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Silymarin abrogates acrylamide-induced oxidative stress-mediated testicular toxicity via modulation of antioxidant mechanism, DNA damage, endocrine deficit and sperm quality in rats

Hissah Ahmed Alturki[1] | Hany Amin Elsawy[1,2] | Ademola Clement Famurewa[3]

[1]Department of Chemistry, College of Science, King Faisal University, Al-Ahsa, Saudi Arabia

[2]Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt

[3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University, Ikwo, Ebonyi State, Nigeria

Correspondence
Hany Amin Elsawy, Department of Chemistry, College of Science, King Faisal University, Al-Ahsa, 31982, Saudi Arabia; Department of Chemistry, Faculty of Science, Tanta University, Tanta, Egypt.
Email: Hmostafa@kfu.edu.sa; hany.mostafa@science.tanta.edu.eg

Ademola Clement Famurewa, Department of Medical Biochemistry, Faculty of Basic Medical, Sciences, College of Medical Sciences, Alex-Ekwueme Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria.
Email: ademola.famurewa@funai.edu.ng

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Abstract

Acrylamide (ACR) is a toxic chemical formed in foods processed at high temperature; it is a food-borne toxicant with increasing public health attention due to its carcinogenic, neurotoxic and reproductive toxicities. However, till date, it is unknown whether silymarin (SIL) could attenuate ACR testicular toxicity. Therefore, the present study investigated the effect of SIL on ACR testiculotoxicity in rats. Rats were randomly divided and administered respective agents in Control group, ACR group, SIL group and ACR + SIL group for consecutive 14 days. Rat exposure to ACR resulted in significant reduction in the level of serum testosterone, whereas FSH and LH levels prominently increased compared to control. Acrylamide induced marked decreases in sperm count and sperm motility followed by a considerable increase in sperm abnormality percentage in the ACR-exposed rats in comparison to control. The testicular activities of glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) were significantly diminished, whereas malondialdehyde (MDA) level considerably increased. Additionally, ACR induced marked DNA fragmentation and histopathological lesions compared to control. Interestingly, the co-treatment of SIL with ACR attenuated the altered reproductive indices and restored antioxidant balance and DNA integrity. Overall, SIL prevents ACR-induced testicular reproductive deficits via modulation of antioxidant mechanism in rats.

KEYWORDS
acrylamide, oxidative stress, silymarin, sperm cell, testis

1 | INTRODUCTION

Acrylamide (ACR) is a highly toxic chemical with ubiquitous applications in plastic and paper production, dyes and water treatment (Radad et al., 2020). It is a monomer used in laboratories for preparation of polyacrylamide gel for electrophoresis and chromatographic techniques (Kirley & Norman, 2020). Strikingly, the discovery of ACR formation in carbohydrate-rich foods processed at high temperature (>120°C) has raised significant human health concerns (Abd-Elsalam et al., 2021). For example, the International Agency for Research on Cancer classifies ACR as a probable human carcinogen (Haidari et al., 2019). Glycinamide, the hepatic metabolite of ACR, binds and forms DNA adduct to cause genetic damage. However, formation of ACR in foods is linked with reactions associated with Millard browning involving carbonyl group of glucose or fructose and amino group of asparagine when foods are

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heated at high temperature (Kandeil et al., 2019). It is formed during grilling, roasting, frying and baking of crackers, potato crisps, cereals, bread, French fries and meat frying (Kandemir et al., 2020). Moreso that cereal grains or potatoes are components of most dry pet foods which are processed at high temperature (Kandeil et al., 2019). Robust body of literature strongly suggests that ACR is a potent chemical inducer of neurotoxicity, hepatotoxicity, nephrotoxicity, genotoxicity, muscle damage and developmental and reproductive derangements in animal models (Al-Karim et al., 2015; Al-Serwia & Ghoneim, 2015; Kandemir et al., 2020; Radad et al., 2020; Sun et al., 2018).

Accumulating evidence shows that environmental chemicals may impair human health especially those that alter endocrine milieu, spermatogenesis, testicular functions and fertility. Acrylamide is one of those environmental chemicals that exert deleterious effects on biological apparatus leading to human infertility. It is a germ cell mutagen and genotoxin that triggers damage to DNA in germ cells (Abd-Elsalam et al., 2021; Nixon et al., 2012; Pourentezari et al., 2014). The study of Farag et al. (2021) shows that ACR induces declined sperm quality, testicular degeneration, epididymis weight loss and aberrant steroidogenic signalling. Previous investigations on male rodents reveal varying degrees of reproductive and endocrine deficits provoked by ACR (Khalil et al., 2014; Wang et al., 2015; Yildizbayrak & Erkan, 2018). However, systematic studies have chiefly implicated oxidative stress in the molecular mechanism of ACR testicular toxicity (Al-Karim et al., 2015; Erdemli et al., 2019; Gül et al., 2021; Kucukler et al., 2020; Radad et al., 2020; Sun et al., 2018). Acrylamide undergoes hepatic metabolism through glutathione conjugation or oxidation yielding glycinamide which promotes membrane peroxidation and generation of reactive oxygen species (ROS) (Haidari et al., 2019). Acrylamide thus induces decline in antioxidant defence milieu to provoke reproductive derangement and DNA damage in delicate organs (Hamdy et al, 2020; Radad et al., 2020; Sengul et al., 2021). The adverse effects of ACR on levels of FSH, LH, testosterone and testicular antioxidant apparatus have been reported (Erdemli et al., 2019; Gül et al., 2021). In the light of the foregoing, it strongly appears that natural products with antioxidant action may play a beneficial role against ACR toxicity.

