Current Topics in Toxicology
Vol. 19, 2023

Mini-Review

Acrylamide-induced reproductive toxicity and its management strategies

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ABSTRACT

Increased consumption of deep-fried and baked food items has escalated acrylamide exposure in humans, eliciting a myriad of toxic clinical effects. Acrylamide (ACR)-triggered reproductive toxicity is widely studied in animal models, with little information available on its impact on humans. Clinical manifestations associated with acrylamide toxicity include decreased body weight, lowered testosterone levels, and testicular impairment, as confirmed by its histopathological evaluation. Several molecules belonging to the domain of nutraceuticals or metabolites are found to mitigate this acrylamide-induced testicular toxicity by virtue of their antioxidant property. The current review discusses the underlying mechanisms of acrylamide intoxication in the male reproductive system and provides a comprehensive knowledge of the mitigation strategies associated with the reported issue.

KEYWORDS: acrylamide, testicular toxicity, antioxidants, phytochemicals, probiotics, mitigation strategies.

1. Introduction

Acrylamide, a food-borne toxicant, is virtually produced during the thermal processing (deep frying, roasting, and baking) of foods. Thermal processing enhances food palatability by enriching its appearance, taste, and crunchiness, which in turn leads to excessive consumption [1, 2]. Research data suggests that some of the widely consumed thermal processed food items like French fries, potato crisps, biscuits, bread, and roasted coffee are loaded with acrylamide ranging between 59-5200 µg/kg. High consumption of these snacks may predispose consumers, predominantly youngsters than adults, to several health ailments [3, 4].

Ingested acrylamide gets metabolized *via* either glutathione-S-Transferase (GST) conjugation or cytochrome P450 oxidation resulting in mercapturic acid and glycinamide metabolites, respectively [5, 6]. Research findings postulate that acrylamide and its metabolites are highly reactive and often form adducts with biomolecules like DNA, protein haemoglobin, etc., thereby eliciting a plethora of toxicological implications like developmental toxicity, neurological toxicity, reproductive toxicity, carcinogenicity, genotoxicity, immunotoxicity, respiratory as well as multiple organ failure [4, 7-15]. Acknowledging the ever-increased consumption of processed food and its alarming adverse effects validated on animal models, the International Agency for Research on Cancer (IARC) has classified acrylamide as a *probable human carcinogen* (based on laboratory data) [3, 16].

Several cohort studies published in the literature elaborate carcinogenic, genotoxic, and neurotoxic effects of acrylamide illustrated *via* human and animal models [5, 17]. Comparatively, limited literature has been published on acrylamide-induced reproductive toxicity. Gradual and cumulative exposure to acrylamide leads to infertility and copulation issues, dictating an efficient management strategy [14].

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Proper selection of raw materials (potato with less starch content), pre-treatment of foodstuffs (soaking, blanching, vacuum incorporation), controlling processing parameters (exposure temperature-time, pH, %moisture), supplementation or fortification of raw materials (asparaginase, cysteine, probiotics) are some of the listed mitigation strategies demonstrating effectiveness in curtailing acrylamide content in food items [18].

The current integrated review attempts to provide insight into acrylamide ingestion and elicited reproductive toxicity, especially testicular toxicity The cited findings will hopefully attract the scientific community toward achieving a substantial solution.

2. Acrylamide (ACR) and reproductive toxicity

Acrylamide has been implicated to cause reproductive toxicity in rats and mice models. The No-observed-adverse-effect-level (NOAEL) of acrylamide for causing reproductive toxicity in rats was evaluated to be 2 to 5 µg/kg/d. Though the reproductive toxicity of ACR in humans has not been studied, the effect of ACR in humans is expected to be qualitatively similar to that in rodents. ACR can readily move across the placental barrier, resulting in its high exposure during the embryonic stage, which ultimately leads to developmental and post-natal defects in rodent offspring [5]. Commonly observed clinical manifestations of acrylamide-induced reproductive toxicity include decreased body weight, impaired sperm morphology, testicular damage, reduced copulation, and infertility in male animal models. However, the effect of acrylamide on female animal models exhibited negligible impact, demonstrating them to be relatively resistant to acrylamide intoxication [19, 5, 20].

Acrylamide toxicity predominantly affects the male reproductive system with minimal effect on the female reproductive system. Acrylamide-induced testicular genotoxicity is observed more in younger male rats than adult male rats implying the predominant impact of acrylamide intoxication on the young population. Data suggests that acrylamide can affect the developmental process by altering gene expression, resulting in neurobehavioral changes [13, 21]. A comprehensive analysis of the literature provides insight into several impaired and altered physiological, biochemical, and chromosomal manifestations in response to acrylamide toxicity; these are discussed in the following sections.

Acrylamide-intoxicated rodents displayed reduced levels of total antioxidant capacity, total testosterone, and free testosterone, while alleviated levels of plasma malondialdehyde (MDA) were observed. mRNA analysis revealed that genes related to testicular hormones are down-regulated, however, immune response-related genes are up-regulated [21]. cDNA microarray analysis of testes isolated from acrylamide-treated rodents displayed altered expression of genes in the testes that had an impact on the sperm concentration and also identified upregulated and downregulated genes that play a key role in nucleic acid-binding, testicular functions, cellular redox, apoptosis, cell growth, and cell cycle [22]. Katen and colleagues, in their study, demonstrated that acute ACR exposure in male rodents resulted in elevated levels of DNA damage in the spermatozoa, which affected fertility and impaired fetal development. Moreover, ACR exposure leads to high levels of enzyme CYP_2E_1 in the spermatozoa of rats and their offspring, which causes more DNA damage [23].

