting from skin exposure is more common in concentrated forms and causes irritation while prolonged contact leads to severe systemic toxicity or even death (Bataller et al., 2000; Marrs and Adjei, 2003).

Paraquat mainly affects the lungs, where it accumulates at up to 6–10 times the plasma concentration, sequestered in pulmonary type I, type II and Clara cells (Krieger and Krieger, 2001; Cope et al., 2004; Shuler et al., 2004; Dinis-Oliveira et al., 2008). Oxygen-free radicals are formed resulting in acute alveolitis 1–3 days’ post-exposure. Tachypnoea, dyspnoea and cyanosis begin from 2 to 7 days’ post-exposure. If the affected animal or human survives, diffuse alveolar septal fibrosis and compensatory type II pneumocyte hyperplasia develop followed by pulmonary fibrosis (chronic phase). Refractory hypoxaemia and eventual death occur from 5 days to several weeks later (Geller and Messonnier, 1998; Cope et al., 2004; Dinis-Oliveira et al., 2008; Gawarammana and Buckley, 2011).

MATERIALS AND METHOD

This study was conducted in the Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State. The animals were acclimatized for two weeks and the actual experimental protocol lasted for 4 weeks.

RESULTS AND DISCUSSION

Table 4.1 shows the effect of ethanolic seed extract Dacryodes edulis on paraquat-induced toxicity on body weight

| Group | Initial Weight (g) | Final Weight (g) | Difference in Weight (g) | Percentage of Weight Difference (%) | P-Value | T-Value |
|-------|-------------------|-----------------|-------------------------|-------------------------------------|---------|---------|
| A     | 150.00 ± 4.80     | 230.00 ± 12.90  | 80.00                   | 53.33                               | 0.02    | -5.06   |
| B     | 213.33 ± 14.52    | 163.33 ± 3.33   | -50.00                  | -23.43                              | 0.10    | 2.88    |
| C     | 175.00 ± 8.66     | 167.50 ± 2.50   | -7.50                   | -4.29                               | 0.49    | -0.79   |
| D     | 167.50 ± 4.78     | 195.00 ± 19.07  | 27.50                   | 16.42                               | 0.12    | -2.20   |
| E     | 167.50 ± 4.78     | 217.50 ± 14.36  | 50.00                   | 29.85                               | 0.01    | -4.62   |
| F     | 170.00 ± 5.77     | 233.33 ± 17.63  | 63.33                   | 37.25                               | 0.04    | -3.45   |
| G     | 152.50 ± 8.53     | 187.50 ± 8.53   | 35.00                   | 22.95                               | 0.03    | -3.65   |
| H     | 150.00 ± 4.08     | 180.00 ± 4.08   | 30.00                   | 20                                  | 0.02    | -4.24   |

Data was analyzed using t-test and values were considered significant at P<0.05. WD= weight difference.
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Result from table 4.1 below showed that there was a significant ($p<0.05$) increase in the body weight in group A, as the final weight was 53.33% greater than the initial weight. Group B showed a decrease in weight that was not significant ($p>0.05$) when the initial weight was compared to the final weight to the tone of 23.43%. Group C showed a decrease in weight that was not significant ($p>0.05$) when the initial weight was compared to the final weight to the tone of 4.29%. Group D showed an increase in weight that was not significant ($p>0.05$) as the final weight was 16.42% greater than the initial weight. Group E showed a significant increase ($p<0.05$) in the weight when the initial weight was compared to the final weight to the tone of 22.95%. Group F showed a significant increase ($p<0.05$) in the weight when the initial weight was compared to the final weight to the tone of 37.25%. Group G showed a significant increase ($p<0.05$) in the weight when the initial weight was compared to the final weight to the tone of 22.95%. Group H showed a significant increase ($p<0.05$) in the weight when the initial weight was compared to the final weight to the tone of 20%.