Silymarin (SIL) is a natural polyphenolic flavonoid found in the seeds and fruits of milk thistle *Silybum marianum*; it has several pharmacological properties and possesses potent free radical scavenging action both in vitro and in vivo (Faezizadeh et al., 2015; Kandemir, Kucukler, et al., 2017; Kandemir, Küçükler, & Çaglayan, 2017). It is known as a safe herbal product with an established hepatoptrotective efficacy at therapeutic doses without side effect (Yardım et al., 2021). It has been reported that the antioxidant nature of SIL prevents membrane lipid peroxidation and oxidative stress (Abd-Eldaim et al., 2021). In fact, a study found that SIL possesses higher antioxidant activity than vitamin E (Das & Mukherjee, 2012). It has been implicated for its modulatory antioxidant effects in various organ diseases, including the prostate, brain, lungs, and kidneys (Abd-Elsalam et al., 2021). However, literature reports a number of antioxidant agents against ACR toxicity; to our knowledge currently, there is a lack of data from studies on the protective effect of SIL against ACR-induced testicular injury. To this end, our study was targeted at evaluating the potential of SIL against ACR testicular toxicity and DNA damage in rats.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Acrylamide was purchased from Sigma Aldrich Chemicals (China). Reagent kits for SOD (SOD: Cat. No. SD 2521), CAT (CAT: Cat. No. CA 2517) and GPx (GPx: Cat. No. GP 2524) activities and MDA (MDA: Cat. No. MD 2529) level were obtained from Diagnostics, Giza, Egypt. Commercial kits for determination of testosterone (T: Cat. No. EIA1559), luteinizing hormone (LH: Cat. No. AKRLH-010) and follicle stimulating hormone (FSH: Cat. No. E-El-M0511) were purchased from DRG Instruments Gmbh, Germany; Shibayagi Co., Ltd., Japan and BIOCODE-HYCEL, Belgium, respectively.

2.2 | Experimental animals

Twenty-four male rats (Wistar strain) with body weight ranging from 200 to 250 g (3 months old) were obtained from the Animal House of King Faisal University, KSA, were housed in rat cages following general conditions (12 hours light /dark cycles at 25 ± 2°C) and were fed pelletized rat food and water and ad libitum during the acclimatization and experimental period. All experimental procedures including the use of animals in the current study were approved by Department of Chemistry, College of Science, King Faisal University, Kingdom of Saudi Arabia.

2.3 | Experimental design

Following acclimatization, all rats were randomly divided into four groups with six rats in each group as follows:

Group I (Control): Rats received daily intraperitoneal administration (i.p.) of normal saline for 14 consecutive days.

Group II (ACR): Rats received a daily dose of ACR (50 mg/kg) (i.p.) for 14 consecutive days.

Group III (SIL): Rats received a daily dose of SIL (160 mg/kg) (i.p.) for 14 consecutive days.

Group IV (ACR + SIL): Rats received a daily dose of ACR (50 mg/kg) (i.p) followed by a daily dose of SIL (160 mg/kg) (i.p.) for 14 consecutive days.

The selected doses of ACR and SIL were according to Mehri et al. (2016). At the end of the experiment, rats were anaethesized by i.p injection of sodium pentobarbital. Blood was obtained from the trunk and used for the separation of serum by centrifugation at 3000*g* for 10 min for serum analyses. Serum samples were thereafter stored at −80°C until use for the assessment of hormone parameters (T, LH and FSH). Rats were sacrificed and testis tissues were removed. Each testis was divided into two parts. One part was stored for assays on testicular activities of SOD, CAT, GPx, levels of MDA, and DNA fragmentation. The testes (1:5 w/v) were homogenized with a potter-Elvehjem homogenizer attached to a Taflon plunger in ice cold phosphate buffer (50 mM, pH 7.5). The homogenates were centrifuged to obtain supernatant samples

(11,000 × g for 20 minutes). The supernatant samples divided into aliquots stored at −80 °C for different assays. The second part was kept in 10% neutral formalin for analysis of histopathological changes.

2.4 | Biochemical assays

2.4.1 | Determination of serum testosterone (T) level

The German DRG Testosterone ELISA Kit (Cat No. EIA1559) was used for level of T determination according to the method of Sakuma (2009). The endogenous T binds to the coated antibody in wells of a microplate in a competitive binding with the testosterone horseradish peroxidase conjugate. After incubation, the colour intensity is inversely proportional to the level of T in the serum sample. The microplate is washed four times after 1-h incubation on a shaker. This was followed by addition of the substrate solution for colour development. The concentration of T is inversely proportional to the absorbance measured.

2.4.2 | Determination of serum LH level

The Japanese Shibayagi Company rat ELISA kit (Cat No. AKRLH-10) was used for the determination of LH in rat serum according to Shioya and Wakabayashi (1998) procedures. The quantitative determination of serum LH followed procedures for LH ELISA Kit in a sandwich ELISA system. After the routine procedures of ELISA, HRP-labelled avidin was added for 30 minutes incubation. HRP-complex remained in wells were reacted with a chromogenic substrate for 20 minutes. Colour development was measured spectrophotometrically at 450 nm.

2.4.3 | Determination of serum FSH level

The Belgian BIOCODE-HYCEL rat ELISA kit (Cat No. E-EI-M0511) was used for the determination of FSH in rat serum according to Teerds et al. (1989) protocols. After the routine ELISA procedures, the HRP- conjugated mouse anti-rFSH monoclonal antibody bound to sample FSH followed by washing of the unbound. Then the chromogen substrate was added for colour development. The intensity of the yellow colour was measured using a spectrophotometer at 450 nm.

2.4.4 | Oxidative stress markers

The activities of GPx, CAT, and SOD and levels of MDA were determined in testis using their commercial kits. Malondialdehyde levels were estimated following procedures of Ohkawa et al. (1979). Testicular activity of SOD was determined by Masayasu and Hiroshi (1979) method. Catalase activity was estimated according to Aebi (1984) method. Glutathione peroxidase (GPx) was determined based on method of Paglia and Valentine (1967).

2.4.5 | Determination of sperm quality

The testis epididymis suspension was used for assessing sperm quality. The suspension was obtained from the minced epididymis in pre-warmed saline (37°C):

Sperm count: The sperms were counted using a haemocytometer following the routine method of Freud and Carol (1964).

Sperm motility: The motility of the sperms was evaluated microscopically within 2–4 min of their isolation and data were expressed as percentage motility (Morrisey et al., 1988). Analyse of 200 motile sperm in 4 different fields was considered using one drop of sperm suspension on a glass slide. The number of non-motile sperm was estimated and then counting of total sperm cells. Sperm motility was expressed as a percent of motile sperm from the total sperm counted.