Administration of ACR in rats causes growth retardation in epididymal sperm reserves [18]. In addition, repeated injections of ACR to male rats caused depressed plasma levels of testosterone and prolactin [24]. Importantly, ACR treatment resulted in reduced fertility in male rodents along with a significant reduction in sperm count. There appears an expected connection between the neurotoxicity and reproductive toxicity of acrylamide because the underlying cause of both neurotoxicity and reproductive toxicity is the disruption of kinesin motor protein function. Kinesin motor proteins are important proteins that are present in the flagella of sperm and in the nervous system and other tissues. Disruption in the normal function of kinesin motor proteins may lead to reduced sperm motility and fertilization events. Protamine is an important and unique protein that is present in the sperm head and tail, and the alkylation of the sulfhydryl groups on these proteins affected rodent reproduction. This could affect sperm penetration and encourage pre-implantation misfortunes, which have been found in some dominant lethal studies [25].

ACR acts as a clastogen that causes major anomaly in various genes, which ultimately results in genotoxicity. In mammalian cells, metabolic conversion of ACR into glycidamide (GA), a powerful mutagen, causes serious deleterious effects at the HPRT locus. It has also been reported that ACR can act as a Michael acceptor, and therefore ACR has unique ability to form adducts with thiol, hydroxyl, or amino groups and nucleophilic centres in the DNA. In a recent study, it was reported that glycidamide significantly induces micronuclei, impairs cell propagation kinetics, and causes drastic decline in overall cell viability when cells are treated with extreme concentrations of glycidamide, and the process does not involve oxidative stress. It further validates that human mammary cells are highly susceptible to GA-induced toxicity [26]. Conversion of acrylamide to its epoxide metabolite, glycidamide, is mediated by cytochrome P450 2E1; glycidamide is considered an active metabolite that plays a key role in acrylamide-induced genotoxicity in experimental animals and humans [6].

Acrylamide (ACR) is quickly converted to glycidamide (GA) in the body by the epoxidation process with CYP2E1 (cytochrome P450 2E) acting as a catalyst. However, both ACR and GA can be readily detoxified *via* direct conjunction with glutathione, mediated by glutathione-S-transferases enzyme, and by hydrolysis of glycidamide to glyceramide [27]. In a study, it was observed that there was a significant increase in the levels of CYP2E1 protein in the spermatocytes of acrylamide-treated mice, and most importantly, enhanced CYP2E1 protein levels were also observed in the progeny of acrylamide-treated mice. It is an important observation since CYP2E1 is the only enzyme that converts acrylamide to its more harmful metabolite, glycidamide [23].

An ACR analog has exhibited potential effectiveness as a novel nitric oxide-independent soluble guanylyl cyclase activator for treating a spread of disorders related to nitric oxide (NO) signalling. A key target of endogenous NO is soluble form of guanylyl cyclase (sGC) enzyme, which is a dimeric protein having an alpha and a beta subunit [25]. Diffusion of NO inside target cells and a concomitant activation of sGC may lead to increase in levels of the second messenger, cyclic guanosine monophosphate (cGMP) [28]. In testis, NOS/sGC/cGMP signalling pathway performs a series of functions, including spermatogenesis, sperm motility, and germ cell apoptosis [26]. It is also important to point out that two types of NOS (eNOS and iNOS, but not nNOS) were implicated to cause germ cell apoptosis [29].

3. Role of antioxidants in acrylamide toxicity

Acrylamide is formed in foodstuffs processed at higher temperatures *via* either acrolein intermediate formation (degradation of lipids, proteins, or sugars), or Maillard-type reaction. Acrylamide exposure damages biological macromolecules and interferes with normal cellular metabolism, which ultimately results in oxidative stress and causes imbalance in antioxidant potential of cell. It elicits several *in-vivo* toxicological implications like neurological, carcinogenic, and reproductive disorders [30]. Thus, curbing oxidative reactions by using antioxidants constitutes one of the significant mitigation strategies for acrylamide toxicity.

For ease of understanding, molecules with similar biochemical nature are grouped and discussed together. They are categorized into:

- Phytochemicals / Nutraceuticals: plant-based phenolics and similar compounds having medicinal properties. E.g., Garlic, Turmeric, Green tea, etc.
- Primary metabolites: compounds chemically distinct from phenolics and secreted by living cells essential for their growth and metabolic requirement. E.g., Amino acids, vitamins, etc.
- Probiotics: living microbes considered safe for human consumption. E.g., *Lactobacillus*, *Bacillus*, etc.
- Miscellaneous: synthetic molecules distinct from phenolics with respect to structure and mode of action are included herein. E.g., minocycline.