Table 4.2 shows the effect of ethanoic seed extract *Dacryodes edulis* on paraquat-induced toxicity on relative organ weight

| Groups | Relative Testicular Weight (g) Mean ± SEM | P-Value |
|--------|-------------------------------------------|---------|
| A      | 0.80 ± 0.01                               | 0.01    |
| B      | 0.57 ± 0.10                               | 0.00    |
| C      | 0.68 ± 0.01                               | 0.21    |
| D      | 0.75 ± 0.11                               | 0.52    |
| E      | 0.64 ± 0.06                               | 0.47    |
| F      | 0.63 ± 0.01                               | 0.54    |
| G      | 0.71 ± 0.02                               | 0.14    |
| H      | 0.72 ± 0.04                               | 0.11    |
| F-Value|                                           | 1.45    |

Data was analyzed using ANOVA, followed by Post Hoc LSD multiple comparism, and data was considered significant at ($p<0.05$).

Result from table 4.2 Results of the relative testicular weight showed an increase in organ weight that was not significant ($p>0.05$) in groups C, D, E, F, G and H, while a significant increase in organ weight ($p<0.05$) in group A when compared to group B.

Table 4.6 shows the effect of ethanoic seed extract *Dacryodes edulis* on paraquat-induced toxicity on LH, FSH, & Testosterone

| Groups | Luteinizing Hormone (m/u/ml) | P-Value | FSH (m/u/ml) | P-Value | Testosterone (ng/ml) | P-Value |
|--------|-----------------------------|---------|--------------|---------|----------------------|---------|
| A      | 2.37 ± 0.13                 | 0.000   | 0.32 ± 0.03  | 0.201   | 4.20 ± 0.03          | 0.986   |
| B      | 1.14 ± 0.12                 | 0.000   | 0.12 ± 0.14  | 0.000   | 4.10 ± 0.03          | 0.683   |
| C      | 2.14 ± 0.12                 | 0.000   | 0.46 ± 0.17  | 0.392   | 4.24 ± 0.16          | 0.000   |
| D      | 1.80 ± 0.01                 | 0.000   | 0.94 ± 0.03  | 0.001   | 7.51 ± 0.10          | 0.000   |
| E      | 2.68 ± 0.09                 | 0.000   | 0.41 ± 0.0   | 0.578   | 8.04 ± 0.07          | 0.000   |
| F      | 3.85 ± 0.08                 | 0.000   | 0.32 ± 0.14  | 1.000   | 11.36 ± 0.28         | 0.000   |
| G      | 3.28 ± 0.06                 | 0.000   | 1.23 ± 0.08  | 0.000   | 7.33 ± 0.08          | 0.000   |
| H      | 3.46 ± 0.18                 | 0.000   | 1.66 ± 0.12  | 0.000   | 9.00 ± 0.05          | 0.000   |
| F-Value| 75.36                       | 25.05   | 687.65       |         |                      |         |

Data was analyzed using ANOVA, followed by Post Hoc LSD multiple comparism, and data was considered significant at ($p<0.05$).

Result from table 4.6 below showed a significant increase ($p<0.05$) in luteinizing hormone level in groups A, C, D, E, F, G, and H when compared to group B. Result of Follicular Stimulating hormone showed a significant ($p<0.05$) increase in group D, G, and H, while an increase that was not significant ($p>0.05$) in groups A, C, and E when compared to group B. Testosterone result showed a significant ($p<0.05$) increase in groups D, E, F, G, and H, and increase that was not significant ($p>0.05$) in groups A and C when compared to group B.
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Table 4.7 shows the effect of ethanoic seed extract *Dacryodes edulis* on paraquat-induced toxicity on Active motility, Sluggish motility, and Non-motile semen.

| Groups | Active Motility (%) | P-Value | Sluggish Motility (%) | P-Value | Non-Motile (%) | P-Value |
|--------|---------------------|---------|-----------------------|---------|---------------|---------|
| A      | 85.00 ± 2.88        | 0.001   | 6.66 ± 1.66           | 0.669   | 8.33 ± 1.66   | 0.000   |
| B      | 20.00 ± 11.45       |         | 11.66 ± 1.66          |         | 68.33 ± 13.01 |         |
| C      | 65.00 ± 2.88        | 0.009   | 10.00 ± 0.00          | 0.886   | 25.00 ± 2.88  | 0.000   |
| D      | 74.66 ± 0.33        | 0.023   | 17.66 ± 1.45          | 0.608   | 7.66 ± 1.45   | 0.000   |
| E      | 56.66 ± 23.33       | 0.029   | 31.66 ± 21.66         | 0.100   | 10.00 ± 0.00  | 0.000   |
| F      | 72.23 ± 4.33        | 0.003   | 12.66 ± 1.45          | 0.932   | 15.006 ± 2.88 | 0.000   |
| G      | 70.00 ± 5.77        | 0.005   | 17.66 ± 1.45          | 0.608   | 12.33 ± 4.33  | 0.000   |
| H      | 61.66 ± 13.64       | 0.015   | 21.66 ± 6.66          | 0.396   | 16.66 ± 7.26  | 0.000   |

