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## RESEARCH ARTICLE

# PROTECTIVE POTENTIAL OF BETULINIC ACID AGAINST GLYPHOSATE-INDUCED TOXICITY IN TESTIS AND EPIDIDYMIS OF MALE WISTAR RATS

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**ABSTRACT**

Betulinic acid (BA), a novel pentacyclic triterpene, widely distributed in plants .This study examined possible protective potentials of Betulinic acid on antioxidant parameters and histology of testis and epididymis of glyphosate-treated rats. Glyphosate significantly elevated Malondialdehyde (MDA) in testes and epididymis by 41.8 % and 49.5%, respectively, compared with controls.BA significantly decreased MDA by 47.8% and 34.0%, respectively, compared with glyphosate group. Superoxide dismutase (SOD) was significantly reduced by 66.7% and 73.7% in testes and epididymis, in glyphosate group, whereas BA supplementation significantly elevated ($p < 0.05$) SOD by 77.8% and 72.2% respectively. Catalase (CAT) activities were reduced in glyphosate group by 29.2% and 21.7% in testes and epididymis, while BA elevated CAT activities by 23.8% and 28. 0% respectively. Glyphosate reduced, Reduced Glutathione (GSH) level by 35.2% in testes, compared with controls, while BA supplementation significantly increased ($p < 0.05$) GSH level by 43.8%, compared with glyphosate group. Glyphosate induced cellular degeneration and deformity in testis and epididymis, and supplementation with BA was observed to reverse these effects. From the results, glyphosate disrupted the antioxidant defense system and histological features of testicular and epididymal tissues, while Betulinic acid exhibited the potential to prevent these toxic effects in male rats.



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## INTRODUCTION

Glyphosate (N-phosphonomethylglycine), the active agent in the herbicide Roundup, is a major herbicide been used throughout the World, not only for agricultural purposes but also for the maintenance lawns. A pharmacokinetic study of ingested glyphosate in humans has shown that small amounts of the ingested glyphosate are metabolized to form aminomethylphosphonic acid (AMPA), while the unmetabolized part is finally eliminated in urine (Williams *et al*., 2000). Although, the manufacturing industry has claimed that this herbicide is almost nontoxic to mammals (Duke and Powles, 2008), and acute studies using rodents have also exhibited no apparent toxicity (Smith and Oehme, 1992), chronic exposures in rodents have been associated with toxicities in both liver and kidney with reduced lifespan, as reported by Séralini *et al.*( 2012). Glyphosate has been reported to suppress the activities of the cytochrome P450 family, including Cyp 450 aromatase enzyme (Richard *et al.,* 2005), which may indicate a possible inhibitory effect on synthesis of sex steroids by the Leydig cells in testes. It has been observed by Samsel and Senef (2013), that glyphosate present in certain western diets could lead to conditions such as diabetes, obesity, gastrointestinal disorder, depression, infertility and Alzheimer's disease (AD). Glyphosate toxicity has also been link with inhibition of the growth of beneficial gut bacteria (Shehata *et al*., 2013), genotoxicity (Poletta *et al*., 2009), cytotoxicity (Benachour *et al.*, 2007) and teratogenicity (Paganelli *et al*., 2010).

Betulinic acid (BA) is a lupane-type triterpenoid widely present in several plants. However, betulinic acid is more widely present than betulin (Hayek *et al*., 1989). Betulin and betulinic acid can be obtained in large amounts from the birch tree (Betula spp. Betulaceae) (O'connell *et al*., 1998; Galgon *et al,*. 1999), Ziziphus spp. (Jagadeesh *et al*. 1998; Schuhly *et al*., 1999), Syzygiumspp (Chang *et al*., 1999), Diospyros Spp. (Higa *et al*., 1998) and *Orthosiphonstamineus* (Family:

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Lamiaceae) (Sriplang *et al*., 2007). Betulinic acid can also be chemically synthesized from betulin (Csuk *et al*., 2006). Intraperitoneal administrations of BA to rats (200 to 400 mg/kg) (Sandberg *et al*., 1987) and mice (doses of 500mg/kg on every fourth day) (Pisha *et al*., 1995) were found to produce no toxicity in the animals. Betulinic acid exhibits very little broad cytotoxicity, if any at relatively high therapeutic doses (Cichewicz and Kouzi, 2004). Betulinic acid (BA) possesses several biological and pharmacological potentials (Alakurtti*e t al.,* 2006), and has been reported BA has been found exhibit cytotoxicity against several tumors that proved resistant to conventional chemotherapeutic drugs (Zuco *et al*., 2002, Ehrhardt *et al*., 2004).

The compound also demonstrated selectivity for metastatic over non-metastatic cancer cells (Rieber and Rieber, 1998), and for cancer cells over normal cells (Selzer *et al*., 2000). The synergy of BA with other anticancer therapies, such as chemotherapeutic drugs e.g. Vincristine (Fulda and Debatin. 2005), ionizing radiation (Selzer *et al*., 2000) and death receptor ligand TRAIL (Fulda *et al*., 2004). The inhibition of prostate cancer and tumor growth by BA has also been associated with proteasome-dependent down-regulation of specificity protein (Sp) transcription factors and many other Sp-regulated genes (Chintharlapalli *et al*., 2007). These Sp transcription factors, including Sp1, Sp3 and Sp4 are usually over expressed in tumor cells, but have relatively low level of expression in non- tumor cells (Chadalapaka *et al*., 2008; Papineni *et al*., 2009; Mertens –Talcott *et al*., 2007). Some of the genes that are regulated by Sp transcription factors include those for proliferation of cancer cells (e.g. epidermal growth factor-EGFR, hepatocyte growth factor receptor-cMET and cyclinD1), cell survival (e.g. Bcl-2 and Survivin) and inflammation (e.g. NF-Kβ p65 subunit) (Chadalapaka *et al*., 2010; Chinthalapalli *et al*., 2011; Pathi *et al*., 2010).

