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Ameliorative potential of Betulinic Acid against Atrazine-induced Hepatic and Testicular Damage in Wistar rats

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Abstract
Atrazine (ATZ) is a selective pre- and post-emergentherbicide for combatingweeds, which however causes endocrine disruption and delays or changes in pubertal development in experimental animals. Since a paucity of information on the effects of Betulinic acid (BA), a triterpene antioxidant on ATZ toxicity was noted in literature, the present work was designed to study the effects of BA on the antioxidant profile and histopathology of liver and testes of rats treated with ATZ. The ATZ significantly increased protein levels in serum (46.2%) and liver (94.8%), while that of testes (55.9%) was significantly decreased, relative to controls. Pretreatment with BA ameliorated the effect in serum and liver compared with ATZ group. The ATZ treatment significantly lowered Superoxide dismutase (SOD) activities by 52.8% and 89.3%, and Catalase (CAT) activities by 56.9% and 53.6% in liver and testes, respectively, relative to controls, whereas the activities were significantly elevated on BA pre treatment. Malondialdehyde (MDA) was elevated by 84.65 and 85.8%, while reduced glutathione (GSH) was decreased by 51.2% and 64.2% in liver and testes, respectively when compared with controls. However, BA ameliorated the effect compared with ATZ group. In the ATZ –treated rats, liver was noted to develop mild hepatocyte degeneration and periportal cell infiltration, while foci tubular distortion and capsular congestion were observed in the testicular tissue, effects which BA pretreatment was able to ameliorate. In conclusion, Betulinic acid improved the antioxidative status of hepatic and testicular tissues against Atrazine intoxication in rats.
Keywords: Atrazine, Betulinic acid, Antioxidant profile, Tissue histology

Introduction

Betulinic acid (3β-hydroxy-lup-20(29)-en-28-oic acid) is a pentacycliclupane – type triterpene, found in the outer bark of the birch tree (*Betula Pendula* Roth) (Cichewicz and Kouzi, 2004), Aziziphus spp. (Jagadeesh*et al*., 1998; Schuhly*et al*., 1999), *Orthosiphonstamineus*(Sriplang*et al*., 2007; Yam *et al*., 2007) and *Eugenia florida* leaves (Alaide*et al*., 2013), Syzygiumspp (Chang *et al*., 1999), Diospyros Spp. (Higa*et al*., 1998) and Paconia Spp. (Lin *et al*., 1998).BA has been found exhibiting cytotoxicity against several tumors (e.g. epithelial cancers, melanomas and primary pediatric acute leukemia) that display resistance to conventional chemotherapeutic drugs (Zuco*et al*., 2002; Ehrhardt*et al*., 2004). BA selectivity has also been established for cancer cells over normal cells (Selzer*et al*., 2000; Zuco*et al*., 2002). Betulinic Acid has been reported to be cytotoxic against melanoma cell lines (Pisha*et al*., 1995) and other human cancers such as leukemia (Ehrhardt*et al*., 2004), carcinoma (cancers of head, neck, colon, liver, lungs, prostate, ovary or kidney) (Thurnher*et al*., 2003) and neuroblastoma (Fulda and Debatin, 2000). An activation of the mitochondrial pathway of apoptosis by BA in cancer cells has been reported (Fulda, 2008).

Atrazine (chemically known as 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a synthetic herbicide widely used agriculturallyagainst broadleaf and grassy weed control in corn, sorghum, sugarcane, cotton, and pineapple vegetation (Cerdeira*et al*., 2005; Dong *et al*., 2009). Atrazine and its metabolite (deethylatrazine) are highly persistent in water and are mostly found in soil especially in farming season (Baker, 1988). The prolonged application of atrazine and its persistence increase the risk of its retention in crops and soils, and may also be transferred from soil surface to ground waters.Costa Silva *et al.*(2010) have reported the maximum contaminant levels of most triazine-based herbicides in drinking water to be about 3 parts per billion (ppb). Due toits chemical characteristics, such aslipophilicity, slow hydrolysis, moderate to low water solubility, high solubility in organic solvents and high degree of absorption by organic matter, clay, and fat tissues, atrazine has been listed among the contaminants to be controlled in some parts of the Europe (Ross *et al.,* 2009).

The pharmacokinetic profile of atrazine in experimental animals has been established by studies conducted by Hayes and Laws (1990) and Bakke *et al*. (1972). They discovered that, 72 hours after oral administration of 0.53 mg/kg of ^{14}C-labeled atrazine, up to 65.5% of the parent compound was detected in urine, while 20.3% and 15.8% were detected in faeces and body tissues, and less than 0.1% was detected in the expired air. The four major types of urinary metabolites identified were 2-hydroxyatrazine, its two mono-dealkylated analogs, 2-chloro-4-amino-6-(ethylamino)-1,3,5-triazine and 2-chloro-4-amino-6-(isopropylamino)-1,3,5-

triazine, and ammeline (Bakke *et al*., 1972). Some later studies identified 2-chloro-4,6-diamino-1,3,5-triazine as another primary urinary atrazine metabolite in adult male Charles River rats(Bradway and Moseman, 1982), deethylatrazine in swine urine (Erickson *et al*., 1979), and 2-chloro-4-amino-6-(ethylamino)-1,3,5-triazineand 2-chloro-4, 6-diamino-1,3,5-triazine in human urine (Catenacci*et al*., 1990). Cytochrome P-450-mediated metabolism has been implicated in*in vitro* studies using hepatic tissues of animals including rats, mice, goats,sheep, pig, rabbit and chicken, to form monodealkyated metabolites of atrazine as reported by Adams and co-workers (1990). These and other workers have also noted the involvement of Glutathione-S-transferase (GST) enzymes in detoxification of atrazine through conjugation this compound and its mono-dealkylated metabolites with reduced glutathione (GSH) (Adams *et al*., 1990; Dauterman and Muecke, 1974).

It has been reported that atrazine intoxication caused morphological and biochemical changes in the heart, liver, lungs, kidney, ovaries as well as endocrine tissues in experimental animals. In furtherance, several reports have noted a positive correlation between testicular degeneration and exposure to toxic compounds especially those which are capable to disrupt endocrine functions (Hayes *et al.,* 2006). In line with this, atrazine and other herbicides have been reported to induce endocrine disruption and disturbance of various hormonal functions, as reported by Rayner and co-workers (2004). Atrazine exposure to male experimental animal models has been associated with reduction in the number and motility of sperm (Kneiwald*et al.,* 2000), delayed sexual maturation (Kneiwald*et al.,* 1987) and reduction in prostate and seminal vesicle weight.The endocrine disruptive effect of atrazine caused demasculinization and feminization of the gonads in male animals by reducing androgen and increasing estrogen levels as observed in fish, amphibians, and reptiles. Reduced testicular testosterone synthesis and poor semen quality in male rats have been found to be caused by atrazine intoxication (Sifakis *et al*., 2011; Hayes *et al*., 2011). Hussainand co-workers (2011) studied the effects of atrazine in male Japanese quail (Coturnix japonica) and discovered smaller testicular size, decreased number of spermatocytes, necrotic nuclei of spermatids, and lesser number or absence of spermatozoa in the birds treated with 500mg/kg. Atrazine has also been established to cause dose-dependent induction of certain marker genes of steroidogenesis such as steroidogenic acute regulatory protein (STAR), cytochrome P450-11A1 and 3-hydroxysteroid dehydrogenase (3-HSD), all indicating toxicity in the Leydig (interstitial) cells of rats (Abarikwu*et al*., 2011; Pogrmic-Maikic*et al*., 2010). In an earlier study by Abarikwu*et al*. (2009), an oral exposure of rats to atrazine (120 mg/kg or 200mg/kg) for 7 and 16 days caused dose-dependent effects on sperm counts, motility, viability, sperm morphology and daily sperm production in the animals. Their observation also included normal testicular morphology and mild tubular degeneration with some defoliated cells. Zaya*et al*. (2011) noticed that atrazine decreased fat body size in tadpoles which culminated in reduced ability to survive stress of metamorphosis and low reproductive potential. The synthesis of heat shock protein-90 (HSP90) is usually triggered during cellular stresses induced by pesticide exposure, and immunohistochemical profiling of 90 KDasubunit of heat shock protein (HSP 90) has established the expression of this protein in the ovary of rats subjected to atrazine exposure (Julian*et al*., 2008). Furthermore, these workers also reported that atrazine sub-acute treatment resulted in reduced folliculogenesis and increased follicular atresia, while the sub-chronic treatment resulted in formation of multioocytic follicle and stress-inducible HSP90 with the attendant reduction in reproductive capacity.