3.1. Role of phytochemicals

Consumed acrylamide generates free oxygen radicals *in-vivo,* which easily traverses lipophilic testicular barrier resulting in cellular toxicity. Medicinal plants and natural herbal products are rich sources of phenolics and flavonoids, which exhibit proven antioxidant and anti-inflammatory properties. Peculiarity in the investigation of

phytochemicals as therapeutic agents lies in their effectiveness without any toxic side effects and adverse effects compared to their synthetic analogs [31]. Classical animal-based models cited in the literature are clubbed, discussed, and compared herein.

Gul *et al*. (2021) have reported a promising effect of crocin against acrylamide-induced testicular toxicity in a rat model when studied for a period of 21 days [32]. Crocin, a phytochemical present in *Crocus sativus*, is well documented in literature for its several pharmacological uses like anti-inflammatory agent, male fertility booster, hypolipidaemic agent, antioxidant [33], etc. Crocin administered at a dose of 50 mg/kg was found to increase the hormonal levels (testosterone, FSH, LH), with improved histopathological testicular changes and oxidant-antioxidant parameters (SOD, CAT, TAS, TOS, MDA, GSH), implying its ameliorative potential against acrylamide-induced testicular oxidative stress.

The effectiveness of turmeric against degenerative testicular changes induced by acrylamide exposure in a mice model was studied by Gouda *et al*. (2011). Findings report that in the acrylamide administered group, around 36% reduction in average sperm count was observed along with irregularity in basement testicular membrane, abnormal vacuolation, and elongated spermatids. All results indicated atrophy of the germinal epithelium of the sperm cells in the acrylamide administered group, whereas the same deformities and atrophy were not seen in control (saline) and curcumin (80 mg/kg body weight per day) administered groups.

An important study by Rajeh and Al-Shehri (2019) documented the mild antioxidant effects of *Ferula hermonis* root extract for the treatment of acrylamide-induced testicular toxicity in adult male virgin Winster rats [34]. Animals were subdivided into four groups: control, Acrylamide fed (60 mg/kg bw), Acrylamide+Freula hermonis root extract fed, and *Ferula hermonis* root extract fed alone groups. Post five-day treatment with *Ferula hermonis* root extract and three days of post-treatment observation, blood, hormonal, and histopathological evaluations were performed. Significant reduction in mean sperm count, serum testosterone concentration, germ cell degeneration, and Leydig cells atrophy was observed in all three groups except the control group. However, no statistically significant histopathological differences were observed in groups treated with Acrylamide and Acrylamide + Ferula hermonis.

Kucukler *et al*. (2020) reported that concurrent administration of Morin alleviates the acrylamide-induced testicular toxicity [35]. In their investigational study on a rat model, co-administration of Acrylamide + Morin at a dose of 38.27+100 mg/kg bw increased titer of oxidative (SOD, CAT, GPx, MDA, GSH), inflammatory cytokines (NF-Kβ, TNF-α, IL-6, 1β, COX-2) and autophagic (Bax/Bcl-2, cytochrome c, caspase 3) titers compared with acrylamide fed group (38.27 mg/kg body weight).

The cytoprotective role of resveratrol and garlic acid against acrylamide-triggered testicular damage was reported by Alturfan *et al*. (2012) and Elghaffar *et al*. (2015), respectively. Individual supplementation of these agents in acrylamide-challenged rats was observed as a promising approach in ameliorating undesirable biochemical and cellular testicular changes.

Green Tea, *Camellia Sinensis,* contains an abundant pool of polyphenolics that exhibits multiple health-promoting properties. The beneficial antioxidant effects of green tea on lung and liver carcinomas are cited in the literature [36]. El-Sweedy *et al*. (2007) have reported the usefulness of green tea in mitigating obesity-associated testicular disorders using an obese Wistar rat model [37]. It was seen that 2% aqueous extract of green tea significantly reduced testicular triglycerides, phospholipids, and nitric oxide levels in an obese rat, thus proving it as a promising drug in alleviating obesity-induced oxidative stress on testicular functioning. Another study done by Yassa *et al*. (2012) demonstrated green tea extract as an antidote for acrylamide toxicity in a study performed on male Sprague-Dawley rats [38]. A significant dose resulted in a proportional decrease in testosterone level and animal body weight, accompanied by increased testicular degenerative histopathological changes in animals fed with acrylamide. Alkylation of SH groups present in sperms or testicular DNA triggers reproductive toxicity. Antioxidant-rich green tea extract inhibits this chemical reaction, thereby eliciting protective effect against acrylamide toxicity.

E. sativa has been traditionally used in the Mediterranean region and Western Asia for its potent aphrodisiac effects and antioxidant properties. Abd-Elsalam *et al*. (2021) investigated the efficacy of Eruca sativa seed extract (ESS) against hypogonadism in acrylamide (ACR)-treated male Wistar rats [39]. ESS extract administration helped in improving the quality of semen, decreased lipid peroxidation, enhancing testicular antioxidant enzyme, restoring serum testosterone level, and reducing testicular degeneration along with preventing Leydig cell death in the ACR-treated rats. It was also observed that testicular B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X proteins (Bax) were elevated due to ESS administration inducing anti-apoptotic effect.