BA generates ROS and hydrogen peroxide, which induce the down regulation of sp-transcription of sp-transcription factors as observed in bladder and pancreatic cancers (Jutooru *et al*., 2010). This growth inhibition by BA through ROS and $H_2O_2$ has been confirmed through co-administration of BA with catalase. The down regulation of the Spproteins was reversed by the catalase, which mopped up hydrogen peroxide (Chintharlapalli *et al*., 2011). It has been suggested that the down regulation of Sp proteins could involve two pathways that result in induction of proteasome –dependent and caspases-dependent degradation of these proteins (Chintharlapalli *et al*., 2007; Chadalapaka *et al*., 2008; Jutooru *et al*., 2010). However, from our review, information on the effect of betulinic acid on glyphosate reproductive toxicity is few in literature, hence the justification for this study.

## MATERIALS AND METHODS

### Chemicals

Betulinic acid (Sigma-Aldrich), and commercial glyphosate were purchased. All other chemicals were of high analytical grades.

### Experimental Design

Twenty male Wistar rats (185 ± 2.5g) rats were purchased and acclimatized for 7 days and then randomly divided into group four groups (A- D) with five rats in each group. Rats in group A served as the control, the rats in group B were administered with betulinic acid (10mg/kg) every other day, group C was treated with glyphosate (100mg/kg) on days 4, 6, 10 and 12, while group D was pretreated with betulinic acid (10 mg/kg) on days 1 and 3, and continued every other day, with glyphosate (100mg/kg) on days 4, 6, 10 and 12. After 14 days of treatment, rats were fasted overnight and weighed. Blood was collected by ocular bleeding and rats were sacrificed by cervical dislocation.

### Preparations of serum and tissue homogenates

Blood was collected into slanting test tubes and then allowed to coagulate for about 2hours. The supernatant was centrifuged at 3000rpm for 10minutes to obtain the serum which was kept under refrigeration ($4^oC$) for biochemical analysis. Testes and epididymis were excised, washed in 1.15% KCl solution (washing buffer) to remove blood and then weighed. The organs were divided into two portions. One portion was fixed in10% formalin (fixative), for preservation prior to histological study. The other portion was homogenized using Teflon homogenizer with a buffer solution (1:4). It was then centrifuged at 3000rpm for 10minutes to obtain supernatant which was used for the determination of biochemical parameters.

### Quantitative determination of total protein

Total protein concentrations of serum, testes and epididymis were determined as described by Lowry *et al.* (1951).

### Determination of Lipid peroxidation (LPO)

The levels of malondialdehyde (MDA) in testes and epididymis homogenates were measured to assess lipid peroxidation. The measurement of Thiobarbituric acid-reacting substances (TBARS) was done as described by Aeschbach *et al*. (1994), measuring the absorbance at 532nm. The MDA concentrations were calculated using a molar extinction coefficient (Ɛ) of 1.56 x $10^5$ $M^{-1}cm^{-1}$(Adam-Vizi andSeregi, 1982).

### Determination of Reduced Glutathione (GSH)

Reduced Glutathione levels in the testes and epididymis homogenates were determined, measuring the absorbance at 412nm as previously described by Mitchell *et al*. (1973).

### Determination of Superoxide dismutase (SOD) activity

Superoxide dismutase activities in testes and epididymis homogenates were determined by the epinephrine method, measuring the absorbance at 480nm as previously described by Mistra and Fridovich, (1975).

### Determination of Catalase (CAT) activity

Catalase activities in testes and epididymis homogenates were spectrophotometrically determined, measuring the absorbance at 240nm according to Aebi (1984).

### Histopathological Analysis

Ultra-thin sections of testes and epididymis, preserved with 10% formalin, were examined under microscope after staining with hematoxyline and Eosin stains.

### Statistical Analysis

Data were analyzed by T- Test using SPSS 17 and expressed in Mean ± Standard Deviation. Level of significance was taken as $p < 0.05$

## RESULTS

### Effects of betulinic acid (BA) on body weights,organ weights and relative weights of rats treated with glyphosate

Table 3.1.1 shows the results of the treatments on the body weight, weights of testes and epididymis, as well as the relative weights of the organs. Significant weight gains were recorded in the treatment groups. Weight gain in glyphosate-treated rats was significantly high ($p<0.005$) (29.9%) compared with the control. On co-administration of BA and glyphosate, a decrease of up to 27.1% was observed compared with glyphosate- treated rats.

### Effects of Betulinic Acid on total protein concentrations of serum, testes and epididymis of rats administered with Glyphosate

As shown in Table 3.2.1, glyphosate treatment exhibited decreases in protein concentrations of serum and testes by 12.1% and 43.9%, respectively, but an increase of about 29.9% in epididymis, when compared with the control rats. In the group treated with BA and glyphosate, protein concentrations in serum and testes were significantly increased ($p < 0.05$) by 23.5% and 36.3%, respectively relative to glyphosate group. The co-administration of BA and Glyphosate was however, observed to lower the protein concentration in epididymis, compared with the glyphosate group.