F-Value 3.24 0.94 12.37

Data was analyzed using ANOVA, followed by Post Hoc LSD multiple comparison, and data was considered significant at (p<0.05).

Result from table 4.7 showed that active motility revealed a significant increase (p<0.05) in-groups A, C, D, E, F, G, and H when compared to group B.

Sluggish motility, result revealed a decrease that was not significant (p>0.05) in groups A and C, while a significant increase (p<0.05) in groups D, E, F, G, and H when compared to group B.

Non-motile sperms showed a significant decrease (p<0.05) in groups A, C, D, E, F, G, and H when compared to group .

Table 4.8 shows the effect of ethanoic seed extract *Dacryodes edulis* on paraquat-induced toxicity on Normal and Abnormal Sperm cells.

| Groups | Normal Sperm Cells (%) | P-Value | Abnormal Sperm cells (%) | P-Value |
|--------|------------------------|---------|--------------------------|---------|
| A      | 85.00 ± 5.00           | 0.00    | 15.00 ± 5.00             | 0.00    |
| B      | 36.66 ± 3.33           |         | 63.33 ± 3.33             |         |
| C      | 70.00 ± 5.77           | 0.00    | 30.00 ± 5.77             | 0.00    |
| D      | 80.00 ± 0.00           | 0.00    | 20.00 ± 0.00             | 0.00    |
| E      | 75.00 ± 2.88           | 0.00    | 25.00 ± 5.00             | 0.00    |
| F      | 65.00 ± 2.88           | 0.00    | 35.00 ± 2.88             | 0.00    |
| G      | 75.00 ± 2.88           | 0.00    | 25.00 ± 2.88             | 0.00    |
| H      | 78.33 ± 3.33           | 0.00    | 21.66 ± 3.33             | 0.00    |

F-Value 17.09

Data was analyzed using ANOVA, followed by Post Hoc LSD multiple comparism, and data was considered significant at (p<0.05).

Result from table 4.8 showed a significant increase (p<0.05) in Normal sperm cells in group A, C, D, E, F, G, and H when compared to group B. Abnormal sperm cell result showed a significant decrease (p<0.05) in group A, C, D, E, F, G, and H when compared to group B.

Table 4.9 shows the effect of ethanoic seed extract *Dacryodes edulis* on paraquat-induced toxicity on Total Sperm Count.

| Total Sperm count (x10^6/ml) | Group A | P-Value | F-value |
|-----------------------------|---------|---------|---------|
|                             | Group A | 6.49 ± 1.17 | 0.00*   |
|                             | Group B | 1.18 ± 0.24 |         |
|                             | Group C | 3.02 ± 0.04 | 0.113   |
|                             | Group D | 5.39 ± 0.74 | 0.00*   | 9.24    |
|                             | Group E | 4.08 ± 1.49 | 0.02*   |
|                             | Group F | 8.59 ± 0.13 | 0.00*   |
|                             | Group G | 2.61 ± 0.62 | 0.21    |
|                             | Group H | 3.90 ± 0.36 | 0.03*   |

Data was analyzed using ANOVA, followed by Post Hoc LSD multiple comparism, and data was considered significant at (p<0.05).
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Result from table 4.9 below showed a significant increase \((p<0.05)\) in the mean total sperm count in group A, D, E, F, and H, and an increase that was not significant \((p>0.05)\) in groupss C and G when compared to group B.