In addition to the atrophy of testicular tissue, atrazine intoxication could also lead to hyperplasia of renal epithelium, squamous metaplasia of urinary bladder epithelium, renal calcium deposition, erythroid hyperplasia of the bone marrow and extramedullaryhematopoiesis in the spleen (Tisdel 1977).The genotoxicor clastogenic potential of atrazine,in the form of chromosomal aberration in mouse bone marrow, has been previously demonstrated by Weisenburger (1988), Loprino*et al*. (1980) and Adler (1980). In the recent time, however, Cavas (2011), has shown that atrazine exposure could produce significant increase in frequencies of micronuclei formation in peripheral blood erythrocytes of a fish, *Carassiusauratus*.

Effects of atrazine treatment on the oxidative stress indices showed that this herbicide could significantly reduce the activities of GST and SOD enzymes in both testis and epididymis, whereas the CAT activity was not significantly changed in testis, but rather reduced in the epididymis of the rats. The level of reduced glutathione (GSH) and glutathione-S-transferase (GST) activity were elevated in the animals with high dose, whereas the activities of superoxide-dismutase (SOD) and catalase (CAT), and levels of ascorbate, malondialdehyde (MDA) and hydrogen peroxide production were unaffected in the testicular tissue after a 7-day atrazine administration as also reported by Abarikwu and colleagues. (2009). It has been observed that atrazine inhibited mitochondrial electron transport and induced oxidative stress in the insect Drosophila melanogaster (Thornton *et al*., 2010).

Materials and Methods

Chemicals

Betulinic acid (Sigma-Aldrich USA), and Atrazine was purchased from commercial Agro-chemical store in Ogbomoso . All other chemicals were of good analytical grades.

Experimental Design

Twenty-four male Wistar rats (153 ± 11.29g) were obtained from the animal house of the University of Ilorin and used for this study. The animals were randomly divided into four groups having six rats per group. They were housed in cages and fed *ad libitum* with rat pellets and distilled water under 12- hours light/dark cycle.The animals were treated and handled based on the general rules on handling of laboratory animals both nationally and as a guide in my institution. The rats were acclimatized for one week. Control group was given normal rat pellets and water. The BA group was given daily oral intubation of Betulinic acid (10 mg/kg) for 14 days.The ATZ group was treated with intraperitoneal injection of Atrazine (5 mg/kg) twice per week, while BA + ATZ group was administered with daily oral intubation of Betulinic acid(10 mg/kg) for 14 days and intraperitonealinjection of Atrazine (5 mg/kg) twice per week. 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. Liver and testes 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 preserved in 10% formalin for histopathological study. The other portion was homogenized with Teflon homogenizer in 0.01M Phosphate buffer solution (pH 7.4). It was then centrifuged at 3000rpm for 10minutes to obtain supernatant which was used for the determination of biochemical parameters.

Total protein estimation

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

Estimation of Superoxide dismutase (SOD) activity

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

Estimation of Catalase (CAT) activity

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

Estimation of Malondialdehyde (MDA) level

The levels of lipid peroxidation in liver and testes were estimated by determining the concentration of malondialdehyde (MDA) in the homogenates. The measurement of MDA involved taking the absorbance of Thiobarbituric acid-reacting substances (TBARS) at 532nm as described by Ohkawa *et al*. (1979). The MDA concentrations were calculated using a molar extinction coefficient (Ɛ) of 1.56×10^5 $M^{-1}cm^{-1}$.

Estimation of Reduced Glutathione (GSH)

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

Histopathological Studies

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

Statistical Analysis

Data are expressed in Mean ± SD.Student T-test analysis was used for statistical comparisonand differences were taken as significant at $p < 0.05$. Descriptive and graphical methods were used to characterize the data.

RESULTS

Monitoring of weights

Table 1 shows thatbody weight gain in the rats treated with ATZ was 34.4% lower ($p < 0.05$) compared with the control rats and there was no significant difference between the ATZ-treated rats and those treated with a combination of ATZ and BA. Treatment with ATZ caused 17.4% increase in liver weight relative to controls, while BA supplementation caused 11.6% reduction compared with ATZ-treated group as also shown in table 1. The relative weight of liver was found to be 22.4% higher ($p < 0.05$) in the ATZ group compared with controls, while only 14.0% decrease was observed in the BA-supplemented rats compared with rats treated with ATZ. The results on testes showed no significant differences in both organ weight and relative weight (Table 1).

Table 1: Changes in body weights, organ weights and relative weights of liver and testes of rats treated with BA and ATZ

Treatments	Weights of rats (g)			Weights of organs (g)		Organs relative weights (%)	
	Initial	Final	Weight gain	Liver	Testes	Liver	Testes
Control	155.59 ±13.60	174.29 ±17.90	18.7 0± 3.20	5.92 ± 1.40	2.11 ± 0.31	3.40 ± 0.02	1.21 ± 0.03
BA	160.89 ±17.10	190.56 ±21.70	29.67 ±1.60	6.16 ± 1.15	2.04 ± 0.81	3.23 ± 0.13	1.01 ± 0.02
ATZ	153.31 ±23.40	167.22± 8.21	13.91 ±2.01*	6.95 ± 1.48*	2.16 ± 0.46	4.16 ± 0.03*	1.29 ± 0.10
BA + ATZ	158.36±11.31	170.70± 13.11	12.34 ± 0.30	6.23 ± 2.10**	2.04 ± 0.63	3.65 ± 0.11**	1.20 ± 0.04

Data expressed as Mean ± S.D, n= 6; *-statistically different ($p< 0.05$) compared with control **- statistically different ($p< 0.05$) compared with ATZ

BA-Betulinic acid, ATZ- Atrazine

Protein determination

Table 2 show the results on the determination of total protein in the serum, liver and testes of the experimental rats. Serum and liver recorded 46.2% and 94.8% increases, respectively, in the levels of total protein relative to the ATZ-treated rats. However, supplementation with BA was found to reduce the levels by 29% and 48.5% in serum and liver, respectively, compared with ATZ group. Testes showed contrasting results in which ATZ treatment caused 55.9% decrease compared with controls, while BA supplementation was of no significant effect.