### Effects of Betulinic Acid on malondialdehyde (MDA) concentrations of testes and epididymis homogenates of rats administered with Glyphosate

Lipid peroxidation was investigated by measuring the concentrations of MDA formed in the homogenates of testes and epididymis of the rats. Figure 3.3.1 shows that glyphosate treatment significantly elevated MDA concentrations in both testes and epididymis by 41.8 % and 49.5%, respectively, compared with controls.

**Table 3.1.1 Effects of betulinic acid (BA) on body weights,organ weights and relative weights of rats treated with glyphosate**

| Treatments | Weightgain of rats (g) | Weights of organs (g) | | Relative weights of organs (g) | |
|---|---|---|---|---|---|
| | | Testes | Epididymis | Testes | Epididymis |
| Control | 27.70 ± 9.2 | 2.11 ± 0.3 | 1.20 ± 0.3 | 1.23 ± 0.3 | 0.7 ± 0.2 |
| BA | 29.67 ± 2.3 | 2.05 ± 0.8 | 1.07 ± 04 | 1.09 ± 0.4 | 0.57 ± 0.2 |
| GLP | 39.54 ± 2.4[a] | 2.76 ± 0.5[a] | 1.79 ± 0.4[a] | 1.53 ± 0.3 | 0.64 ± 0.1 |
| BA + Glyphosate | 28.81 ± 1.4[b] | 1.92 ± 0.5[b] | 1.12 ± 0.6[b] | 1.19 ± 0.1[b] | 0.61 ± 0.2 |

Data expressed as M ± SD, n= 5; [a]-statistically different ($p< 0.05$) compared with control [b]- statistically different ($p< 0.05$) compared with GLYP-group. BA- Betulinic acid, GLYP-Glyphosate

**Table 3.2.1 Effects of Betulinic Acid on total protein concentrations of serum, testes and epididymis of rats administered with Glyphosate**

| Treatments | Serum (µmol/L) | Testes (µmol/L) | Epididymis (µmol/L) |
|---|---|---|---|
| Control | 7.46 ± 2.6 | 4.35 ± 2.1 | 1.83 ± 0.8 |
| BA | 9.07 ± 2.2 | 3.10 ± 1.2 | 2.31 ± 1.4 |
| Glyphosate | 6.56 ± 0.1 | 2.44 ± 0.7[a] | 2.61 ± 0.7[a] |
| BA+ Glyphosate | 8.57 ± 2.9[b] | 3.83 ± 0.7[b] | 1.74 ± 0.2 |

Data expressed in M ± SD, n= 5, [a]-statistically different ($p< 0.05$) compared with control [b]- statistically different ($p< 0.05$) compared with GLYP-group. BA- Betulinic acid, GLYP-Glyphosate

The weights of testes and epididymis were observed to increase in glyphosate group by 23.6% and 33.0%, respectively, relative to the control. BA supplementation was found to significantly reduce ($p < 0.05$) the organs weights by 30.4% and 37.4%, respectively, compared with the glyphosate-treated rats. The relative weight of testes was observed to increase in glyphosate group by 19.6%, compared with the control, while BA supplementation lowered it significantly by 22.2%, compared with the glyphosate group. There was no significant difference observed in the relative weight of epididymis.

On pretreatment and co-administration with BA, the concentrations were observed to be significantly decreased by 47.8% and 34.0%, respectively, compared with the glyphosate group.

### Effects of Betulinic Acid on Superoxide dismutase (SOD) activity of testes and epididymis homogenates of rats administered with Glyphosate

From the results shown in Figure 3.4.1, glyphosate was found to significantly ($p < 0.05$) reduce the SOD activities

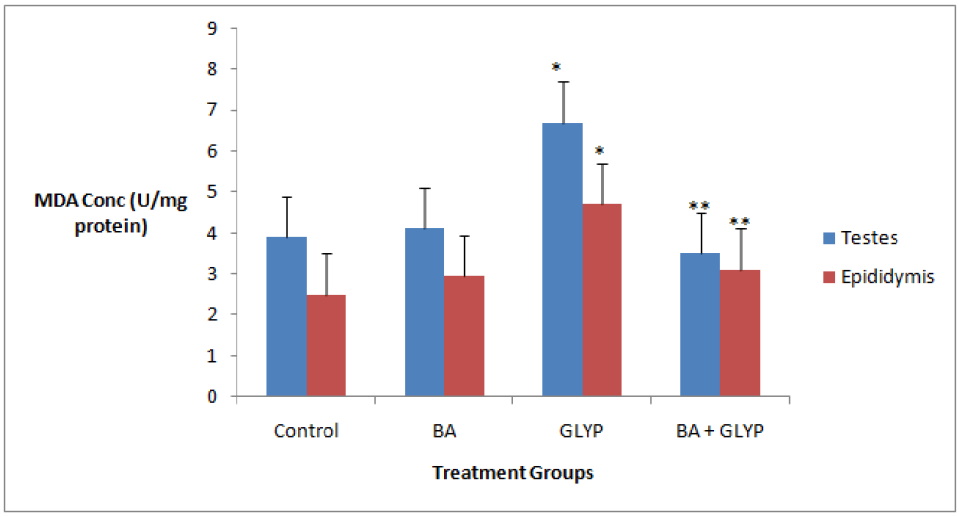


**Fig 3.3.1Effects of Betulinic Acid on malondialdehyde (MDA) concentrations of testes and epididymis homogenatesof rats administered with Glyphosate**

Data expressed in M ± SD, n=5, *-statistically different (p< 0.05) compared with control,**- statistically different (p< 0.05) compared with GLYP-group. BA- Betulinic acid, GLYP-Glyphosate