Sperm abnormality: The number of morphologically abnormal sperm was obtained by Evans and Maxwell (1987) method. Briefly, a drop from the sperm suspension stained with Eosin-nigrosin slide film was used in the determination of the number of live and dead spermatozoa at 37° C. The smear was air-dried and examined using a high power (100×) oil immersion objective. About 200 sperm cells were examined to determine the live and dead spermatozoa. The unstained head of sperm cells was regarded as live cells whereas partially or completely red stained heads were classified as dead. The dead/live ratio was calculated and the percentage abnormality was determined.

2.4.6 | DNA fragmentation analysis

The DNA fragmentation was assayed by agarose gel electrophoresis. The DNA was isolated from the testis using Wizard Genomic DNA Purification Kit (Promega Corporation Company, WI, USA) following manufacturer's instructions. The extracted DNA was electrophoresed on 2% agarose gel, and stained with ethidium bromide. DNA fragmentation pattern was photographed using a gel documentation system (Sambrook et al., 1989).

TABLE 1 Effect of silymarin on serum T, LH, and FSH levels in ACR-intoxicated rats

Group	T (pg/ml)	LH (ng/ml)	FSH (ng/ml)
Group I	4.90 ± 0.13	1.12 ± 0.08	0.47 ± 0.01
Group II	1.02 ± 0.05[a]	2.25 ± 0.09[a]	1.23 ± 0.04[a]
Group III	4.66 ± 0.12	1.12 ± 0.06	0.42 ± 0.01
Group IV	2.01 ± 0.19[b]	1.58 ± 0.06[b]	0.75 ± 0.01[b]

Notes: Values are mean ± SEM (6 rats/group).
Abbreviations: FSH, follicle stimulating hormone; LH, leutenizing hormone; T, Testosterone.
[a]p <0.05: significant compared to group I (control) in the same column.
[b]p <0.05: significant compared to group II (ACR) in the same column.

2.5 | Histopathological studies

Testis specimens were fixed in 10% buffered formalin solution for 48 hours. Graded ethanol was used for dehydration and embedded in paraffin. Sections of the testis were prepared by rotary microtome and stained with haematoxylin and eosin (H and E) for microscopic histopathological alterations (Bancroft & Gamble, 2002). The slides were examined under light microscope. The four tissue sections were scored and average value determined as showing: no changes (0), mild (1), moderate (2), and severe (3) following extensive alterations according to their histopathological findings.

2.6 | Statistical analysis

Data were compared using one-way ANOVA followed by LSD multiple range test. Significant differences were obtained at $P < 0.05$. Statistical tests were performed using SAS statistical software (SAS v.9.2, SAS Institute, Inc).

3 | RESULTS

3.1 | Effect of silymarin on serum hormones of ACR-intoxicated rats

Table 1 presents the effect of silymarin administration on serum levels of T, LH and FSH in rats exposed to ACR. We observed that ACR significantly reduced ($p < 0.05$) serum T level, whereas the levels of LH and FSH significantly increased compared to group I control rats. In contrast, the co-treatment of SIL with ACR considerably increased ($p < 0.05$) serum T level while the levels of LH and FSH markedly reduced compared to ACR group II. Administration of SIL alone in group III did not affect the endocrine indices compared to control group.

3.2 | Effect of silymarin on sperm quality of ACR-intoxicated rats

Table 2 depicts sperm quality characteristics consisting of sperm count, motility and abnormality. There was a significant decrease ($p < 0.05$) in sperm count as well as sperm motility while there was a significant increase in percentage of sperm abnormality in the rats intoxicated with ACR compared to group I normal control. In group IV (ACR + SIL) co-treatment, these altered indices were prominently attenuated by SIL compared to the group II (ACR). Treatment with SIL alone in group III did not affect the indices when compared with the control group I.

3.3 | Effect of silymarin on oxidative stress markers of ACR-intoxicated rats

3.4 | DNA fragmentation

The qualitative changes in the integrity of the testicular genomic DNA is represented in Figure 1. ACR (lane 2) induced marked increase in DNA fragmentation as compared to the control group (lane 1). On the other hand, Group IV (ACR + SIL) revealed that SIL had insignificant improvement on the DNA degradation (lane 4) compared to ACR in lane 2. DNA extracted from the control group (lane 1) as well as from the SIL group (lane 3) showed no specific DNA degradation.

3.5 | Effect of silymarin on histology of testis

The changes observed in the testis specimens were showed in Figure 2. Control group with normal seminiferous tubules (Figure 2a, b). In ACR group, there were alterations consistent with wide interstitial space (arrow) between irregular tubules (S) and depletion of sperms (star) (Figure 2c,d). SIL group showed healthy tubules (S) and

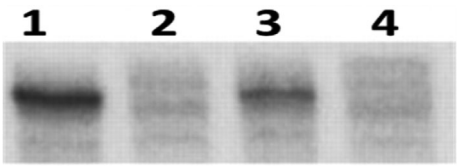

FIGURE 1 Effect of silymarin on testicular DNA fragmentation in acrylamide-intoxicated rats. 1) Control group showed thick bound of intact DNA; 2) ACR group showed dispersed DNA fragment without thick bound; 3) SIL group with thick bound; 4) ACR + SIL group with mild DNA fragment

TABLE 2 Effect of silymarin on sperm quality indices in ACR-intoxicated rats

Group	Sperm count (million/ml)	Sperm motility (%)	Sperm abnormality (%)
Group I	92.80 ± 1.62	88.0 ± 0.95	6.40 ± 0.60
Group II	25.20 ± 1.50[a]	45.8 ± 1.39[a]	34.0 ± 1.87[a]
Group III	91.0 ± 1.14	86.6 ± 1.21	6.60 ± 0.51
Group IV	52.8 ± 1.02[b]	66.0 ± 1.10[b]	15.60 ± 0.81[b]

Notes: Values are mean ± SEM (6 rats/group).
Abbreviations: FSH, follicle stimulating hormone; LH, leutenizing hormone; T, Testosterone.
[a]p <0.05: significant compared to group I (control) in the same column.
[b]p <0.05: significant compared to group II (ACR) in the same column.