Farag *et al*. (2021) have investigated the beneficial role of *Portulaca oleracea* seeds' extract (POS) for treating acrylamide-induced testicular toxicity in male Wistar rats [40]. ACR-administered rats showed epididymides weight loss, increased testicular lesion scoring, testicular oxidative stress, and testicular degeneration. They also showed a significant decline in serum testosterone level and expression of steroidogenic genes such as CYP11A1 and 17b3-HSD. Subsequently, treatment of ACR-treated rats with POS extract showed marked improvement in the serum testosterone levels and in the expression levels of the aforementioned steroidogenic genes.

Abd Al Haleem *et al*. (2021) provided a detailed mechanistic understanding of how anti-inflammatory and antioxidant pathways manage ACR-induced oxidative stress in testes [41]. Their experimental illustration was based upon two phytochemicals *viz*, thymoquinone and capsicin, which were administered to rats alone and in combination with acrylamide, and their effects on male reproductive system were compared against control and acrylamide fed group. Reversal of acrylamide-induced oxidative stress mechanism involving downregulation of occluding expression, and increased expression of NF-Kβ/p65 by phytochemicals was postulated.

3.2. Primary metabolites

3.2.1. Amino acids

L-cysteine (L-cys) is a sulphur-containing semi-essential amino acid with proven anti-oxidant and anti-inflammatory properties. Kacar *et al*. (2018) investigated the efficacy of L-cys against acrylamide (ACR)-induced testicular toxicity in the Sprague-Dawley rat model [42]. Their findings displayed a decrease in body and testis weights, seminiferous tubule diameter, and proliferating cell nuclear antigen expression. In contrast, an increase was observed in Bax protein expression and the percentage of seminiferous tubules containing multinucleated giant cells in the ACR-induced rats. However, Group IV (L-cys treated ACR administered rats) showed a decrease in multinucleated giant cells along with a reduced rate of decrease of proliferating cell nuclear antigen expression; no effect was seen in reducing, restoring, or reversing any of the other damages. Thus, it was observed that L-cys afforded partial protection against testicular damage and further studies are needed to prove its exact effects. Shahrzad *et al*. (2020) conducted an investigation to assess the protective effects of N-acetyl cysteine (NAC) on testicular tissue and serum levels of pituitary-gonadal axis hormones in ACR-treated adult rats [43]. The acrylamide-fed group (50 mg/kg body weight) showed reduced FSH and testosterone levels, alleviated LH levels, and decreased spermatogenic effects/process and Leydig cell count. The animal group fed only with NAC (40 mg/kg body weight) showed no impact on hormonal levels and spermatogenesis. However, when ACR and NAC were co-administered in a dose-dependent manner, marked improvement was seen in serum levels of FSH, LH, and testosterone; there was an increase in the number of spermatogenic events and recovery of disrupted Leydig cells. Thus, it was observed that NAC administration in rats helped in the recovery of spermatogenesis process and pituitary-testicular axis hormones. However, this study is limited due to various aspects, including its short duration. Hence, for better results, duration needs to be increased along with evaluation of different sperm parameters.

3.2.2. Enzymes

Coenzyme Q10 (CoQ10) is a proven antioxidant. Taking into consideration the protective properties of CoQ10, Mazen & Elnegris (2013) investigated its benefits against acrylamide (ACR)-induced histological test changes in albino rats [44]. These

rats, in experiment, were divided into three groups: SubGroup Ia (control group) was administered orally with distilled water, SubGroup Ib (control group) with an intraperitoneal injection of corn oil (solvent media of CoQ10), Group II orally with ACR (15 mg/kg bw daily for 8 consecutive weeks) and Group III orally with ACR (15 mg/kg bw daily for 8 consecutive weeks) and intraperitoneal injection of CoQ10 (10 mg/kg bw daily for 8 consecutive weeks). ACR administration resulted in tubular affection, shrunken tubules with disorganized germinal epithelium, cellular vacuolations, and sloughed spermatogenic cells into lumen. Further, it was observed that the interstitium of the testis has widened with congested capillaries, interstitial hyperplasia and eosinophilic material. There was a significant deterioration of proliferating cell nuclear antigen immunoreactive spermatogonia and spermatocytes, whereas the number of inducible nitric oxide synthase, immunoreactive spermatogonia and spermatocytes showed alleviation. In the group where CoQ10 was administered with ACR, the results clearly showed that CoQ10 afforded protection to testes against damages, as mentioned earlier.

3.2.3. Vitamins

The proven antioxidant role of Vitamin E has been prophylactically and therapeutically exploited in the management of several health ailments like cancer, aging, etc. Acrylamide ingestion elicits testicular oxidative degeneration, which could be partially limited or absolutely controlled by masking the free oxygen radicals. El Kotb *et al*. (2020) have reported the cytoprotective role of Vitamin C and E against monosodium glutamate-triggered testicular toxicity in a rat model [45]. Fewer reports are available in literature citing the potential of vitamins in alleviating acrylamide-induced testicular toxicity. Rajeh and Khayyat (2017) investigated the protective role of vitamin E on adult Wistar rats, which were categorically sub-divided into seven groups as control, single drug fed group-acrylamide (ACR), Vitamin E, 5-amino salicylic acid (5-ASA) and Co-administered groups; ACR + 5-ASA, ACR + Vitamin E, ACR + 5-ASA + Vitamin E at an ACR, 5-ASA and Vitamin E dose of 45 mg/kg (bw)/day, 25 mg/kg (bw)/day, 200 mg/kg (bw)/day, respectively [46]. After feeding program, animals were sacrificed, and their testicular biochemical and histopathological evaluation was performed. Supplementation of both 5-ASA and vitamin E showed promising reversal of ACR-induced testicular degeneration. A similar study in this context was done by Randagale *et al*. (2012), wherein they demonstrated that during active feeding of acrylamide and vitamin E in rats, the antioxidant activity of vitamin E was not observable [47]. However upon completion of the feeding strategy, testicular recovery in vitamin E-administered group was significantly faster than in untreated group. Thus, from the experimental study, it can be concluded that vitamin E facilitates post-acrylamide-induced cytoprotective effect, and demonstrates therapeutic efficacy in the management of ACR-induced reproductive toxicity.