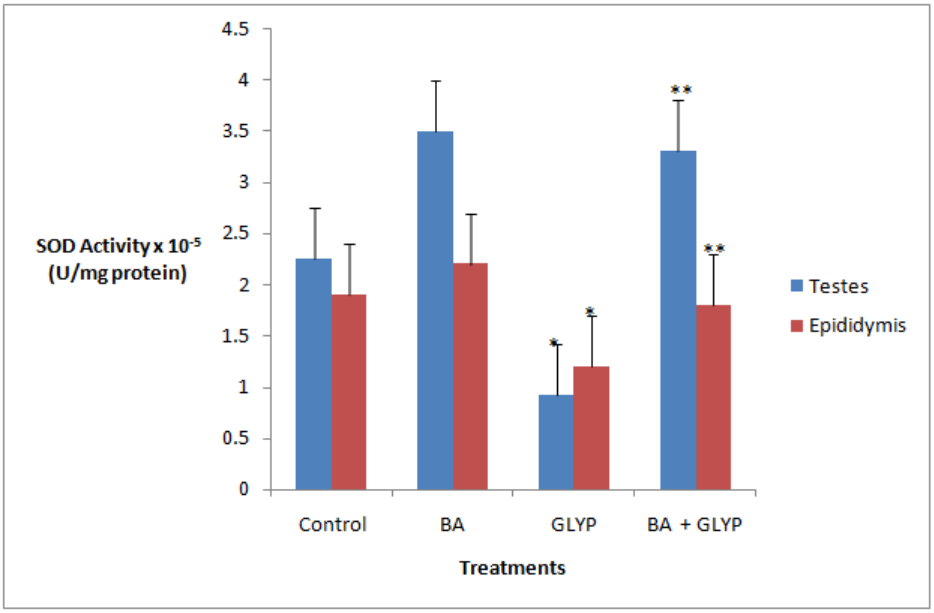


**Fig. 3.4.1 Effects of Betulinic Acid on Superoxide dismutase (SOD) activity of testes and epididymis homogenates of rats administered with Glyphosate**

Data expressed in M ± SD, n=5,*-statistically different (p< 0.05) compared with control, **- statistically different (p< 0.05) compared with GLYP-group.BA-Betulinic acid, GLYP-Glyphosate

by 66.7% and 73.7% in testes and epididymis, respectively, relative to control rats. However, when BA was co-administered with glyphosate, the activities were significantly elevated (p< 0.05) by 77.8% and 72.2% in testes and epididymis, respectively, in comparison with the glyphosate group.

**Effects of Betulinic Acid on Catalase (CAT) activity of testes and epididymis homogenates of rats administered with Glyphosate**

On investigating the CAT activities in the homogenates, there were reductions in the glyphosate group by 29.2% and 21.7% in testes and epididymis, respectively, compared with controls (Fig. 3.5.1). On co-administration, BA was able to elevate the CAT activities by 23.8% and 28. 0% in testes and epididymis, respectively, compared with the glyphosate group (Fig 3.5.1).

**Effects of Betulinic Acid on reduced glutathione (GSH) concentration of testes and epididymis homogenates of rats administered with Glyphosate**

Figure 3.6.1 presents the results on the effects of the treatments on the GSH concentrations. Glyphosate treatment reduced the GSH level by 35.2% in testes, compared with controls, while BA supplementation significantly increased the GSH level by 43.8%, compared with the glyphosate group. In the epididymis, GSH levels were not significantly affected by the treatments.

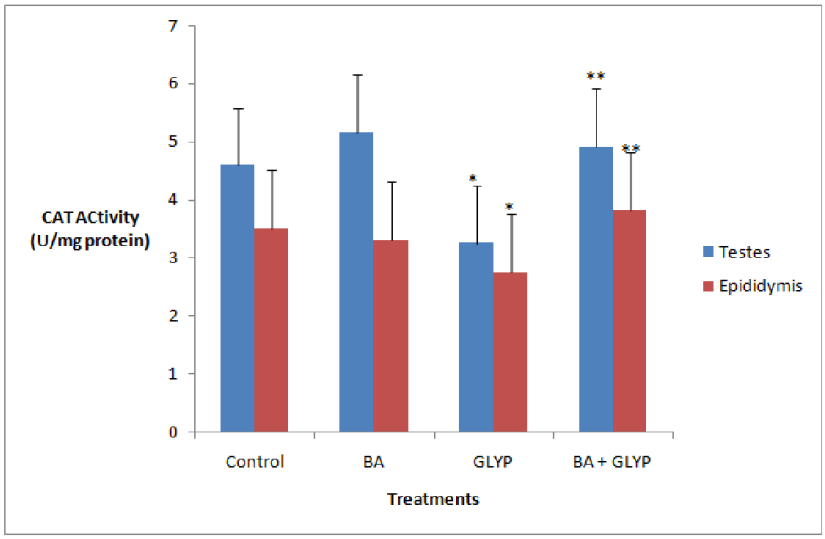


**Fig 3.5.1Effects of Betulinic Acid on Catalase (CAT) activity of testes and epididymis homogenates of rats administered with Glyphosate**

Data expressed in M ± SD, n=5, *-statistically different (p< 0.05) compared with control,**- statistically different (p< 0.05) compared with GLYP-group. BA-Betulinic acid, GLYP-Glyphosate