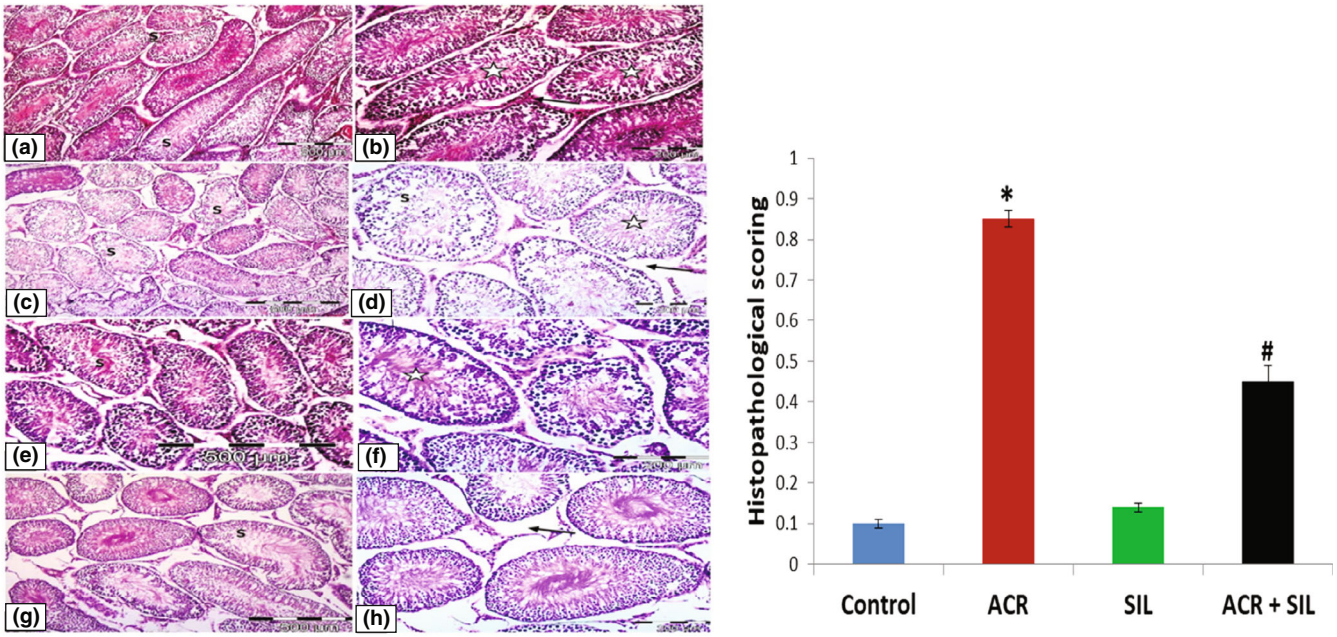

FIGURE 2 Representative photomicrographs of section of rat testis administered with silymarin and acrylamide (H & E stain). Control (a,b), ACR (c,d), SIL (e,f), ACR + SIL (g,h). Control showed normal histologic appearance of seminiferous tubules (star); ACR showed altered interstitial space (arrow) between irregular tubules (S) and depletion of sperm cells (star); SIL control showed normal histology; ACR + SIL showed mild improvement in the seminiferous tubules (S) with wide interstitial space (arrow) and restoration of spermatogenesis (star) (g, h). Values are expressed as mean ± SEM ($n = 6$). *Significant when compared to control ($p < 0.05$); #significant compared to ACR group

TABLE 3 The effect of silymarin on testicular antioxidant enzyme activities and lipid peroxidation marker

Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (mU/mg protein)	MDA (nmol/g tissue)
Group I	67.80 ± 1.07	38.40 ± 1.12	59.80 ± 1.28	7.04 ± 0.09
Group II	32.40 ± 0.93[a]	16.20 ± 0.66[a]	24.40 ± 0.60[a]	29.0 ± 1.30[a]
Group III	65.0 ± 0.71	37.60 ± 0.86	61.40 ± 1.08	6.58 ± 0.11
Group IV	39.0 ± 1.0[b]	29.80 ± 1.39[b]	36.40 ± 1.21[b]	17.60 ± 1.07[b]

Notes: Exposure to ACR induced significant depression ($p < 0.05$) in the activities of SOD, CAT, and GPx enzymes, whereas MDA level increased markedly relative to the control group. Conversely, there was a significant increases ($p < 0.05$) in activities of SOD, CAT and GPx along with noticeable decrease in MDA level in group IV (ACR + SIL) co-treatment group. Values are mean ± SEM (6 rats/group).
[a]p <0.05: significant compared to group I (control) in the same column.
[b]p <0.05: significant compared to group II (ACR) in the same column.

normal number of sperms (star) (Figure 2e,f). ACR + SIL group showed mild improvement in the seminiferous tubules (S) with wide interstitial space (arrow) and restoration of spermatogenesis (star) (Figure 2g,h).

4 | DISCUSSION

Acrylamide exposure is unavoidable due to global consumption of foods processed with high temperature (Farag et al., 2021). Acrylamide is formed during roasting, baking and frying; it is not basically formed in microwaved or boiled foods. It is a heat-induced toxicant usually found in bakery products like bread, and fried potato, potato crisps, French fries, and biscuits (Capuano & Fogliano, 2011; Haleem et al., 2021). Due to the testicular toxicity reported for ACR and its documented carcinogenic potential which may induce a lifelong harm, the present investigation has evaluated the possible protective effect of SIL against ACR-induced testicular toxicity.