Table 2 Effects of BA on total protein concentrations of serum, liver and testes of rats administered with ATZ

Treatments	Serum (µmol/dL)	Liver (µmol/dL)	Testes(µmol/dL)
Control	5.33 ± 0.49	4.59 ± 0.51	4.35 ± 2.16
BA	6.42 ± 1.09	7.67 ± 3.01	3.10 ± 1.15
ATZ	7.79 ± 2.69*	8.94 ± 2.30*	2.79 ± 1.29*
BA+ ATZ	6.04 ± 0.15**	6.02 ± 0.2**	2.95 ± 0.86

Data expressed in M ± S.D, n = 6, *-statistically different ($p< 0.05$) compared with control,
**- statistically different ($p< 0.05$) compared with ATZ.
BA- Betulinic acid, ATZ-Atrazine

Oxidative Stress markers

Treatment with ATZ significantly ($p < 0.05$) decreased the superoxide dismutase (SOD) activities in liver and testes by 52.8% and 89.3%, respectively, compared with control rats. The activities were however observed to be increased by 42.5% and 162.1% on BA supplementation in the respective tissues (Table 3). The activities of catalase (CAT) were noted to be significantly lowered by 56.9% and 53.6% in liver and testes, respectively, of ATZ group relative to controls, while the activities were significantly elevated by 50.6% and 108.1%, respectively in BA-supplemented rats compared with ATZ-treated rats as shown in table 3.

Table 3 Effects of BA on Superoxide dismutase (SOD) and Catalase (CAT) activities in liver and Testes of rats treated with ATZ

Treatments	SOD (U/mg protein)		CAT (U/mg protein)	
	Liver	Testes	Liver	Testes
Control	10.45 ± 2.56	2.65 ± 2.18	213.54± 12.05	45.92± 0.24
BA	12.97 ± 1.18	3.56 ± 2.89	229.67 ± 10.77	51.98± 0.01
ATZ	6.84 ± 3.03*	1.40 ± 1.03*	136.13 ± 21.11*	29.90± 0.51*
BA + ATZ	9.75 ± 6.50**	3.67 ± 3.13**	205.04 ± 16.91**	62.22± 0.20**

Data expressed in Mean ± S.D, n=6, *-statistically different ($p < 0.05$) compared with control,
**- statistically different ($p < 0.05$) compared with ATZ, BA- Betulinic acid, ATZ- Atrazine

In figure 1, treatment with ATZ caused the level of Malondialdehyde (MDA) to significantly increase ($p < 0.05$) by 84.6% and 85.8% in both liver and testes, respectively, when compared with BA-supplemented group. However, when BA was combined with ATZ treatment, the levels were found to be significantly lowered by 47.2% and 46%, respectively relative to the ATZ-treated rats. It was observed from the study that ATZ significantly reduced the concentrations of reduced glutathione (GSH) in liver and testes by 51.2% (Figure 2) and 64.2% (Figure 3), respectively compare with control rats. On supplementation with BA, it was noted that GSH concentrations were significantly increased by 47.1% (Figure 2) and 90.1% (Figure 3), respectively compared with ATZ-treated rats.

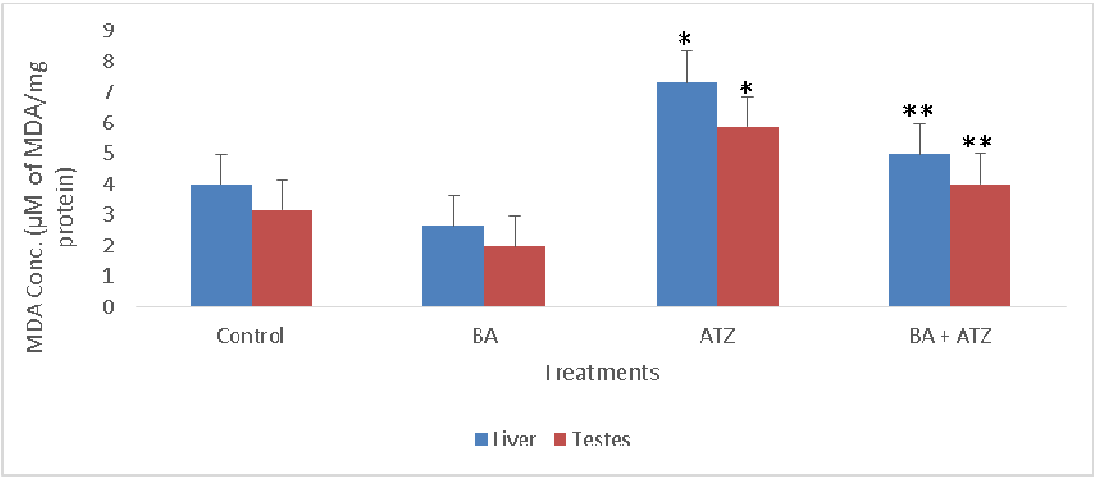


Figure 1. Effects of Betulinic Acid on Malondialdehyde (MDA) concentrations in liver and testes of rats treated with ATZ

Data expressed in M ± SD, n = 6, *-statistically different ($p < 0.05$) compared with control,

**- statistically different ($p < 0.05$) compared with ATZ.

BA- Betulinic acid, ATZ-Atrazine

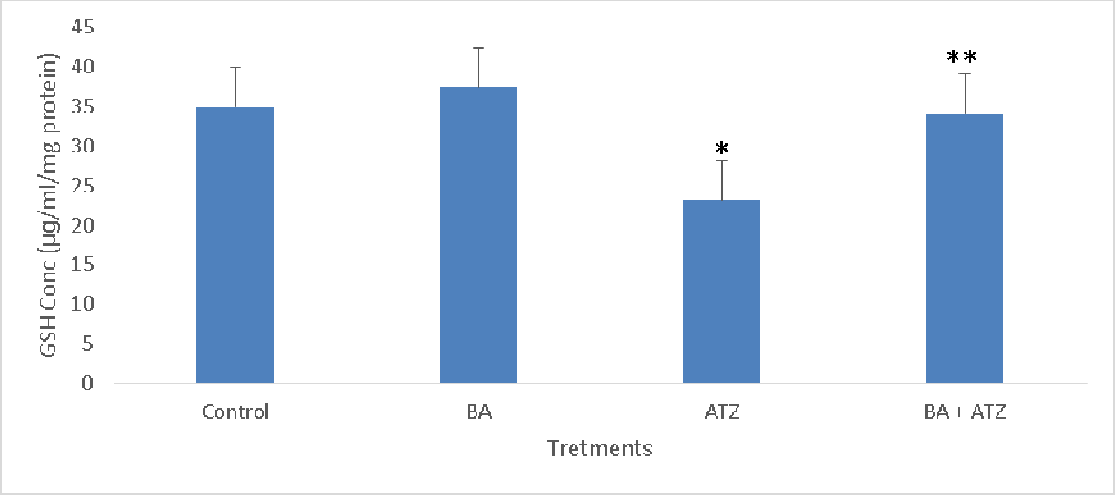


Figure 2. Effects of Betulinic Acid on reduced Glutathione (GSH) concentration in liverof rats treated with ATZ

Data expressed in M ± SD, n = 6, *-statistically different (p< 0.05) compared with control, **- statistically different (p< 0.05) compared with ATZ.