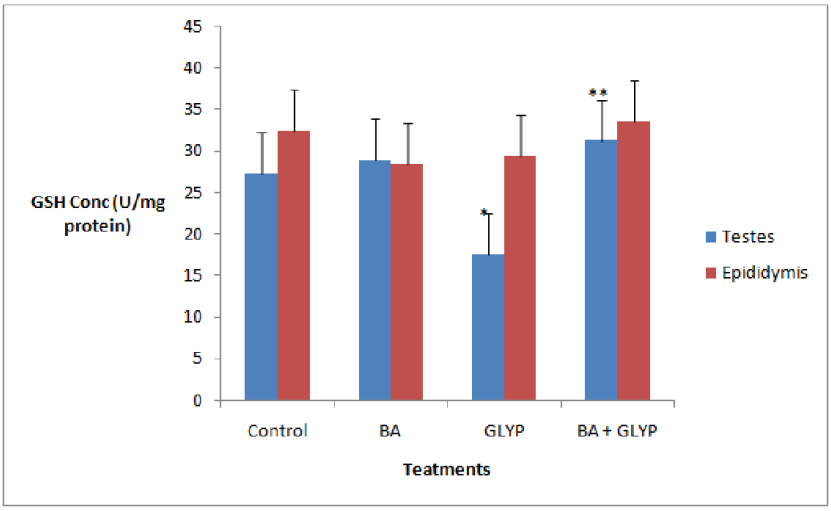


**Fig 3.6.1Effects of Betulinic Acid on reduced Glutathione (GSH) concentrations of testes and epididymis homogenates of rats administered with Glyphosate**

Data expressed in M ± SD, n=5, *-statistically different (p< 0.05) compared with control,**- statistically different (p< 0.05) compared with GLYP-group. BA-Betulinic acid, GLYP-Glyphosate

## Histological Results

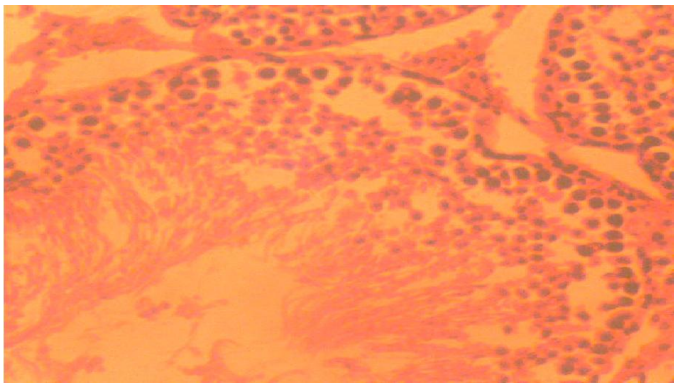

**Fig 3.7.1a Control (Testes) - Normal interstitial cells, connective tissues, spermatogonia, spermatocyte and spermatids. (X200)**