In the present study, ACR triggered endocrine disruption underscored by the altered levels of T, LH and FSH. Published papers have implicated ACR as an endocrine disruptor that impairs steroidogenesis and spermatogenesis (Matoso et al., 2019). We observed that ACR exposure markedly reduced T, while LH and FSH prominently increased compared to control (Table 1). The hormonal dysregulation herein suggests adverse effect of ACR on testicular synthesis of T which has been reported in earlier publications (Abd-Elsalam et al., 2021; Erdemli et al., 2019; Farag et al., 2021). However, the decreased T level implicates toxicity of ACR on T synthesis apparatus, including LH, FSH, 3β-hydroxyl steroid dehydrogenase (3β-HSD) and 17β-HSD in the Leydig cells (Sharma et al., 2018). Although our study

did not explore 3β-HSD and 17β-HSD changes, studies suggest decrease in T levels during reduction in Leydig cell steroidogenic enzyme activities of 3β-HSD and 17β-HSD due to testicular toxicity (Hu et al., 2013; Sharma et al., 2018). In addition, the increased levels of LH and FSH could suggest a positive feedback mechanism on the pituitary gland system in this study which is in agreement with the report of a study that, decreased testosterone after cypemethrin exposure caused increased levels of FSH and LH (Hu et al., 2013). Interestingly, the concomitant administration of SIL, a natural flavonoid, inhibited the ACR-induced hormonal dysregulation in this study. The beneficial effects of natural products on testicular functions have been published in the existing literature (Belhan et al., 2021; Enebeli et al., 2021; Famurewa et al., 2020; Okkay et al., 2021). Silymarin is a natural products that has shown beneficial effect via exerting endocrine balance efficacy in several organs (Hussein et al., 2021; Yardım et al., 2021). In this study, SIL was observed to increase T level, while LH and FSH levels were restored comparable to normal control. In previous studies, SIL inhibits testicular toxic damage and enhances T production (Belhan et al., 2021; Faraji et al., 2019). The effect of SIL may be associated with its prowess to stabilize the cell membrane and promotes steroidogenesis in Leydig cells (Faraji et al., 2019).

Accumulating evidence indicates that ACR exerts deleterious effect on spermatogenesis. Studies have shown that ACR reduces sperm quality and quantity (Abd-Elsalam et al., 2021; Farag et al., 2021; Kucukler et al., 2020). In this study, ACR exposure impaired spermatogenesis as observed by significant reduction in epididymal sperm count, motility and considerably increased sperm abnormalities percentage compared to control (Table 2). Conceivably, these impairments may result from endocrine deficit and gonadotropin imbalance observed in this study. In consistent with our findings however, a previous study indicates that ACR-induced decreases in T may result in hypospermatogenesis (Farag et al., 2021). Furthermore, the physiological level of FSH is critical for normal spermatogenesis; it regulates proliferation and other physiological functions of Sertoli cells for germ cells maintenance (Alotaibi et al., 2020). Therefore, the altered levels of T and FSH in this study might have induced the markedly low sperm count. Our histopathological lesion from the testes showed irregular tubules which may also contribute to the low sperm count in this study as earlier indicated by Christina and Daniel (2015). Moreover, damage by peroxidation to sperm membrane rich in unsaturated fatty acids and kinesin motor proteins in the sperm flagella could be associated with the impaired motility (Aitken et al., 2013). In contrast, the administration of SIL significantly improved the reproductive indices, sperm count, sperm motility and abnormalities in this study. The beneficial effects of SIL on the levels of T, LH and FSH could be associated with the restoration of the sperm indices in this study (Moshtaghion et al., 2013). In addition, there was an alleviation of testicular histopathological changes in rats intoxicated by ACR and co-treated with SIL. These results reveal fertility action of SIL against ACR toxicity corroborating previous studies (Belhan et al., 2021; El-Hanbuli et al., 2017; Faraji et al., 2019; Jahromi et al., 2016).

Cellular redox homeostasis is maintained by endogenous antioxidant system detoxifying reactive oxygen species (ROS) from physiological metabolisms (Abd-Elsalam et al., 2021). Toxicants that inflict damage on delicate organs, including the testis are inducers of ROS generation. Generation of ROS in biological systems is well known to incapacitate antioxidant mechanism resulting into oxidative stress and lipid peroxidation. Herein, ACR, a food-borne toxicant, generates ROS and impaired antioxidant milieu in the testes of rats. This was manifested, in this study, by considerable depressions in the testicular activities of SOD, CAT and GPx, while MDA level conspicuously increased, which would consequently result in testicular oxidative stress (Table 3). SOD, CAT and GPx are antioxidant enzymes that collectively detoxify ROS preventing oxidative damage to cells. By implication, ACR is an oxidant capable of inducing damage on lipids, proteins, carbohydrates and nucleic acids. In fact, our study observed DNA fragmentation by ACR (Figure 1) in consistent with earlier findings (Abdel-Daim et al., 2020; Nixon et al., 2012; Sengul et al., 2021). Growing number of evidences suggest that ACR-induced toxicity in the testis, liver, brain and kidney occurs chiefly via oxidative stress (Al-Serwia & Ghoneim, 2015; Erdemli et al., 2019; Farag et al., 2021; Kandemir et al., 2020; Triningsih et al., 2021). Studies show that ACR-induced oxidative stress is related to Michael-type adduct formation that promotes depression in the cysteine moiety of glutathione, an intracellular tripeptide antioxidant. The formation of this ACR-glutathione adduct exacerbates GSH depletion leading to subsequent aggravation of ROS generation and lipid peroxidation (Triningsih et al., 2021). The increased MDA level suggests enhanced peroxidation and failure of the antioxidant mechanisms to prevent the production of testis ROS. Therefore, the ACR-induced ROS and lipid peroxidation might have overwhelmed the activities of GPx, SOD, CAT, and cause DNA damage in the testes, hence, the depressed activities found in this study. Thus, our results are consistent with the literature reports that ACR impairs antioxidant system and causes oxidative stress (Abd Elghaffar et al., 2015; Abd-Elsalam et al., 2021; Farag et al., 2021; Omar et al., 2015; Triningsih et al., 2021). Strikingly, our results showed significant decreases in MDA level and marked increases in CAT, SOD and GPx activities in testis of rats treated with ACR and SIL. However, the prevention of DNA fragmentation by SIL was not obviously evident as seen in Figure 1. The oxidative lesions in the histology of testis were also ameliorated in the current study (Figure 2). These are in conformation with the published reports by Faraji et al. (2019), Noureen et al. (2017) and Sajedianfard et al. (2016) that SIL decreases lipid peroxidation and increase antioxidant enzyme activities of CAT, SOD, GPx in testis of rats treated with cadmium, carbon tetrachloride and busulfan, respectively. Furthermore, the antioxidant efficacy of SIL against oxidative stress has also been reported in the liver, kidney and brain (Kandemir, Kucukler, et al., 2017; Kandemir, Küçükler, & Çaglayan, 2017; Mehri et al., 2016; Yardım et al., 2021). In corollary, it could be suggested that the improvement of reproductive function, sperm quality and testis histology observed in rats treated with ACR and SIL may be attributed to the antioxidant activity of SIL which has ameliorating effect on the hypothalamus–pituitary–testis axis leading to restorative levels of T, LH and FSH.