BA- Betulinic acid, ATZ-Atrazine

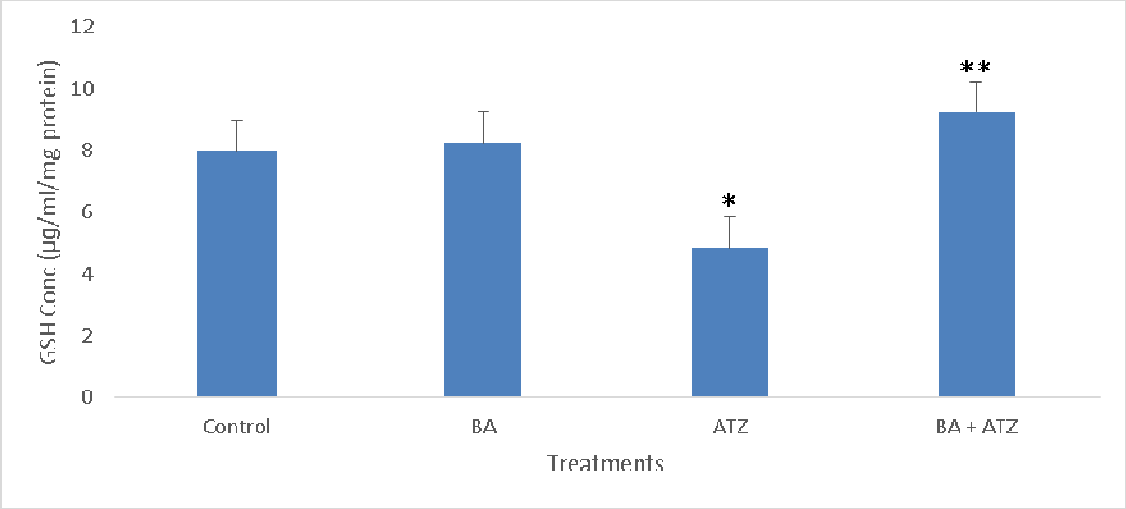
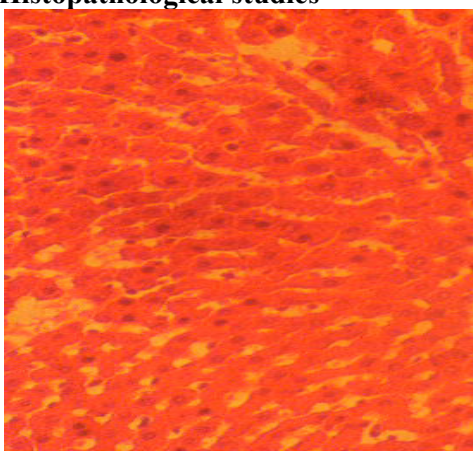


Figure 3. Effects of Betulinic Acid on reduced Glutathione (GSH) concentration in testes of rats treated with ATZ

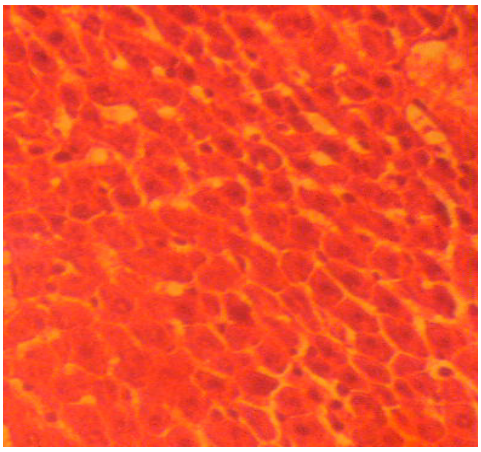
Data expressed in M ± SD, n = 5, *-statistically different (p< 0.05) compared with control, **- statistically different (p< 0.05) compared with ATZ.

BA- Betulinic acid, ATZ-Atrazine

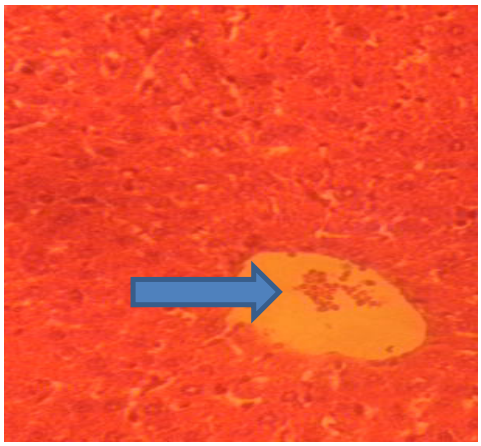
Histopathological studies



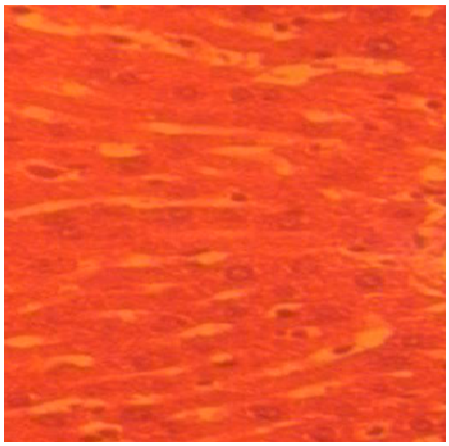
Control (Liver)- No visible lesion observed



BA (Liver) - No visible lesion observed

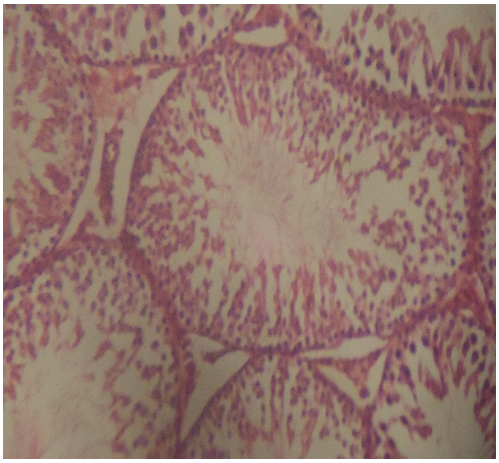


ATZ- (Liver)-Mild hepatocyte degeneration and periportal cell infiltration (Blue Arrow) observed

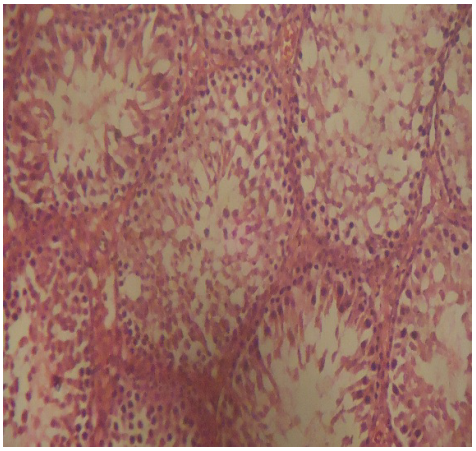


BA + ATZ (Liver) - No visible lesion

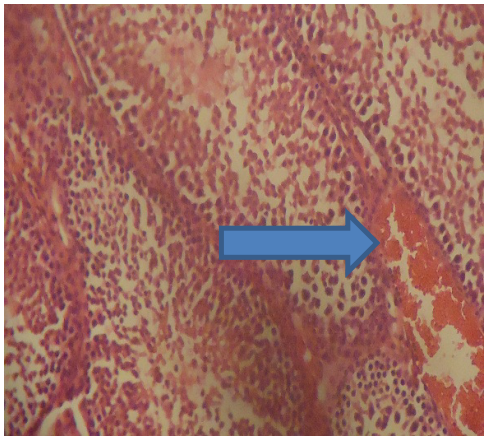
Figure 4. Effects of Betulinic acid on histology of liver of rats treated with Atrazine (x 100)