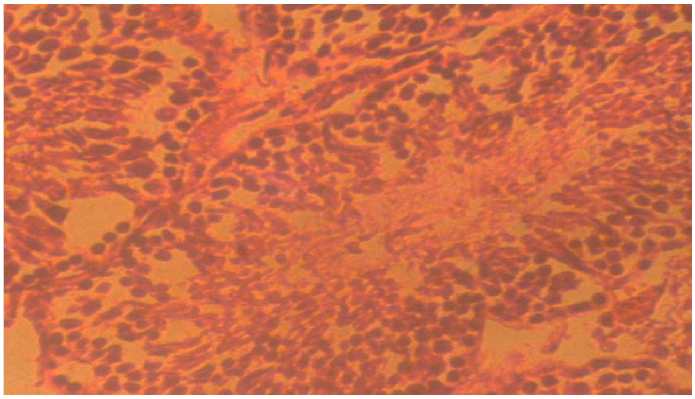

**Fig 3.7.1b Betulinic acid (Testes) - Normal interstitial cells and connective tissues (X200)**

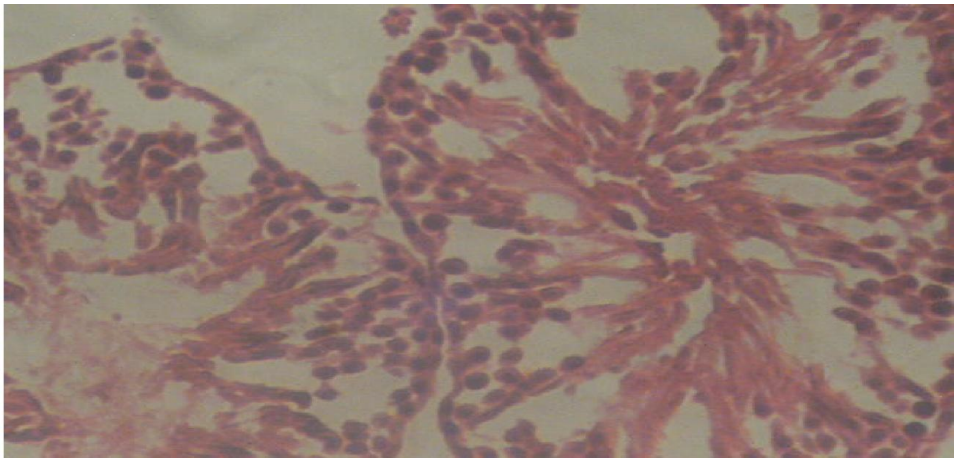

**Fig 3.7.1c Glyphosate (Testes) - Cellular degeneration and tubule deformity (X200)**

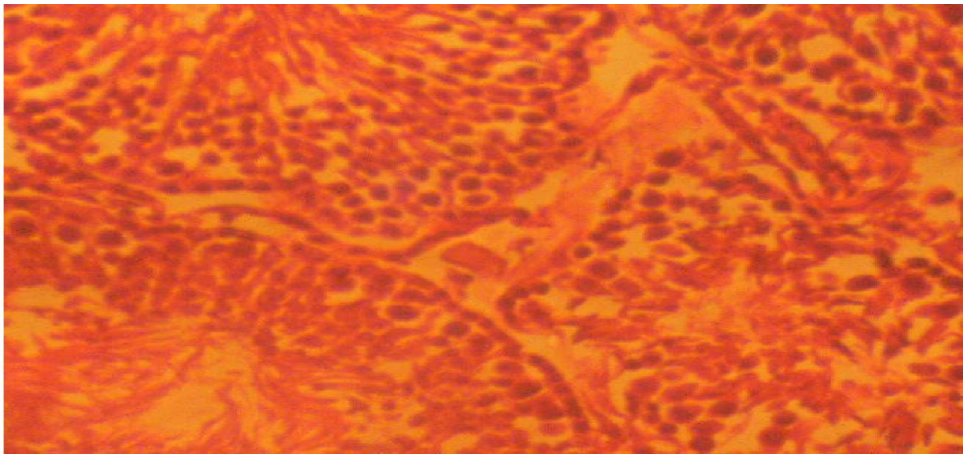

**Fig 3.7.1dBetulinic acid + Glyphosate (Testes) – Moderate tubule deformity and normal spermatids (X 200)**

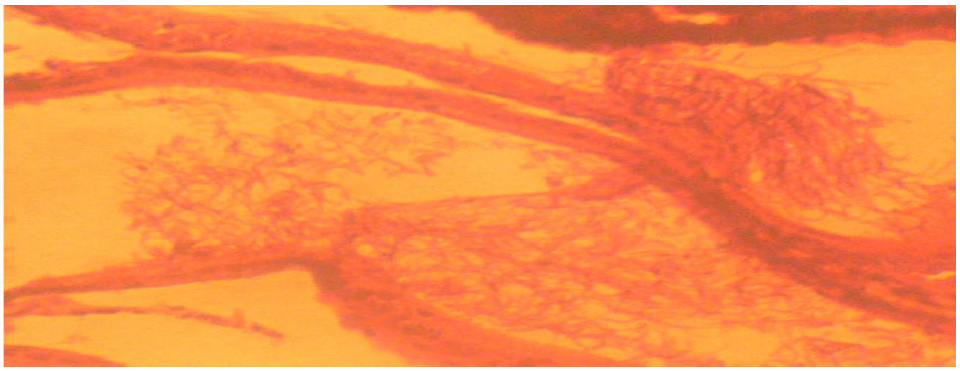

**Fig 3.7.2a Control (Epididymis) - Normal coily epididymis tubules with sperm cells (x 200)**

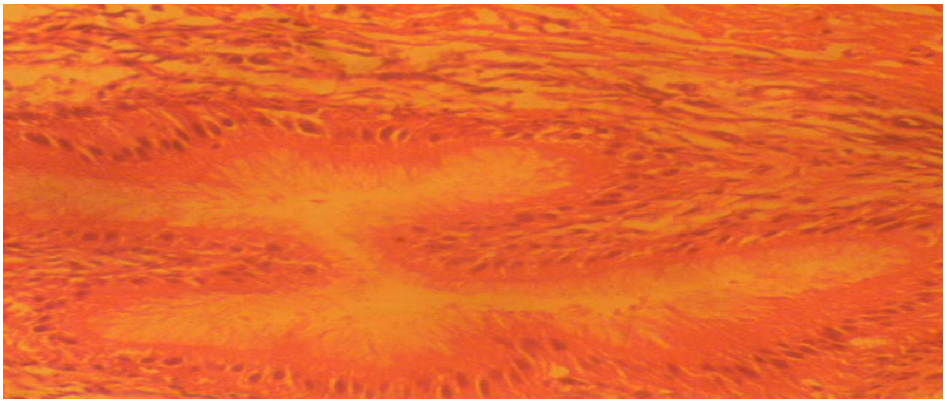

**Fig 3.7.2bBetulinic acid (Epididymis) - Normal vas deferens (x 200)**

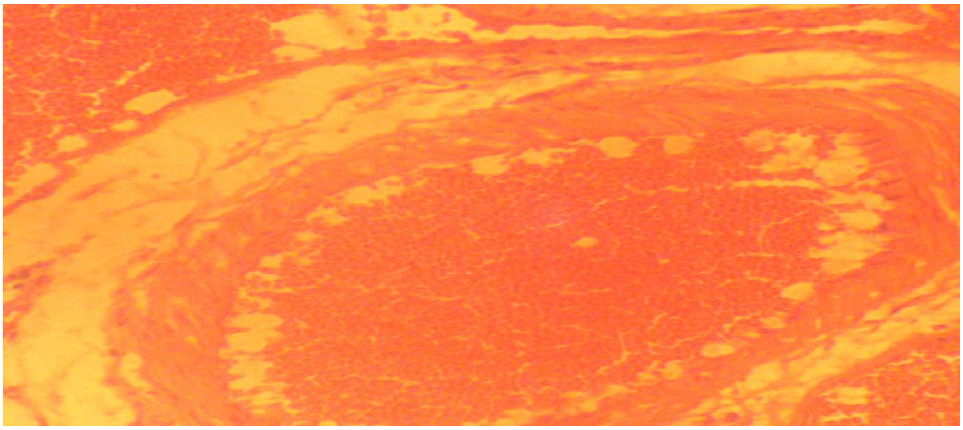

**Fig 3.7.2c Glyphosate (Epididymis) –Cellular degeneration of epididymis (x 200)**

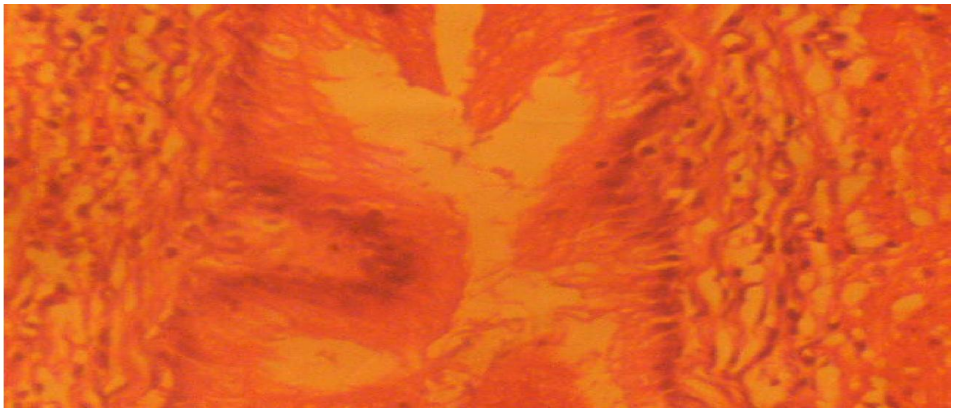

**Fig 3.7.2d Betulinic acid+ Glyphosate (Epididymis) - Normal epididymis (x 200)**

## DISCUSSION

### Monitoring of weights

Glyphosate has been, non-selectively, applied for the destruction of most herbaceous plants, and it is harmful to non-targeted plants, animals and the environment (Simonsen *et al.,* 2008). However, in animal experiments, glyphosate toxicity has been associated with inhibition of the growth of beneficial gut bacteria (Shehata *et al*., 2013), genotoxicity (Poletta *et al*., 2009), cytotoxicity (Benachour *et al.*, 2007), as well as, teratogenesis(Paganelli *et al*., 2010). Betulinic acid (BA), a lupane-type pentacyclic triterpene has beenreported to exhibit several biological and pharmacological potentials (Alakurtti *et al.,* 2006), including cytotoxicity against several tumors cell lines that are resistant to conventional anticancer drugs (Zuco *et al*., 2002, Ehrhardt *et al*., 2004) .The present study has been undertaken to examine the possible effects of betulinic acid on the antioxidant status and cytological features of testes and epididymis of rats treated with glyphosate.

Monitoring of weights of the experimental rats shows that significant weight gains were recorded in all the treatment groups (Table 3.1.1). Weight gain in glyphosate-treated rats was significantly high ($p<0.005$) (29.9%) compared with the control, while co-administration of BA and glyphosate, led to 27.1% reduction, compared with glyphosate group. Glyphosate also caused up to 23.6% and 33.0% in the weights of testes and epididymis, respectively, compared with the control. BA supplementation was found to significantly