5 | CONCLUSION

In conclusion, ACR is a food-borne toxicant that induced depressed sperm quality, endocrine dysregulation, testicular oxidative stress and DNA fragmentation in the current study. We report herein, for the first time, the antioxidant efficacy of SIL enhancing sperm quality following restoration of endocrine balance and testicular antioxidant mechanism in rats. Therefore, SIL could protect the reproductive apparatus from ACR toxicity and also enhance fertility. However, future detailed mechanistic investigations are needed to support our findings before a dietary application could be considered.

AUTHOR CONTRIBUTIONS

Hissah Ahmed Alturki: Conceptualization, experimental design, results/data generation. **Hany Amin Elsawy**: Conceptualization, experimental design, analyses, writing of first draft, approval of final and full manuscript. **Ademola Clement Famurewa**: Experimental design, writing of drafts, writing of full manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Ademola Clement Famurewa https://orcid.org/0000-0002-8795-9964

REFERENCES

Abd Elghaffar, S. K., Fiedan, I. O., Ahmed, E. A., & Omar, H. E. (2015). Acrylamide induced testicular toxicity in rats: Protective effect of garlic oil. *Biomarkers Journal*, *1*, 1–5.

Abd-Eldaim, M. A., Barakat, E. R., Alkafafy, M., & Abd-Elaziz, S. A. (2021). Antioxidant and anti-apoptotic prophylactic effect of silymarin against lead-induced hepatorenal toxicity in rats. *Environmental Science and Pollution Research*, *28*(41), 57997–58006.

Abdel-Daim, M. M., Abo El-Ela, F. I., Alshahrani, F. K., Bin-Jumah, M., Al-Zharani, M., Almutairi, B., Alyousif, M. S., Bungau, S., Aleya, L., & Alkahtani, S. (2020). Protective effects of thymoquinone against acrylamide-induced liver, kidney and brain oxidative damage in rats. *Environmental Science and Pollution Research*, *27*, 37709–37717.

Abd-Elsalam, R. M., El Badawy, S. A., Ogaly, H. A., Ibrahim, F. M., Farag, O. M., & Ahmed, K. A. (2021). Eruca sativa seed extract modulates oxidative stress and apoptosis and up-regulates the expression of Bcl-2 and Bax genes in acrylamide-induced testicular dysfunction in rats. *Environmental Science and Pollution Research*, *28*, 53249–53266.

Aebi, H. (1984). Catalase. In H. U. Bergmeyer (Ed.), *Methods in enzymatic analysis* (pp. 276–286). Academic Press.

Aitken, R. J., Smith, T. B., Lord, T., Kuczera, L., & Koppers, A. J. (2013). On methods for the detection of reactive oxygen species generation by human spermatozoa: Analysis of the cellular responses to catechol oestrogen, lipid aldehyde, menadione and arachidonic acid. *Andrology*, *1*(2), 192–205.

Al-Karim, S., El-Assouli, S., Ali, S., Ayuob, N., & ElAssouli, Z. (2015). Effects of low dose acrylamide on the rat reproductive organs structure, fertility and gene integrity. *Asian Pacific Journal of Reproduction*, *4*(3), 179–187.

Alotaibi, M. F., Al-Joufi, F., Seif, H. S., Alzoghaibi, M. A., Djouhri, L., Ahmeda, A. F., & Mahmoud, A. M. (2020). Umbelliferone inhibits spermatogenic defects and testicular injury in lead-intoxicated rats by suppressing oxidative stress and inflammation, and improving Nrf2/HO-1 signaling. *Drug Design, Development and Therapy*, *14*, 4003–4019.

Al-Serwia, R. H., & Ghoneim, F. M. (2015). The impact of vitamin E against acrylamide induced toxicity on skeletal muscles of adult male albino rat tongue: Light and electron microscopic study. *Journal of Microscopic and Ultrastructure*, *3*, 137–147.

Bancroft, D., & Gamble, M. (2002). *The theory and practice of histology technique* (5th ed., p. 75). Churchill Living Stone.

Belhan, S., Yildirim, S., Kayikci, C., Kömüroğlu, A. U., Özdek, U., & Kuşcu, Y. (2021). Investigation of the effect of silymarin on oxidative DNA damage and inflammatory markers in ischemia/reperfusion injury following experimental testicular torsion/detorsion in rats. *Turkish Journal of Zoology*, *45*, 267–276.

Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *LWT- Food Science and Technology*, *44*, 793–810.

Christina, O. U., & Daniel, U. O. (2015). Effects of acrylamide on the reproductive hormones and sperm quality in male rats. *International Journal of Science Research*, *6*(7), 5–8.

Das, S. K., & Mukherjee, S. (2012). Biochemical and immunological basis of silymarin effect, a milk thistle (*Silybum marianum*) against ethanol-induced oxidative damage. *Toxicology Mechanisms and Methods*, *22*, 409–413.

El-Hanbuli, H. M., Abo-Sief, A. F., & Mostafa, T. (2017). Protective effect of silymarin on the testes of rats treated with anabolic androgenic steroid: A biochemical, histological, histochemical and immunohistochemichal study. *Journal of Histology and Histopathology*, *4*, 1–7.

Enebeli, B., Nwangwa, E. K., Nwogueze, B. C., Nzenegu, A., Agbonifo-Chijiokwu, E., Omeru, O., & Ebuwa, E. I. (2021). *In vivo* attenuation of alcohol- and cadmium chloride–induced testicular toxicity modulated by silymarin in male wistar rats. *Biology Trace Element Research*. https://doi.org/10.1007/s12011-021-02944-3

Erdemli, Z., Erdemli, M. E., Turkoz, Y., Gul, M., Yigitcan, B., & Bag, H. G. (2019). The effects of acrylamide and vitamin E administration during pregnancy on adult rat testis. *Andrologia*, *51*(7), e13292.