3.2.4. Lipoic acid

Smaller molecular size coupled with a higher lipophilic index supports ease of accessibility and reactivity towards reactive oxygen species across biological membranes. These features of lipoic acid make it a promising natural therapeutic agent against oxidative stress [48, 49]. Reports citing the therapeutic application of lipoic acid in cardiovascular disorders [50] and xenobiotic toxicity [51] are abundant in the literature. Lipoic acid has a protective role in the case of acrylamide-induced testicular and epididymal damage in a rat model, which was demonstrated in the study performed by Lebda *et al*. (2014) [52]. 1% lipoic acid supplementation facilitated reversal of acrylamide-induced testicular oxidative stress *via* attenuation of free oxygen reactive species, restoration of hormonal levels and re-establishment of healthy seminiferous tubular structure.

3.3. Probiotics

Acrylamide detoxification by probiotics occurs either by cell wall interaction, molecular adsorption, or metabolite secretion. The therapeutic and prophylactic role of probiotics in cellular management and homeostasis is well established and commercialized for metabolic disorders. Involvement of probiotics as an antioxidant and anti-inflammatory agent in cellular and reproductive disorders is an upcoming research area [53-55].

Poutahidis *et al*. (2014) demonstrated a potential impact of probiotics in restoring male reproductive

balance against aging and oxidative stress [56]. Genetically outbred mice, CD-I, were subdivided into diet-based four groups viz, control (AIN-76A), New Westernized diet (High fat, low fiber, and substandard vitamin D), Probiotic alone (*L. reuteri*), and co-administered (New westernized + probiotic). These groups were started with their pre-planned diet plan at the age of 8 weeks and were continued till the animals attained the cohort five months, seven months, nine months, and twelve months of age, followed by humane euthanasia. Effect of diet was studied across the different mice groups at varied ages for their anti-inflammatory markers, serum testosterone titer, sperm quality and quantity, histopathology, and immunohistochemistry. The results obtained are depicted in Table 1. It suggests that probiotic supplementation offers a promising alternative in restoring reproductive fitness by overcoming age and diet barrier.

Zhao *et al*. (2020) published systematic experimental evidence of probiotics upon acrylamide-induced oxidative damage in rat model [57]. In their investigation, 48 adult male rats were categorized into 8 groups (n = 6), *viz*. as control, preventive and therapeutic groups. Acrylamide-treated group (40 mg/kg/day) exhibited a significant reduction in body weight gain, varying degrees of tissue injury, decreased levels of superoxide dismutase, catalase, and glutathione, and increased lipid peroxidation. *L. plantarum* $1*10^9$ CFU/mL administered to preventive and therapeutic groups were observed to limit acrylamide-induced tissue damage.

Table 1. Restorative effect of probiotic on acrylamide-induced toxicity.

Sr. No.	Study parameter	Experimental findings on probiotic diet consumption against control and westernized diet
1	Morphology	Increased testes weight
2	Biochemical	Increased testosterone concentration
3	Histopathology and Immunohistochemistry	1. Increased seminiferous tubules 2. Increased seminiferous tubule cross-sectional area 3. Increased germ cell nuclear volume 4. Increased area of the testicular interstitium and Leydig cell area and volume 5. Increased cellular proliferation in Leydig cells
4	Immunological implication	6. Upregulation of IL-10 7. Decreased IL-17

Table 2. Comparative dynamic biological variations upon acrylamide and antioxidant administration.

Parameter	Detail	ACR exposed	Antioxidant administered
Quality and quantity of sperms	Relative testes weight Sperm count Head and tail abnormalities	↓ 35% ↓ 42% ↓ 79%	↑ 45% ↑ 65 ↑ 380
Serum sex hormones	Testosterone FSH LH	↓ 52% ↑ 82% ↑ 73%	↑ 98% ↓ 44% ↓ 40
Oxidative stress markers	GSH MDA SOD CAT LDH-X	↓ 71% ↑ 418% ↓ 91% ↓ 83% ↓ 48%	↑ 111 ↓ 58 ↑ 266 ↑ 236 ↑ 31
Histopathological	Nuclear factor in testicular region Occludin detection	↑ ↓ 55%	↓ ↑ 99

Table 3. Consolidated summary of acrylamide and antioxidant administration on animal models studied.