reduce ($p < 0.05$) the organs weights by 30.4% and 37.4%, respectively, compared with the glyphosate-treated rats (Table 3.1.1). The relative weight of testes was observed to increase in glyphosate group by 19.6%, compared with the control, whereas, supplementation with BA reduced it significantly by 22.2%, compared with the glyphosate group. Epididymis however showed no appreciable changes in the relative weight (Table 3.1.1). Chan and Mahler (1992) observed no significant changes in the testicular and epididymal weights in rats treated with glyphosate. Baillie-Hamilton (2002) has suggested that the increasing anthropogenic synthesis of several organic and inorganic chemicals may be associated with body weight gain, culminating in the present global trend of obesity epidemics. The high level of weight gain recorded in the present study may be explained based on the tryptophan-depleting effects of glyphosate, which causes depletion of serum tryptophan, and that of serotonin and melatonin in the brain (Zimmermann *et al.,* 1993). Serotonin which is derived from tryptophan is a potent appetite suppressant, and its deficiency may lead to overeating and possibly obesity (Breisch *et al*., 1976). A suppression of serotonin signaling therefore enhances increase in body weight. Glyphosate has also been described as an endocrine disruptor (Gasnier *et al.*, 2009). Inhibition of both amylase and pancreatic lipase, leading to impaired carbohydrate and lipid metabolism, has been associated with weight loss and anti-obese action, as observed with pentacyclic

triterpenes, such as oleanolic acid, ursolic acid and betulinic acid (Ali *et al*., 2006; Jang *et al*., 2008).

**Total protein concentrations**

Glyphosate treatment exhibited caused decrease in protein concentrations of serum and testes by 12.1% and 43.9%, respectively, while an increase of up to 29.9% was found with epididymis, when compared with the controls (Table 3.2.1). The decrease in serum and testes protein concentration observed in the present study may be due to some proteolytic processes that occur during toxicity according to Remia *et al.,* (2008).However, it was noticed that BA, co-administered with glyphosate, significantly increased the protein concentrations in serum and testes by 23.5% and 36.3%, respectively relative to glyphosate group. The co-administration of BA and Glyphosate was observed to reduce the protein concentration in epididymis, compared with the glyphosate group.