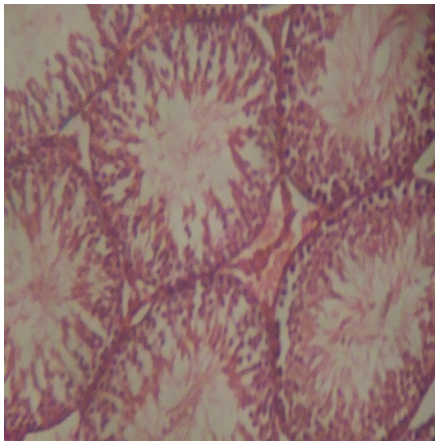
Control (Testis) - No visible lesion observed



BA (Testis) – No visible lesion observed



ATZ (Testis)- Foci tubular distortion and capsular congestion (Blue Arrow) observed



BA + ATZ (Testis) - No visible lesion

Figure 5. Effects of Betulinic acid on the histology of testes of rats treated with Atrazine (x 400)

Discussion

Atrazine is a selective pre- and post-emergentherbicide used to control broad leaf and grassy weeds.This herbicide is also used for selective weed controlin conifers, primarily Christmas trees and ornamentals (Meister, 1998; WSSA, 1994).Betulinic acid is a naturally occurring pentacyclictriterpenoid and has been shown to exhibit a myriad of biological activities and medicinal properties. Betulinic acid has also been reported to selectively inhibit proliferation of cancer cells, without affecting normal cells (Chintharlapalli*et al.,* 2011; Fulda, 2008). The non-cytotoxicity of BA has been demonstrated in human astrocytes, human dermal fibroblasts, peripheral blood lymphoblasts and animal studies (Faujan*et al.,* 2010;Mashitoh*et al.,* 2012).The present study has been undertaken to examine the possible ameliorative effects of Betulinic acid on the oxidativestress elicited in liver and testes of experimental Wistar rats treated with atrazine.

Table 1 shows that body weight gain in the rats treated with ATZ was 34.4% lower compared with the control rats and there was no significant difference between the ATZ-treated rats and those treated with a combination of ATZ and BA. Treatment with ATZ caused 17.4% increase in liver weight relative to controls, while BA supplementation caused 11.6% reduction compared with ATZ-treated group as also shown in table 1. This result is consistent with a study conducted by Santa Maria *et al*. (1987), who reported that orally ingested atrazine by male Wistar rats for 14 days, caused bodyweight to decreasein a dose-dependent manner. The reduction in body weight may be due to decrease in food intake. In a study,it has been found out that a chronic exposure of rats to low dose (30 or 300µg/kg of atrazine could induce increase in body weight (Gojmerac*et al*., 1995), which may be result from increase in insulin resistance, lipogenesis and fat storage in the adipose tissue

(Lim *et al*., 2009). The relative weight of liver was found to be 22.4% higher in the ATZ group compared with controls, while only 14.0% decrease was observed in the BA-supplemented rats compared with rats treated with ATZ. The results on testes showed no significant differences in both organ weight and relative weight (Table 1). Mainiero*et al*. (1987) indicated that atrazine decreased body weightsand increased the relative weights of testes in experimental rats. The findings of Rudzki*et al*. (1992) showed that atrazine exposure could decrease the weights of testes, epididymis and prostate by 29%, 29% and 22%, respectively. A recent study conducted in our laboratory on the effects of another herbicide, glyphosate, in rats also noted significant increase in body weights of the animals relative to controls, which Betulinic acid was able to ameliorate (Adeleke *et al*., 2015). We also observed that glyphosate caused significant elevation in the weights of testis and epididymis without any significant effects on their relative weights.Gojmerac*et al.* (1995) observed increased body weights in rats following a chronic administration of atrazine at low concentrations of 30 or 300 μg/kg.The increase in body weight gain in the atrazine-treated animals could be due to insulin resistance, since atrazine has been established as an endocrine disruptor according to Rayner *et al*. (2004).

Table 2 shows the results on determination of total protein in the serum, liver and testes of the experimental rats. Serum and liver recorded 46.2% and 94.8% increase, respectively, in the levels of total protein in the atrazine-treated rats relative to control rats. However, supplementation with BA was found to reduce the levels by 29% and 48.5% in serum and liver, respectively, compared with ATZ group. Yuanxiang*et al*. (2011) have noted that after administration of 10 and 1000 µg/L of atrazine for 14 days to Zebrafish, there were changes in protein regulation, which may be due to responses to various biological processes, such as oxidative stress and even inflammation. Testes,however, showed a contrasting result, in which atrazine treatment caused 55.9% decrease compared with controls, while BA supplementation was of no significant effect.

Treatment with ATZ significantly ($p < 0.05$) decreased the superoxide dismutase (SOD) activities in liver and testes by 52.8% and 89.3%, respectively, compared with control rats. The activities were however observed to be increased by 42.5% and 162.1% on BA supplementation in the respective tissues (Table 3). The activities of catalase (CAT) were noted to be significantly lowered by 56.9% and 53.6% in liver and testes, respectively, of ATZ group relative to controls, while the activities were significantly elevated by 50.6% and 108.1%, respectively in BA-supplemented rats compared with ATZ-treated rats as shown in table 3. In figure 1, treatment with ATZ caused the level of Malondialdehyde (MDA) to significantly increase ($p < 0.05$) by 84.6% and 85.8% in both liver and testes, respectively, when compared with BA-supplemented group. However, when BA was combined with ATZ treatment, the levels were found to be significantly lowered by 47.2% and 46%, respectively relative to the ATZ-treated rats. It was observed from the study that ATZ significantly reduced the concentrations of reduced glutathione (GSH) in liver and testes by 51.2% (Figure 2) and 64.2% (Figure 3), respectively compare with control rats. On supplementation with BA, it was noted that GSH concentrations were significantly increased by 47.1% (Figure 2) and 90.1% (Figure 3), respectively compared with ATZ-treated rats.In a related investigation of the antioxidant defence system, atrazine treatment significantly reduced the activities of GST and SOD enzymes in testicular and epididymal tissues, while the CAT activity was not significantly affected in testis, but rather reduced in the epididymis of the rats. The level of reduced glutathione (GSH) and glutathione-S-transferase (GST) activity were elevated in the animals with high dose, whereas the activities of superoxide-dismutase (SOD) and catalase (CAT), and levels of ascorbate, Malondialdehyde (MDA) and hydrogen peroxide were unaffected in the testicular tissue after a 7-day atrazine administration as also reported by Abarikwu and colleagues. (2009).Exposure to pesticides is knownto induce lipid peroxidation which may induce toxic biological effects (El-Demerdash*et al.,* 2004). Mechanism of pesticide toxicity in testis has been usually associated with increased lipid peroxidation as shown by Wafa*et al.*(2013). A similar exposure of rats to glyphosate was found to elevate the level of MDA and lower the activities of SOD and CAT in testis and epididymis, while the GSH level was lowered only in testicular tissue. These effects were ameliorated by Betulinic acid pretreatment as established by Adeleke *et al*.(2015).