Sr. No.	Antioxidant (reference)	Animal model & number of animals	Groups	Conc.	Animal body weight (mg) / Testis weight* (mg) / Sperm count#		Testosterone level	Histopathological observation	Biochemical observations
					Initial	End	Initial		
1	Green Tea (Yassa *et al.* 2013)	Sprague Dawley rats (n = 10)	Control	Saline	182±2.5*	223±0.61*	3.95±0.87 ng/ml	Thickened tubular endothelium, sperm cells degeneration, multinucleated giant cells in seminiferous tubules	NM
			ACR	30 mg/kg	179±0.23*	183.2±1.4*	0.79±0.32 ng/ml		
			ACR + Drug	30 mg/kg + 70 mg/kg	180±1.9*	224.2±1.9*	3.90±0.75 ng/mL		
			Drug	70 mg/kg	177±1.8*	219.9±1.9*	3.94±0.88 ng/ml		
2	Crocin (Gul *et al.* 2021)	Wistar Albino rats (n = 10)	Control	Saline	NS	NS	50.94±3.82 nmol/l	Testicular atrophy, germ cell degeneration, heterochromatic Leydig cells with degenerated vascular structures	↑OSI, Malondialdehyde, Total OS with decreased conc. of TAS, GSH, CAT, SOD in ACR-treated group
			ACR	25 mg/kg	NS	NS	45.05±3.43 nmol/l		
			ACR + Drug	25 mg/kg + 50 mg/kg	NS	NS	65.05± 2.29 nmol/l		
			Drug						
3	Curcumin (Gouda *et al.* 2011)	Albino mice (n = 7)	Control	Distilled water	NS	NS	NS	Atrophy and exfoliation of the germinal epithelium of seminiferous tubule, thickening of the basement membrane, higher percentage of abnormal sperms	
			ACR	60 mg/kg	NS	NS	NS		
			ACR + Drug	60 mg/kg +80 mg/kg	NS	NS	NS		
			Drug						
4	Ferula (Rajeh & Al-Shehri, 2019)	Wistar Rats (n = 4)	Control	Normal drinking water	650x $10^{6\#}$	650x $10^{6\#}$	16 ng/ml	Reduction in sperm count with ACR-treated group. No significant histopathological difference observed in ACR and ACR + Ferula-treated group implying it to be a weak antioxidant.	

Table 3 continued..

			ACR	60 mg/kg	650x $10^{6\#}$	300x $10^{6\#}$	3 ng/ml		
			ACR + Drug	60 mg/kg + Ferula root extract	650x $10^{6\#}$	400x $10^{6\#}$	2 ng/ml		
			Drug		650x $10^{6\#}$	400x $10^{6\#}$	7 ng/ml		
5	L-cysteine	Sprague Dawley rats (n = 7)	Control	Saline	285.1±31.0*; 3.22±0.18	292.6±38.2*; 3.22±0.18	34.74	ACR induced significant decrease in seminiferous tubular diameter, loss of germ cells, increased formation of multinucleated giant cells	Decreased PCNA expression, increase in Bax protein expression in ACR-treated group as against control and drug alone groups. The L-cysteine co-treated group showed sustained PCNA and Bax protein expression.
			ACR	40 mg/kg bw	279.1±8.5*; 3.22+0.18	220.3±19.5*; 2.82±0.26	9.64		
			ACR + Drug	40 +150 mg/kg bw	280.0±9.6*; 2.87±0.15	231.1±36.8*; 2.87±0.15	14.73		
			Drug	150 mg/kg bw	271.4±21.8*; 3.25±0.14	279.7±24.2*; 3.25±0.14g	21.61		
6	Q10 (Mazen and Elnegris, 2012)	Albino rats (n = 10)	Control	Distilled water/ Corn oil	NS	NS	3.92±0.77 ng/ml	Distorted and disorganized seminal tubules, wide interstitium with numerous interstitial cells and congested capillaries observed in ACR-induced group as against control and drug group	Significant reduction in the number of immunoreactive spermatogonia and spermatocytes; Marked increase in the iNOS immunoreactivities in the spermatids and spermatocytes
			ACR	15 mg/kg bw	NS	NS	0.78± 0.22 ng/ml		
			ACR+Drug	10 + 60 mg/kg bw	NS	NS	3.01± 0.3 ng/ml		
			Drug	NM	NS	NS			
7	Satica seed extract (Abd-Elsalam *et al.* 2021)	Wistar Albino rats (n = 7)	Control	2% Tween in Distilled water	NS	NS	4.2 ng/ml	ACR-induced group displayed the following against other groups; Decreased weight of testes and epididymis Decreased motility and viability of spermatozoa Increased percentage of abnormal spermatozoa Reduced sperm count	ACR-induced group displayed the following against other groups; Reduction in testicular SOD, GSH conc. Increased lipid peroxidation Increased MDA levels

Table 3 continued..

			ACR	10 mg/kg bw	NS	NS	1 ng/ml		
			ACR+Drug	10+200 mg/kg bw	NS	NS	3.5 ng/ml		
			Drug	200 mg/kg bw	NS	NS	4 ng/ml		

NM: Not mentioned; NS: Not specified; *denotes testis weight; # denotes sperm count

In a nutshell, dynamic biological variations observed with acrylamide exposure and concurrent administration of antioxidants in male reproductive system are consolidated in Table 2. Another study of thymoquinone co-administration and a comprehensive log of antioxidant studies concerning its dose, animal model, and protective biological effect are demonstrated in Table 3.