**Antioxidant profile**

Lipid peroxidation was investigated by measuring the concentrations of MDA formed in the homogenates of testes and epididymis of the rats. Figure 3.3.1 shows that glyphosate treatment significantly elevated MDA concentrations in both testes and epididymis by 41.8 % and 49.5%, respectively, compared with controls. The elevated level of MDA may be due to the generation of reactive oxygen species (ROS) according to Ahmad *et al*. (2000), who reported that ROS could interact with membrane lipids to form lipid peroxidation products, such as MDA and 4-hydroxynonenal (4-HNE), causing oxidative stress (Hermes-Lima, 2004). Many of the products of lipid peroxidation are electrophilic and interact with DNA and proteins to form adduct which initiate mutagenesis (Marnett, 1999). BA supplementation was observed to significantly decrease MDA levels by 47.8% and 34.0%, in testes and epididymis, respectively, compared with the glyphosate group. This therefore indicates the MDA-lowering effect of Betulinic acid, hence suppressing *in vivo* oxidative stress. The SOD–CAT system is the first line of defense against oxygen toxicity, which brings about inhibition of free radicals (Pandey *et al*., 2003), and these enzymes are frequently used as biomarkers of oxidative stress (Monteiro *et al*., 2006). From the results shown in figure 3.4.1, glyphosate was found to significantly ($p < 0.05$) reduce the SOD activities by 66.7% and 73.7% in testes and epididymis, respectively, relative to control rats (Fig 3.4.1). However, when BA was co-administered with glyphosate, the activities were significantly elevated ($p < 0.05$) by 77.8% and 72.2% in testes and epididymis, respectively, in comparison with the glyphosate group. On investigating the CAT activities in the homogenates, there was reduction in the glyphosate group by 29.2% and 21.7% in testes and epididymis, respectively, compared with controls (Fig. 3.5.1).

On co-administration, BA was able to elevate the CAT activities by 23.8% and 28. 0% in testes and epididymis, respectively, compared with the glyphosate group. The reduction in superoxide dismutase activity after exposure of the rats to glyphosate may be related to the production of oxidants such as free radicals. It is known that there is a complex pathway of interaction among the enzymes involved in mammalian antioxidant system and that the activity of one enzyme may influence those of other enzymes. The products of one antioxidant enzymes can also influence the activities of others. For instance, an excess of hydrogen peroxide may reduce SOD activity, while superoxide anion may be responsible for lowered CAT activity (Bagnyukova *et al*., 2006). This may be supported by the decrease (although not significant) in CAT activity, observed in this study, in the rats exposed to glyphosate, enhancing accumulation of hydrogen peroxide in the tissues. The reduction in CAT activity may be due to insufficient neutralization of superoxide anions by SOD activity. It has earlier been mentioned that glyphosate could lead to tryptophan depletion, causing that of serotonin and melatonin (Zimmermann *et al.,* 1993). Melatonin has been reported to be a potent antioxidant and regulator of redox reactions (Pandi-Perumal *et al.,* 2013), and its neuroprotective role in aging and many neurodegenerative conditions such as Alzheimer's disease and Parkinson's disease could likely be due to its antioxidant effects (Borah and Mohanakumar, 2009). It may therefore be suggested that the reported lowering-effect of glyphosate on tryptophan-melatonin system could also result in increased oxidative stress.

Reduced glutathione (GSH), a major non-protein thiol of cells, is involved in the cellular defense against the toxic action of oxyradicals (Schuliga *et al*., 2002). This low molecular weight thiol can be easily oxidized and serves as a sink for free radicals and other reactive species (Hermes-Lima, 2004). Protective and adaptive potentials of GSH against oxidative stress have well been established through several studies by Regoli and Principato (1995), and Otto and Moon (1995). Figure 3.6.1 shows the effects of the treatments on the GSH concentrations. From the present study, it was observed that glyphosate significantly reduced the GSH level by 35.2% in testes, compared with controls, while BA supplementation significantly increased the GSH level by 43.8%, compared with the glyphosate group. In the epididymis, however, GSH levels were not significantly affected by the treatments. An impairment of the antioxidant system by glyphosate in testicular and epididymal tissues has been suggested by Ikpeme *et al*. (2012). It could therefore be suggested from our results that BA was able to compensate for the antioxidant system impairment caused by glyphosate, as observed in this study.

**Histological Studies**

Investigation of the histological effects on the testes and epididymis of the experimental rats shows that glyphosate induced cellular degeneration and tubular deformity in testes (Fig 3.7.1c), compared with the normal interstitial cells, spermatocytes and spermatids in the control rats (Fig 3.7.1.a). A supplementation with BA caused moderate tubular deformity with normal spermatids in testes, as shown if fig 3.71d. In epididymis, glyphosate treatment was observed to cause cellular degeneration (Fig 3.7.2a), as compared with the normal epididymis tubules observed in both control and BA-supplemented groups. Although, the present study did not investigate sperm count and motility, Chan and Mahler (1992) established that glyphosate caused up to 20% decrease in sperm count and no significant effect on epididymal sperm motility. It

has been observed that glyphosate caused testicular damage, including tubular necrosis and interstitial congestion, in rats, according to Ikpeme *et al*. (2012).An earlier study carried out by Ikpeme *et al*. (2010) has established the adverse effect of glyphosate administration on the hormones involved in spermatogenesis, hence its potential to induce infertility in male mammals. Recently, it has been observed that glyphosate led to oxidative stress and necrosis in rat testis, as a result of calcium overload, occurring through the opening of L-type voltage- dependent calcium pump and calcium influx (de Liz Oliveira Cavalli *et al.,*2013; Samsel and Senef, 2013).

**Conclusion**

From the data presented in this study, glyphosate disrupted the antioxidant defense system and histological features of testicular and epididymal tissues, whereas Betulinic acid exhibited the potential to reverse these toxic effects of the herbicide in male rats. This is therefore suggestive of its possible protective potential against glyphosate-induced reproductive toxicity.

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