Figure 4 shows that ATZ treatment caused mild degeneration of hepatocytes and periportal cell infiltration in the hepatic tissue, while BA pretreatment was found to ameliorate the features. Santa Maria *et al*. (1987) observed increase in total serum lipids, alkaline phosphatase (ALP) and alanine aminotransferase (ALT) in rats treated with daily oral gavage of atrazine, indicating liver toxicity. Although, there were no significant ultra-structural changes in hepatocytes up to 100 mg/kg/day, they noted a dose-dependent proliferation and degeneration of smooth endoplasmic reticulum (ER), lipid accumulation, mitochondrial malformation as well as alteration of bile canaliculi of the hepatocytes.

Atrazine and its related herbicides have been reported not to be primarily involved in carcinogenesis, but rather associated in endocrine responses in connection with reproduction and development in humans (Jowa and Howd, 2011). Figure 5 shows the result on investigation of the histopathological changes in the testes of experimental rats. Foci tubular distortion and capsular congestion in testicular tissue were observed in the ATZ-treated rats, which were observed to be ameliorated with BA-pretreatment.In an earlier study, Tisdel (1977) have reported that atrazine induced testicular atrophy, while Osterloh*et al*. (1983) observed that sperm morphology

showed no cytotoxic or genotoxic changes in animals treated with 600mg/kg/day of atrazine. Administration of 50, 100 and 200mg/kg/day to male Wistar rats for 31 days was observed to induce delay in sexual maturity, reduced serum levels of prolactin (PRL), leutenizing hormone (LH) and testosterone, while estradiol level was elevated (Stoker *et al*., 2000). However, when 400ppm of atrazine was administered to female Sprague-Dawley rats, the effects on the reproductive hormones included elevated plasma prolactin and estradiol, and reduced progesterone (Thakur, 1991a; McConnell, 1995). In a study reported by Capien (1996), an administration of 1000ppm of atrazine was found to cause testicular interstitial cell tumour.Atrazine exposure with male experimental animal models has been associated with reduction in the number and motility of spermatozoa (Kneiwald*et al.,* 2000), delayed sexual maturation (Kneiwald*et al.,* 1987) and reduction in prostate and seminal vesicle weight.According to Sifakis *et al*.(2011) and Hayes *et al*.(2011), atrazine has been found to cause low testicular testosterone synthesis and poor semen quality in male rats. A studyconducted on the effects of atrazine in male Japanese quail (*Coturnix japonica*) discovered smaller testicular size, decreased number of spermatocytes, necrotic nuclei of spermatids, and lesser number or absence of spermatozoa in the birds treated with 500mg/kg (Hussain*et al*., 2011). A dose-dependent induction of certain marker genes connected with steroidogenesis, includingsteroidogenic acute regulatory protein (STAR), cytochrome P450-11A1 and 3-hydroxysteroid dehydrogenase (3-HSD) has been noticed with atrazine administration, all indicating toxicity in the Leydig (interstitial) cells of rats (Abarikwu*et al*., 2011; Pogrmic-Maikic*et al*., 2010). In our earlier study using glyphosate, we noticed that this herbicide induced cellular degeneration and deformation of tubules in the testes of rats, and Betulinic acid exhibited an ameliorative potential against it (Adeleke *et al*., 2015).

Conclusion

The data presented from the present study have revealed that Betulinic acid elevated the activities of superoxide dismutase and catalase, while the levels of reduced glutathione and malondialdehyde were increased and decreased, respectively. The histology showed the ameliorative potential of Betulinic acid against hepatocyte degeneration and cell infiltration in liver, and tubular distortion and capsular congestion in testes, against Atrazine-induced toxicity. The properties thus exhibited by Betulinic acid in the present study are indicative that, this compound may serve as a potential template or agent in alleviating the toxic effects of environmental toxicant such as atrazine due to the antioxidant properties exhibited by this compound.

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REFERENCES

Abarikwu S. O., Adesiyan A. C., Oyeloja T. O., Oyeyemi M. O., Farombi E. O. (2009). Changes in Sperm Characteristics and Induction of Oxidative Stress in the Testis and Epididymis of Experimental Rats by a Herbicide, Atrazine. Arch Environ ContamToxicol., 58 :874–882.

Abarikwu S.O., Farombi E.O., Kashyap M. P., Pant A. B. (2011). Atrazine induces transcriptional changes in marker genes associated with steroidogenesis in primary cultures of rat Leydig cells. Toxicol In Vitro., 12: 361- 390.

Adams, N. H., Levi, P., and Hodgson, E. (1990). *In vitro* studies of the metabolism of atrazine, simazine, and terbutryn in several vertebrate species. J. Agric. Food Chem. *38*: 1411-1417.

Adeleke G.E., Adedosu O.T., Badmus J.A., Adaramoye O.A., Olajutemo T.O., Oluomo R.S. (2015). Protective potential of Betulinic acid against Glyphosate-induced toxicity in testes and epididymis of male Wistar rats. Int. J. Curr Res. 7 (6): 16650-16660.

Adler, I. (1980). A review of the coordinated research effort on the comparison of the test systems for the detection of mutagenic effects. Sponsored by the European Economic Community. Mutat. Res. 74:77-93. cited*in*: US EPA, 1990. Drinking Water Criteria

Document for Atrazine. PB91-142794. May 1990. Prepared by Dynamac Corporation for the Criteria and Standards Division, Office of Drinking Water. United States Environmental Protection Agency, Washington D.C.

Aebi H. (1984). Catalase in vitro. In: Packer L. Editor. Methods in Enzymology. Orlando FL: Academic Press; pp 121-126.

AlaideBS.,Glaucio FD., Claudia-Cristina DNH., Carolina LS., Bruno DL., Carine DF., Leandro DS., Ana ACF., Anthonio SC. (2013). Seasonal evaluation and quantitative analysis of Betulinic acid in ethanolic extracts of *Eugenia florida* leaves through the use of HPLC-UV and GC-FID. Academia Journal of Biotechnology. 1 (2): 021-026.

Anderson ME (1985). “Tissue glutathione”, In: Greenwald RA, ed Handbook of Methods for Oxygen Radical Research (CRC Press, Boca Raton).

Baker, D.B. (1988). Sediment, Nutrient and pesticide transport in selected great lakes tributaries. Great Lakes

National Program Office, U.S. Environmental Protection Agency, Region 5.

Bakke, J., Larson, J. D., and Price, C. E. (1972). Metabolism of atrazine and 2-hydroxyatrazine by the rat. J. Agric. Food Chem. *20*: 602-607.

Bradway D. E, and Moseman R. F. (1982). Determination of urinary residue levels of the *N*-dealkyl metabolites of triazine herbicides. J. Agric. Food Chem. *30*: 244-247.

Capen, C.C., (1996). Toxic Responses of the Endocrine System, pp. 617-640 in Casarett and Doull's Toxicology-the Basic Science of Poisons.Ed. C.D. Klaasen, 5th.Ed McGraw-Hill, NY.

Catenacci G, Maroni M, Cottica D, and Pozzoli L (1990). Assessment of human exposure to atrazine through thedetermination of free atrazine in urine. Bull. Environ. Contam. Toxicol. *44*: 1-7.

Cavas T. (2011). In vivo genotoxicity evaluation of atrazine and atrazine-based herbicide onfishCarassiusauratususing the micronucleus test and the comet assay. Food ChemToxicol., 49(6): 1431-1435.