4. Conclusion

Foodstuff rich in sugars and amino acids, when heated at a temperature of 120 °C or above, undergo a Maillard-type reaction leading to the formation of a food-borne toxicant, acrylamide. Complete elimination of this acrylamide *via* diet is inevitable. Hence, it is imperative to understand associated toxic effects and probable mitigation strategies for acrylamide intoxication. In this review, it has been shown that oxidative stress impairs biochemical pathways, cellular redox potential, gene regulations, and chromosomal aberrations, which are underlying pathways elicited due to acrylamide exposure. Interestingly, natural products and biological metabolites exhibiting antioxidant properties can dramatically not only decrease but also reverse clinical manifestations induced by acrylamide as studied and observed in animal models. Additionally, research insights in understanding the effects of acrylamide on human system and effectiveness of the antioxidant need further scientific research and understanding of topic.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST STATEMENT

None.

REFERENCES

1. Sun, Y., Liu, B., Snetselaar, L. G., Robinson, J. G., Wallace, R. B., Peterson, L. L. and Bao, W. 2019, bmj, 364.
2. Tucker, G. and Featherstone, S. 2021, Essentials of thermal processing. John Wiley & Sons.
3. Mucci, L. A. and Wilson, K. M. 2008, J. Agric. Food Chem., 56, 6013.
4. Rifai, L. and Saleh, F. A. 2020, Int. J. Toxicol, 39, 93.
5. Dearfield, K. L., Abernathy, C. O., Ottley, M. S., Brantner, J. H. and Hayes, P. F. 1988, Mutation research/reviews in genetic toxicology, 195, 45
6. Pruser, K. N. and Flynn, N. E. 2011, Front Biosci (Schol Ed.), 3, 41.
7. Besaratinia, A. and Pfeifer, G. P. 2007, Carcinogenesis, 28, 519.
8. Field, E. A., Price, C. J., Sleet, R. B., Marr, M. C., Schwetz, B. A. and Morrissey, R. E. 1990, Toxicol. Sci., 14, 502.
9. Gadiraju, T. V., Patel, Y., Gaziano, J. M. and Djoussé, L. 2015, Nutrients, 7, 8424.
10. Ghamdi, A., Alenezi, F., Algoferi, M., Alhawas, M., Farga, A. A. and Afifi, M. 2020, Biomed. J. Sci. Technol. Res., 27, 20638.
11. Guo, J., Yu, D., Lv, N., Bai, R., Xu, C., Chen, G. and Cao, W. 2017, Environ. Pollut., 225, 506
12. Kalaivani, M., Saleena, U. V., Katapadi, K. G. K., Kumar, Y. P. and Nayak, D. 2018, J. Clin. Diagnostic Res, 12, 11.
13. Koyama, N., Yasui, M., Kimura, A., Takami, S., Suzuki, T., Masumura, K., Nohmi, T., Masuda, S., Kinae, N., Matsuda, T. and Imai, T. 2011, Mutagenesis, 26, 545.
14. Ma, Y., Shi, J., Zheng, M., Liu, J., Tian, S., He, X., Zhang, D., Li, G. and Zhu, J. 2011, Toxicol. Ind. Health, 27, 617.
15. Pennisi, M., Malaguarnera, G., Puglisi, V., Vinciguerra, L., Vacante, M. and Malaguarnera, M. 2013, Int. J. Environ. Res., 10, 3843.
16. Timmermann, C. A. G., Mølck, S. S., Kadawathagedara, M., Bjerregaard, A. A., Törnqvist, M., Brantsaeter, A. L. and Pedersen, M. 2021, Toxics, 9, 155.
17. Yang, H. J., Lee, S. H., Jin, Y., Choi, J. H., Han, C. H. and Lee, M. H. 2005, J. Vet. Sci., 6, 103.
18. Friedman, M. 2015, Food Funct., 6, 1752.
19. Chapin, R. E., Fail, P. A., George, J. D., Grizzle, T. B., Heindel, J. J., Harry, G. J., Collins, B. J. and Teague, J. 1995, Fundam Appl. Toxicol., 27, 9.
20. Fennell, T. R. and Friedman, M. A. 2005, In Chemistry and Safety of Acrylamide in food, Springer, Boston, MA.
21. Seale, S. M., Feng, Q., Agarwal, A. K. and El-Alfy, A. T. 2012, Pharmacol Biochem Behav., 101, 77.