Evans, G., & Maxwell, W. M. (1987). Handling and examination of semen. In W. M. C. Maxwell (Ed.), *Salamon's artificial insemination of sheep and goats* (pp. 93–106). Butterworths.

Faezizadeh, Z., Gharib, A., & Godarzee, M. (2015). *In-vitro* and ***in-vivo*** evaluation of silymarin Nanoliposomes against isolated methicillin-resistant *Staphyloccus aureus*. *Iranian Journal Pharmaceutical Research*, *14*, 627–633.

Famurewa, A. C., Ekeleme-Egedigwe, C. A., Onwe, C. S., Egedigwe, U. O., Okoro, C. O., Egedigwe, U. J., & Asogwa, N. T. (2020). Ginger juice prevents cisplatin-induced oxidative stress, endocrine imbalance and NO/iNOS/NF-κB signalling via modulating testicular redox-inflammatory mechanism in rats. *Andrologia*, *52*, e13786. https://doi.org/10.1111/and.13786

Farag, O. M., Abd-Elsalam, R. M., El Badawy, S. A., Ogaly, H. A., Alsherbiny, M. A., & Ahmed, K. A. (2021). *Portulaca oleracea* seeds' extract alleviates acrylamide-induced testicular dysfunction by promoting oxidative status and steroidogenic pathway in rats. *BMC Complementary and Medical Therapy*, *21*, 122–136.

Faraji, T., Momeni, H. R., & Malmir, M. (2019). Protective effects of silymarin on testis histopathology, oxidative stress indicators, antioxidant defence enzymes and serum testosterone in cadmium-treated mice. *Andrologia*, *e13242*, e13242.

Freud, M., & Carol, B. (1964). Factors affecting haemocytometer count of sperm concentration in human semen. *Journal of Reproduction and Fertility*, *8*, 149–152.

Gül, M., Kuştepe, E. K., Erdemli, M. E., Altınöz, E., Bağ, H. G., Gül, S., & Göktürk, N. (2021). Protective effects of crocin on acrylamide-induced testis damage. *Andrologia*, *00*, e14176. https://doi.org/10.1111/and.14176

Haidari, F., Mohammadshahi, M., Zarei, M., & Fathi, M. (2019). Protective effect of citrus lemon on inflammation and adipokine levels in acrylamide-induced oxidative stress in rats. *Brazilian Journal of Pharmaceutical Sciences*, *55*, e18285.

Haleem, E. N., Hasan, W. Y., & Arafa, H. M. (2021). Therapeutic effects of thymoquinone or capsaicin on acrylamide-induced reproductive toxicity in rats mediated by their effect on oxidative stress, inflammation, and tight junction integrity. *Drug and Chemical Toxicology*. https://doi.org/10.1080/01480545.2021.1942485

Hu, J. X., Li, Y. F., Li, J., Pan, C., He, Z., Dong, H. Y., Dong, H. Y., & Xu, L. C. (2013). Toxic effects of cypermethrin on the male reproductive system: With emphasis on the androgen receptor. *Journal of Applied Toxicology*, *33*(7), 576–585.

Hussein, R. M., Sawy, D. M., Kandeil, M. A., & Farghaly, H. S. (2021). Chlorogenic acid, quercetin, coenzyme Q10 and silymarin modulate Keap1-Nrf2/heme oxygenase-1 signaling in thioacetamide-induced acute liver toxicity. *Life Sciences*, *277*, 119460.

Jahromi, H., Abedi, H., Hashemi, S., Jashni, H., Jahromi, Z., & Pourahmadi, M. (2016). The effect of silymarin on spermatogenesis process in rats. *International Journal of Medical Research and Health Science*, *6*, 146–150.

Kandeil, M. A., Hassanin, K. M., Arafa, M. M., Abdulgawad, H. A., & Safwat, G. M. (2019). Pomegranate peels ameliorate renal nitric oxide synthase, interleukin-1β, and kidney injury molecule-1 in nephrotoxicity induced by acrylamide in rats. *Egypt Pharmaceutical Journal*, *18*, 368–376.

Kandemir, F., Küçükler, S., & Çaglayan, C. (2017). Beneficial effects of silymarin and naringin against methotrexate-induced hepatotoxicity in rats. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, *12*, 167–177.

Kandemir, F. M., Kucukler, S., Caglayan, C., Gur, C., Batil, A. A., & Gülçin, I. (2017). Therapeutic effects of silymarin and naringin on methotrexate-induced nephrotoxicity in rats: Biochemical evaluation of anti-inflammatory, antiapoptotic, and antiautophagic properties. *Journal of Food Biochemistry*, *41*, e12398.

Kandemir, F. M., Yildirim, S., Kucukler, S., Caglayan, C., Darendelioglu, E., & Dortbudak, M. B. (2020). Protective effects of morin against acrylamide-induced hepatotoxicity and nephrotoxicity: A multi-biomarker approach. *Food and Chemical Toxicology*, *138*, 111190.

Khalil, W., Ahmed, H., Hanan, F., Aly, H., & Eshak, M. (2014). Toxicological effects of acrylamide on testicular function and immune genes expression profile in rats. *Journal of Pharmaceutical Science and Review Research*, *24*(1), 143–151.

Kirley, T. L., & Norman, A. B. (2020). Ligand binding to a humanized anti-cocaine mAb detected by non-reducing SDS-PAGE. *Biochemistry and Biophysics Report*, *23*, 100795.

Kucukler, S., Caglayan, C., Darendelioğlu, E., & Kandemir, F. M. (2020). Morin attenuates acrylamide-induced testicular toxicity in rats by regulating the NF-κB, Bax/Bcl-2 and PI3K/Akt/mTOR signaling pathways. *Life Sciences*, *261*, 118301.

Masayasu, M., & Hiroshi, Y. (1979). A simplified assay method of superoxide dismutase activity for clinical use. *Clinica Chimica Acta*, *92*(3), 337–342.

Matoso, V., Bargi-Souza, P., Ivanski, F., Romano, M. A., & Romano, R. M. (2019). Acrylamide: A review about its toxic effects in the light of developmental origin of healthand disease (DOHaD) concept. *Food Chemistry*, *283*, 422–430.