Cerdeira A.L., Dornelas-DeSouza M., Bolonhezi D., Queiroz S.C.N., Ferracini V.L.,Ligo M.A.V., Pessoa M.C.P.Y., Smith Jr, S. (2005). Effects of Sugar CaneMechanical Harvesting Followed by No-Tillage Crop Systems on Leaching ofTriazine Herbicides in Brazil. *Bull. Environ. Contam. Toxicol,* 75: 805-812.

Chang CW, Wu TS, Hsieh YS, Kno SC, Chao PDL (1999). Triterpenoids of Syzygiumformosanum J Nat prod, 62:327-328.

Chintharlapalli S, Papineni S, Lei P, Pathi S, Safe S (2011). Betulinic acid inhibits colon cancer cell and tumor growth and induces proteasomedependent and -independent downregulation of specificity proteins (Sp) transcription factors. BMC Cancer, 11:371–383.

Cichwicz RH and kouzi SA (2004). Chemistry, biological activity and chemotherapentic potential of BA for the prevention and treatment of cancer and HIV infection. Medicinal Research Reviews 24 (1), 90-114.

Costa Silva, R.G.C., Vigna, C.R.M, Bottoli, C.B.G., Collins, C.H., Augusto, F. (2010). Molecularly imprinted silica as a selective SPE sorbent for triazine herbicides. *J. Sep.Sci.*, Vol. 33, pp. 1319–1324.

Dauterman, W. C., and Muecke, W. (1974). *In vitro* metabolism of atrazine by rat liver. *4*, 212-219. Determination of *s*-triazine herbicide residues in urine: Studies of excretion and metabolism in swine as a model to human metabolism. J. Agric. Food Chem. *27*, 743 746.

Dong X, Zhu L, Wang J, Xie H, Hou X and Jia W (2009). Effects of atrazine on cytochrome P450 enzymes of zebrafish (Daniorerio). Chemosphere; 77: 404-412.

Ehrhardt H, Fulda S, Fuhrer M, Debatin KM, Jeremias I (2004). Betulinic acid induced apoptosis in leukemiacells. Leukemia, 18:1406-1412.

El-Demerdash F. M., Yousef M.I., Kedwany F.S, Baghdadi H. (2004). "Role of α-tocopherol and β-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters, and semen quality of male rats," Journal of Environmental Science and Health. Part BPesticides, Food Contaminants, and Agricultural Wastes, 39(3): 443–459.

Erickson M.D., Frank C. W., and Morgan D. P. (1979). Determination of *s*-triazine herbicide residues in urine: Studies of excretion and metabolism in swine as a model to human metabolism. J. Agric. Food Chem. *27*: 743-746.

Faujan N.H, Alitheen N.B, Yeap S.K, Ali A.M, Muhajir A.H, Ahmad F.B.H (2010). Cytotoxic effect of betulinic acid and betulinic acid acetate isolated from Melaleuca cajuput on human myeloid leukemia (HL-60) cell line. A J Bio, 9:6387–6396.

Fulda S. (2008). Betulinic acid for cancer treatment and prevention. Int. J. Mol. Sci; 9:1096-1107.

Fulda S and Debatin K.M. (2000). Betulinic acid induces apoptosis through a direct effect on mitochondria in neuroectodermaltumors. Med PediatrisOncol; 35:616-618

Gojmerac T., Kartal B., Zuric M.,Curic S., Mitak M. (1995). "Serum biochemical and histopathological changes related tothe hepatic function in pigs following atrazine treatment,"*Journal of Applied Toxicology*, vol. 15, no. 3, pp. 233–236.

Hayes T. B., Anderson L. L., Beasley V. R., de Solla S. R., Iguchi T., Ingraham H., KestemontP., Kniewald J., Kniewald Z., Langlois V. S., Luque E. H., McCoy K. A., Muñoz-de-Toro M., Oka T., Oliveira C. A., Orton F., Ruby S., Suzawa M., Tavera-Mendoza L.E., Trudeau V. L., Victor-Costa A. B., Willingham E. (2011). Demasculinization andfeminization of male gonads by atrazine: Consistent effects across vertebrateclasses. *J Steroid Biochem Mol Biol.,* 3: 100-115.

Hayes TB, Stuart AA, Mendoza M, Collins A, Noriega N, Vonk A, Johnston G, Liu R and Kp-odzo D (2006). Characterization of atrazine-induced gonadal malformations in African clawed frogs (Xenopuslaevis) and comparisons with effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17beta-estradiol): Support for the demasculinization/feminization hypothesis. Environ Health Per-spect; 114 Suppl 1: 134-141.

Hayes WJ, Laws ER. (1990). Handbook of pesticide toxicology. Classes of pesticides, vol. 3. New York:

Academic Press, Inc.
Higa M, ogihara K, Yogis N. (1998). Bioactive naphthoquinone derivatives from dispyros maritime blume. Chem. Pharm bull: 46:1189-1193
Hussain R., Mahmood F., Khan M. Z., Khan A., Muhammad F. (2011). Pathological and genotoxic effects of atrazine in male Japanese quail (Coturnix japonica). Ecotoxicology, 20: 1–8.
Jagadeegh SG, Krupadanam GLD, Srimannarayana G, Murth SS, Kaur A, Raja SS. (1998). Tobacco caterpillar antifeedant from the gotti stem wood triterpene betulinic acid. J.Agric Food Chem. 46:2797-2799.
Juliani, C.C.; Silva-Zacarin, E.C.M.; Carvalho, D., Boer, P. A. (2008). Effects of atrazine on female Wistar rats: morphological alterations in ovarian follicles and immunocytochemicallabeling of 90 kDa heat shock protein. Micron, 30: 607-616.
Jowa L., Howd R. (2011). Should atrazine and related chlorotriazines be considered carcinogenic for human health risk assessment? J Environ Sci Health C EnvironCarcinogEcotoxicol Rev., 29(2):91-144.
Kniewald J., Jakominić M., Tomljenović A., Simić B., Romać P., Vranesić D., Kniewald Z (2000). "Disorders of male rat reproductive tract under the influence of atrazine", J. Appl. Toxicol., 20: 61-68.
Kniewald J., Peruzović M., Gojmerac T., Milković K., Kniewald Z. (1987). Indirect influence of s-triazines on rat gonadotropic mechanism at early postnatal period. J Steroid Biochem. 27(4-6):1095-100
Lim S., Ahn S. Y., Song I. C., Chung M. H., Jang H. C., Par K. S., Lee K., Pak Y. K., Lee H. K. (2009). Chronic Exposure to the Herbicide Atrazine Causes Mitochondrial Dysfunction and Insulin Resistance. *PLOS ONE*, Vol. 4, No 4, pp. 1-11
Lin H.C, Ding H.Y, Wu Y.C (1998). Two novel compounds from paconiasuffruticosa. J. Nat prod. 61:343-346. aurva SK, Devis, Pandey VB (1989). Fitother, 60:468
Loprieno, N., Barale R., Mariani L., Presciuttini S., Rossi A Sbrana I., Zaccaro L.,
AbbondandoloA., Bonnatti A.,(1980). Results of Mutagenicity Tests on the Herbicide
Atrazine. Mutat. Res. 74: 250-?.cited*in*: US EPA, 1990. Drinking Water Criteria Document for Atrazine. PB91-142794. May 1990. Prepared by Dynamac Corporation for the Criteria and Standards Division, Office of Drinking Water. United States Environmental Protection Agency, Washington D.C.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. J. boil. Chem. 193, 265-275.
Mainiero, J., Youreneff M., GiknisM., Yau E.(1987). Atrazine Technical: A Two
Generation Reproduction Study in Rats (MIN 852063). Ciba-Geigy Corporation. Study No. 87076. DPR Vols. 220-101.
Mashitoh AR, Yeap SK, Ali AM, Faujan A, Suhaimi M, Ng MK, Lam HY, Alitheen NB (2012). Immunomodulatory effects of betulinic acid isolation from the bark of Melaleuca cajuputi. Pertanika J Trop Agric Sci, 35:293–305.
McConnell R.F.(1995). A histomorphologic reevaluation of the ovaries, uterus, vagina,mammary gland, and pituitary gland from Sprague-Dawley and Fischer-344 female rats treated with atrazine. DPR Vol. 220-376.
Meister, R. T. (1998). Pesticide Dictionary; Atrazine. In Farm Chemicals Handbook '98, R. T. Meister, ed. (Willoughby, OH: Meister Publishing Company), pp. C34-35.
Misra H.P. and Fridovch J. (1975). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.
Mitchell J.R., Jollow D.J, Potter W.Z., Davis D.C., Gillette J.R., Brodi B.B. (1973). Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J. PharmacolExer Therap. 187: 185-194.
Ohkawa H., Ohishi N., Yagi K. (1979). Assay for lipid peroxides in animal tissues by Thiobarbituric acid reaction. Anal Biochem. 95: 351-358.
Osterloh, J., G. Letz, S. Pond and C. Becker, 1983. An Assessment of the Potential Testicular Toxicity of 10 Pesticides Using the Mouse-Sperm Morphology Assay. Mutat. Res.116:407-415. cited*in*: US EPA, 1990. Drinking Water Criteria Document for Atrazine.PB91-142794. May 1990. Prepared by Dynamac Corporation for the Criteria and StandardsDivision, Office of Drinking Water. United States Environmental Protection Agency,Washington D.C.
Pisha E, Chai H, Lee Is, chagwedera TE, Farnsworth NR, cordell AC, Beecher CWW, Frong HHS, kinghorn AD, Brown DM, Wani MC, Wall ME, Hieken TJ, Das hupta TK, Pezzuto JM (1995). Discovery of betubinic acid as a selective inhisitor of human melanoma that functions by induction of apDptosis. Nat med: 1:1046-1051.
Pogrmic-Majkic K., Fa S., Dakic V., Kaisarevic S., Kovacevic R. (2010). Upregulation of Peripubertal Rat Leydig Cell Steroidogenesis Following 24 h In Vitro and In Vivo Exposure to Atrazine. Toxicol.Sci., 118(1): 52-60.
Rayner JL, Wood C and Fenton SE (2004). Exposure parameters necessary for delayed puberty and mammary gland development in Long-Evans rats exposed in utero to atrazine. ToxicolApplPharmacol; 195: 23-

34
Ross, M. K., Jones, T. L., Filipov, N. M. (2009). Disposition of the Herbicide 2-Chloro-4- (ethylamino)-6-(isopropylamino)-s-triazine (Atrazine) and Its Major Metabolites in Mice: A Liquid Chromatography/Mass Spectrometry Analysis of Urine, Plasma, and Tissue Levels. *Drug Metabolism and Disposition,* 37: 776-786.
Rudzki, M.W., G. Batastini and A.T. Arthur. 1992. G-30033 13-Week Study in Dogs. Ciba-Geigy. Study No. MIN 902187. DPR Vol. 220-212.
Santa Maria, C., J. Moreno and J. Lopez-Campos. 1987. Hepatotoxicity Induced by the Herbicide Atrazine in the Rat. J. Appl. Toxicol. 7: 373-378.
Schuhly W, Heilmann J, Calis I, Sticher O (1999). New triterpenoids with antibacterial activity from zizyphusjoazeiro. Planta Med., 65:740-743
Selzer E., Pimentel E., Wacheck V., Schlegel W., Pehamberger H., Jansen B., Kodym R. (2000). Effects of betulinic acid alone and in combination with irradiation in human melanoma cells. J. Invest Dermatol; 114: 935-940.
Sifakis S., Mparmpas M., Soldin O. P., Tsatsakis A. (2011). Pesticide Exposure and Health Related Issues in Male and Female Reproductive System. In: Pesticides - Formulations, Effects, Fate. Margarita Stoytcheva, pp495-526.
Sriplang K., Adisawattana S., Rungsipipat A., Yibook A.S (2007). Effects of orthhosiphonstamineus aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin induced diabetic rats. J. Ethnopharmacol. 109: 510-514.
Stoker T.E., Guidici D.L., McElroy W.K., Laws S.C., Cooper R.L.(2000). The Effect of Atrazine (ATR) on Puberty in Male Wistar Rats. Tox. Sci 54:366.
Thakur A.(1991a). Determination of Hormone Levels in Sprague-Dawley Rats Treated with Atrazine Technical. Hazleton Washington Report No. 483-278. DPR Vol. 220-167.
Thuruher D, Turhani D, Pelzmann M, Wannemacher B, Knerer B, Formanek M, Wacheck V, Selzer E (2003). Betulinic acid: a new cytotoxic compound against malignant head and neck cancer cells. Head Neck, 25:732-740
Thornton B. J., Elthon T. E., Cerny R. L., Siegfried B. D. (2010). Proteomic analysis of atrazineexposure in *Drosophila melanogaster* (Diptera: Drosophilidae). Chemosphere,81(2): 235-41.
Tisdel M. (1977). 90-Day Subacute Feeding Study of Atranex in Rats.Agan Chemical Manufacture Ltd. Study No. T-636. DPR Vol. 220-044.
Wafa T, Nadia K, AmelN et al (2013). "Oxidative stress, hematological and biochemical alterations in farmers exposed topesticides," Journal of Environmental Science and Health—PartB Pesticides, Food Contaminants, and Agricultural Wastes, 48 (12): 1058–1069.
Weisenburger D., JoshiS., Hickman T., Walker B., Lawson T.,(1988). Mutagenesis Tests of Atrazine and N-Nitrosoatrazine. Proceedings of the American Association for Cancer Research.29:106.
WSSA. (1994). Atrazine. In Herbicide Handbook, 7th ed., W. H. Ahrens, ed. (Champaign, IL: Weed Science Society of America), pp. 20-23.
Yam MF, Basir R, Asmawi MZ and Ismanl Z (2007) Antioxidant and hepatoprotective effects of orthosiphonstamineus. Benth. Standardized extracts Am J. Chinese med. 35-115-126
Yuanxiang J., Xiangxiang Z., Dezhao L., Zhengwei F. (2011). Proteomic Analysis of HepaticTissue in Adult Female Zebrafish (*Daniorerio*) Exposed to Atrazine. Arch Environ ContamToxicol., DOI 10.1007/s00244-011-9678-9700
Zaya R. M., Amini Z., Whitaker A. S., Kohler S. L., Ide C. F. (2011). Atrazine exposure affectsgrowth, body condition and liver health in Xenopuslaevistadpoles. AquatToxicol., 104(3-4): 243-253.
Zuco V, Supino R, Righetti SC, Cleris K, Marchesi E, Gambacorti-Passerini C, Formelli F (2002). Selective cytotoxicity of betulinic acid on tumor cell lines, but not normal cells. Cancer Lett: 175:17-25.