22. Khalil, W. K. B., Ahmed, H., Hanan, F., Aly, H. and Eshak, M. 2014, J. Pharm. Sci. Rev. Res., 24, 143.
23. Katen, A. L., Chambers, C. G., Nixon, B. and Roman, S. D. 2016, Biol. Reprod., 95, 86.
24. Wexler, P. 2014, Encyclopedia of Toxicology (3rd ed.). Academic Press.
25. Wang, H., Huang, P., Lie, T., Li, J., Hutz, R. J., Li, K. and Shi, F. 2010, Reprod. Toxicol., 29, 225.
26. Lee, N. P. and Cheng, C. Y. 2004, Biol. Reprod., 70, 267.
27. Duale, N., Bjellaas, T., Alexander, J., Becher, G., Haugen, M., Paulsen, J. E., Frandsen, H., Olesen, P. T. and Brunborg, G. 2009, Toxicol. Sci., 108, 90.
28. Shi, F. and Wang, T. 2005, J. Androl., 26, 258.
29. Zini, A., Abitbol, J., Girardi, S. K., Schulsinger, D., Goldstein, M. and Schlegel, P. N. 1998, Arch. Androl., 41, 57.
30. Trevisan, M., Browne, R., Ram, M., Muti, P., Freudenheim, J., Carosella, A. M. and Armstrong, D. 2001, Am. J. Epidemiol., 154, 348.
31. Sak, K. 2012, Chemother. Res. Pract., 2012, 282570.
32. Gül, M., Kayhan Kuştepe, E., Erdemli, M. E., Altınöz, E., Gözükara Bağ, H. G., Gül, S. and Göktürk, N. 2021. Andrologia, 53(9), p.e14176.
33. Sefidgar, S. M., Ahmadi-Hamedani, M., Javan, A. J., Sani, R. N. and Vayghan, A. J. 2019, Avicenna J. Phytomedicine, 9, 347.
34. Rajeh N. A., Al-Shehri A. M. 2019, Indian J. Exp. Biol., 57,138.
35. Kucukler, S., Caglayan, C., Darendelioğlu, E. and Kandemir, F. M. 2020, Life Sci., 261, 118301.
36. Wang, Z. Y., Hong, J. Y., Huang, M. T., Reuhl, K. R., Conney, A. H. and Yang, C. S. 1992, Cancer Res., 52, 1943.
37. El-Sweedy, M., Abdel-Hamid, N. and El-Moselhy, M. 2007, J. Appl. Biomed., 5.
38. Yassa, H. A., George, S. M., Refaiy, A. E. R. M. and Moneim, E. M. A. 2014, Environ. Toxicol., 29, 1155.
39. Abd-Elsalam, R.M., El Badawy, S. A., Ogaly, H. A., Ibrahim, F. M., Farag, O. M. and Ahmed, K. A. 2021, Environ. Sci. Pollut Res., 28, 53249.
40. Farag, O. M., Abd-Elsalam, R. M., El Badawy, S. A., Ogaly, H. A., Alsherbiny, M. A. and Ahmed, K. A. 2021, BMC complement. Med. Ther., 21, 1.
41. Abd Al Haleem, E. N., Hasan, W. Y. S. and Arafa, H. M. M. 2021, Drug Chem. Toxicol, 45(5), 2328.
42. Kaçar, S., Şahintürk, V., Betül, C. A. N. and Musmul, A. 2018, Balkan Med J., 35, 311.
43. Shahrzad, E., Shariati, M., Naimi, S. and Edalatmanesh, M. A. 2020, Adv. Hum. Biol., 10, 16.
44. Mazen, N. F. and Elnegris, H. M. 2013, Egypt. J. Histol., 36, 164.
45. El Kotb, S. M., El-ghazouly, D. E. S. and Ameen, O. 2020, Alexandria J. Med., 56, 134.
46. Rajeh, N. A. and Khayyat, D. 2017, J. Taibah Univ. Medical Sci., 12, 445.
47. Rahangadale, S., Jangir, B. L., Patil, M., Verma, T., Bhandarkar, A., Sonkusale, P. and Kurkure, N. 2012, Toxicol. Int., 19, 158.
48. Aly, H. A., Lightfoot, D. A. and El-Shemy, H. A. 2009, Chem. Biol. Interact., 182, 112.
49. Suzuki, Y. J., Tsuchiya, M. and Packer, L. 1991, Free Radic. Res. Commun., 15, 255.
50. Guimarães, S. B., Santos, J. M. V., Aragão, A. A., de Sandes Kimura, O., Barbosa, P. H. U. and de Vasconcelos, P. R. L. 2007, Nutrition, 23, 76.
51. El-Beshbishy, H. A., Aly, H. A. and El-Shafey, M. 2013, Toxicol. Ind. Health., 29, 875.
52. Lebda, M., Gad, S. and Gaafar, H. 2014, Mater. Socio Med., 26, 208.
53. Dos Reis, S. A., da Conceição, L. L., Siqueira, N. P., Rosa, D. D., da Silva, L. L. and Maria do Carmo, G. P. 2017, Nutr Res., 37, 1.
54. Mohammadi, R., Sohrabvandi, S. and Mohammad Mortazavian, A., 2012, Eng. Life Sci., 12, 399.
55. Setbo, E., Campbell, K., O'Cuiv, P. and Hubbard, R. 2019, J. Nutr. Health Aging, 23, 364.
56. Poutahidis, T., Springer, A., Levkovich, T., Qi, P., Varian, B. J., Lakritz, J. R., Ibrahim, Y. M., Chatzigiagkos, A., Alm, E. J. and Erdman, S.E. 2014, PLoS One, 9, e84877.
57. Zhao, S., Zhao, X., Liu, Q., Jiang, Y., Li, Y., Feng, W., Xu, H. and Shao, M. 2020, Appl. Biol. Chem., 63,1.