Mehri, S., Dadesh, Q., Tabeshpour, J., Hassani, F. V., Karimi, G., & Hossein, H. (2016). Evaluation of the neuroprotective effect of silymarin on acrylamide-induced neurotoxicity. *Jundishapur Journal of Natural Pharmaceutical Products*, *11*(14), e37644.

Morrisey, R. E., Schwctz, B. A., James, C., Monica, D. R., Teague, J. L., & Moris, R. W. (1988). Evaluation of rodent sperm, vagina cytology and reproductive organ weight from National Toxicology Programme-13weeks studies. *Fundamental and Applied Toxicology*, *11*, 343–358.

Moshtaghion, S.-M., Malekinejad, H., Razi, M., & Shafie-Irannejad, V. (2013). Silymarin protects from varicocele-induced damages in testis and improves sperm quality: Evidence for E2f1 involvement. *Systems Biology in Reproductive Medicine*, *59*(5), 270–280.

Nixon, B. J., Stanger, S. J., Nixon, B., & Romon, D. (2012). Chronic exposure to acrylamide induces DNA damage in male germ cells of mice. *Toxicological Sciences*, *129*(1), 135–145.

Noureen, F., Khan, M. R., Shah, N. A., Khan, R. A., Naz, K., & Sattar, S. (2017). *Pistacia chinensis*: Strong antioxidant and potent testicular toxicity amelioration agent. *Asian Pacific Journal of Tropical Medicine*, *10*(4), 380–389.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Annals of Biochemistry*, *95*, 351–358.

Okkay, U., Ferah Okkay, I., Cicek, B., Aydin, I. C., Ertugrul, M. S., Bayram, C., Senyayla, S., Sezen, S., Mendil, A. S., Guven, L., & Hacimuftuoglu, A. (2021). *Achillea millefolium* alleviates testicular damage in paclitaxel-intoxicated rats via attenuation of testicular oxido-inflammatory stress and apoptotic responses. *Andrologia*, *53*, e14028.

Omar, H. E., Abd-elghafar, S. K., Fiedan, I. O., & Ahmed, E. A. (2015). L-cysteine ameliorated testicular toxicity induced by acrylamide in rats. *European Journal of Biological Research*, *5*(2), 1–8.

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*, *70*, 158–169.

Pourentezari, M., Talebi, A., Abbasi, A., Khalili, M. A., Mangoli, E., & Anvari, M. (2014). Effects of acrylamide on sperm parameters, chromatin quality, and the level of blood testosterone in mice. *Iranian Journal of Reproductive Medicine*, *12*, 335–342.

Radad, K., El-Amir, Y., Al-Emam, A., Al-Shraim, M., Bin-Jaliah, I., Krewenka, C., & Moldzio, R. (2020). Minocycline protects against acrylamide-induced neurotoxicity and testicular damage in Sprague-Dawley rats. *Journal of Toxicologic Pathology*, *33*, 87–95.

Sajedianfard, J., Nazifi, S., Izadi, A., Chahardahcherik, M., & Honarmand, M. (2016). Effect of various doses of silymarin on the oxidative stress induced by busulfan administration in the different organs of rats. *Turkish Journal of Pharmaceutical Science*, *13*(2), 233–240.

Sakuma, Y. (2009). Gonadal steroid action and brain sex differentiation in the rat. *Journal of Neuroendocrinology*, *21*(4), 410–414.

Sambrook, J., Fritsch, F., & Maniatis, T. (1989). *Molecular cloning: A labolatory manual*. CSHL press.

Sengul, E., Gelen, V., Yildirim, S., Tekin, S., & Dag, Y. (2021). The effects of selenium in acrylamide-induced nephrotoxicity in rats: Roles of oxidative stress, inflammation, apoptosis, and DNA damage. *Biological Trace Element Research*, *199*(1), 173–184.

Sharma, P., Khan, I. A., & Singh, R. (2018). Curcumin and quercetin ameliorated cypermethrin and deltamethrin-induced reproductive system impairment in male wistar rats by upregulating the activity of pituitary-gonadal hormones and steroidogenic enzymes. *International Journal of Fertility and Sterility*, *12*(1), 72–80.

Shioya, N., & Wakabayashi, K. (1998). In vivo bioactivities and kinetic parameters of rat luteinizing hormone components: Discrepancy between in vitro and in vivo assays. *Endocrine Journal*, *45*, 307–314.

Sun, J., Li, M., Zou, F., Bai, S., Jiang, X., Tian, L., Ou, S., Jiao, R., & Bai, W. (2018). Protection of cyanidin-3-O-glucoside against acrylamide-and glycidamide-induced reproductive toxicity in leydig cells. *Food and Chemical Toxicology*, *119*, 268–274.

Teerds, K. J., Closset, J., Rommerts, R. F. G., De Rooij, D. G., Stocco, D. M., Colenbrander, B., Gwensing, J., & Hennen, G. (1989). Effects of pure FSH and LH preparations on the number and function of Leydig cells in immature hypophysectomized rats. *Journal of Endocrinology*, *1989*(120), 97–106.

Triningsih, D., Yang, J.-H., Sim, K. H., Lee, C., & Lee, Y. J. (2021). Acrylamide and its metabolite induce neurotoxicity via modulation of protein kinase C and AMP-activated protein kinase pathways. *Toxicology In Vitro*, *72*, 105105.

Wang, E.-T., Liu, H., Yan, H.-Y., & Yuan, Y. (2015). Protective effect of allicin against glycidamide-induced toxicity in male and female mice. *General Physiology and Biophysics*, *34*(2), 177–187.

Yardım, A., Kucukler, S., Ozdemir, S., Çomaklı, S., Caglayan, C., Kandemir, F. M., & Çelik, H. (2021). Silymarin alleviates docetaxel-induced central and peripheral neurotoxicity by reducing oxidative stress, inflammation and apoptosis in rats. *Gene*, *769*, 145239.

Yildizbayrak, N., & Erkan, M. (2018). Acrylamide disrupts the steroidogenic pathway in Leydig cells: Possible mechanism of action. *Toxicology and Environmental Chemistry*, *100*(2), 235–246.

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