Histopathological Findings

PLATE 1 (Group A) Testes: Photomicrographs of testes tissue show normal histology. Seminiferous tubules are intact with active spermatocytes (white short arrow) and spermatogonium (white long arrow) (H&E x 400 x100).

Plate 2 (Group B: Paraquat Only) Testes: Photomicrographs of testes tissue show mild spermatogenic arrest (white arrow). Seminiferous tubules are intact with deactivated spermatocytes and spermatogonium (Arrow head) (H&E x 100 x400).
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Plate 3 (Group C: administered with paraquat for two weeks only). Photomicrographs of testes tissue show normal histology. Seminiferous tubules are intact with mild active spermatocytes and spermatogonium. There is no injury (H&E x 100 x400).

Plate 4 (Group D: administered with paraquat for 2 weeks and treated with H.D of edulis). Photomicrographs of testes tissue show normal histology. Seminiferous tubules are intact with active spermatocytes and spermatogonium (Arrow head). There is no injury (H&E x 400 x100).
Plate 5 (Group E: Paraquat 2-weeks and discontinued). Photomicrographs of testes tissue show normal histology. Seminiferous tubules are intact with active spermatocytes and spermatogonium. There is no injury (H&E x 100 x400).

Plate 6 (Group G: L.D of *Dacryodes edulis*). Photomicrographs of testes tissue show normal histology. Seminiferous tubules are intact with active spermatocytes and spermatogonium. There is no injury (H&E x 100 x400).
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Findings from table 3 revealed a significant increase (p<0.05) in LH in groups (A, C, D, E, F, G, & H), FSH (group D, G, & H) and testosterone level in groups D, E, F, G, & H when compared to paraquat control (group B). The mechanism of action in the significant increase in the hormonal level in the treated groups following administration of Dacryodes edulis, which contains flavonoids and polyphenols compounds present, thus attenuating oxidative damages caused by free radicals. However, there was a significant decrease (p<0.05) in paraquat control when compared to normal control (group A). This is attributed to the generation of reactive oxygen species resulting from oxidative stress by PQ intoxication.

The findings of this study as shown in table 4 showed a significant increase (p<0.05) in active motility in group C, D, E, F, G, & H when compared to paraquat control (group B). Non-motile sperm showed a significant decrease (p<0.05) in group C, D, E, F, G, & H when compared to group B. The precise mechanism of action is due to the presence of flavonoids and polyphenolic compounds present in Dacryodes edulis attenuating oxidative damages caused by PQ intoxication. Although, in-group E, PQ intoxication showed a reverse effects of semen motility changes. However, paraquat control group when compared to normal control showed a significant decrease (p<0.05) in normal sperm cell and significant increase (p<0.05) in abnormal sperm. This is attributed to generation of ROS production by PQ intoxication. This study agrees with Chen et al., (2017) who reported a significant decrease in sperm viability following paraquat administration. Eduardo et al., (2018) findings agrees with report of this present study on viability of normal sperm cell.

Findings from table 6 showed a significant increase (p<0.05) total sperm count in groups D, E, F, & H when compared to paraquat control (group B). This is attributed to polyphenols and flavonoids present in Dacryodes edulis. However, when paraquat control was compared to normal control, there was a significant decrease (p<0.05) in total sperm count. This is present of ROS generation by PQ intoxication. This study agrees with Chen et al., (2017) who reported a significant decrease in sperm count following paraquat administration. Eduardo et al., (2018) findings agrees with report of this present study on sperm count, which showed a significant decrease following paraquat administration.

Plate 7 (Group H: H.D of Dacryodes edulis). Photomicrographs of testes tissue show normal histology. Seminiferous tubules are intact with active spermatocytes and spermatogonium. There is no injury (H&E x 100 x400).
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Testicular histology showed a mild spermatogenic arrest, with the seminiferous tubules intact with deactivated spermatocytes and spermatogonium as observed in-group B. This study is in line with Shanker et al., (2011); Atashpour et al., (2017); while there was an increase in spermatogenesis in the treated groups.

CONCLUSION
This study showed that the ethanolic seed extract of Dacryodes edulis was able to protect the Testes histoarchitecture, and reduce enzymes activities caused by PQ intoxication.

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