Attenuating effect of Chlorella Vulgaris against acrylamide intoxication on thyroid gland

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Abstract—Oral administration of Acrylamide in our food produces adverse health effects through different vital systems. This study examined the effect of simultaneous oral supplementation of unicellular algae Chlorella vulgaris (CV) on thyroid gland disruption induced by intoxication with Acrylamide (ACR). Healthy male rats (n=60) distributed in six groups: -ve control, +ve control (CV 300 mg/kg), low dose Acrylamide (LACR, 5 mg/kg), high dose Acrylamide (HACR, 50 mg/kg), pre-administration of Chlorella Vulgaris with Acrylamide CV+ LACR (5mg/kg), and with Acrylamide CV+HACR (50 mg/kg). All groups received treatments orally for 30 days. ACR treated groups (LACR, HACR) demonstrated a significant elevation in oxidative stress biomarkers Malondialdehyde (MDA), 4-Hydroxynonenal (4-HNE), Nitric Oxide (NO) and pro-inflammatory cytokines C-reactive protein (CRP), Tumor Necrosis Factor alpha (TNF-α), Interleukin 6 (IL-6) and Interleukin 1 beta (IL-1β). In relation to antioxidant biomarkers Glutathione (GSH) and Glutathione peroxidase (GPx), GSH recorded significant reduction while GPx indicated significant elevation as compared to control and +ve control group at (p<0.05). Thyroid gland biomarkers showed reduction in Thyroid-Stimulating hormone (TSH) in ACR groups whereas Thyroxine (T4) and Triiodothyronine (T3) presented a significant elevation. On the other hands pre-administration of CV with LACR and HACR indicated potent effects over alteration changes induced by ACR. It was clearly obvious
through remarkable improvement in the levels of MDA, 4-HNE and NO as well as each of pro-inflammatory cytokines CRP, TNF-α, IL-6 and IL-1β. Meanwhile, GSH and GPx were recorded improvement nearly to be more or less reached to level of control. However, CV supplemented groups have increased TSH level and decreased T3 and T4 levels, it should be noted that ameliorating effect of CV was much more presented in CV+ LACR groups. Pre-administration of Chlorella Vulgaris orally have a potent counteracting effect against Acrylamide-induced disturbance on thyroid gland along with all above mentioned parameters.

**Keywords**—Acrylamide, Chlorella Vulgaris, unicellular Algae, oral toxicity, thyroid, oxidative stress.

**Simple Summary:** In this study, 60 male albino rats were allocated to the control group (GI) and were given orally 1 mL of distilled water; G II were treated orally with 300 mg/kg of CV; G III were treated orally with 5 mg/Kg of ACR; G IV were treated orally with 50mg/Kg of ACR; G V were pre-supplemented with 300 mg/kg of CV followed by 5 mg/kg ACR; G VI were pre-supplemented with 300 mg/kg of CV followed by 50 mg/kg ACR. ACR. A significant elevation in serum level of MDA, 4-HNE, NO, CRP, TNF-α, IL-6 and IL-1β was recorded individually in the groups treated with low and high doses of ACR. Also an elevation was indicated in GPx activities where the GSH content decreased. Thyroid hormones, T3 and T4 were significantly increased, TSH levels recorded a significant reduction. On the other hand, pre-supplemented groups with CV presented obvious improvements over all parameter to be more or less nearly reached to control and +ve control levels especially in groups treated with low dose of ACR. In conclusion, exposure to acrylamide disrupt antioxidants, pro-inflammatory mediators, and thyroid hormones. Furthermore, the data from this investigation support the idea that pre-supplementation of Chlorella Vulgaris in our daily life works as general defense system enhancer against exposure to the deleterious effects of ACR and other toxicants.

**Introduction**

Acrylamide (ACR) is an odorless, white crystalline, water-soluble solid with chemical formula C3H5NO [1]. It is mainly produced as a result of Millard reaction between free asparagine and reducing sugars during heating cooking processes at temperature greater than (120°C) through the following: frying, roasting, baking or toasting [2]. Due to its rabid biotransformation to highly reactive metabolites, the concerns of its potential toxicity have increased about life-long exposure even to low dietary intake [3]. Once acrylamide is administrated orally, it is absorbed from gastrointestinal tract and then distributed to all body tissues followed by immediate metabolism [4]. One of it is metabolite called Glycidamide, which is contributed to cause adverse effects on nervous system, pre- and post-natal development, reproduction, gene expressions and cancer initiation based on animal studies [5]. The National Toxicology Program discussed the acrylamide susceptibility as carcinogen for human being [6]. Food Additives Expert Committee of Joint Food and Agriculture Organization & World Health
Organization (JECFA) concluded that ACR considers as human health concern and recommended more long-term studies [7]. There is adequate evidence that ACR is a rodent carcinogen [8], International Agency for Research on Cancer (IARC) labelled it as a (Group 2A) probable carcinogen for human [9]. Various ACR adverse effects have been demonstrated via repeated dose toxicity studies on animals. ACR toxic effects in rodents are apparent in form of mutagenicity, neurotoxicity, male reproductive system disturbance, prenatal mortality and endocrine disruptor [4]. Oral administration of 50 mg/kg body weight of ACR for 5, 21 and 56 days, involved in the induction of oxidative stress in brain tissues leading to neurotoxicity [10-12], disruption of pituitary gonadal hormones of testicular tissues and reproductive impairment in male rats for 56 and 28 days, respectively [13, 14]. Also a 28 days exposure to the mentioned dose resulted in disturbance in serum level of Progesterone, Luteinizing hormone (LH), Follicle stimulating hormone (FSH) and [15]. Recent studies have confirmed the hepatic and renal dysfunction exhibited by elevated level of serum alanine transferase, aspartate transferase, alkaline phosphatase and increasing renal function markers including: urea, creatinine and uric acid [16] accompanied with raising and reducing renal & hepatic lipid peroxidation, antioxidant enzyme activities, respectively [17].

Thyroid is a small gland, shaped like a butterfly, which located in the middle of the lower neck. It plays principle role in our body as it controls body metabolic rate (Which is body’s vital duties at cellular level). The control is reached through secretion of thyroid hormones that regulates body’s energy utilization. A well-functioning thyroid will produce the appropriate amount of hormones to maintain the body’s metabolism at a satisfactory manner. The thyroid secretes replacement hormones as the originals are depleted. The pituitary gland which located at the base of the brain regulates and monitors the amount of thyroid hormones in the bloodstream via adjusting the release of Thyroid Stimulating Hormone (TSH) whenever it senses either depletion or elevation of Triiodothyronine (T3) and Thyroxine (T4) [18]. Generating severe disorders is associated with thyroid hormone disruption will lead to immune system decline and induces a numerous of diseases [19]. A previous study confirmed the negative effect of Acrylamide for long-term on thyroid reported changes in gene expression of thyroid gland and its hormones, labelled a significant increase of Triiodothyronine (T3) and Thyroxine (T4) levels [20].

A long time ago microalgae has been known with many beneficial effects in food products, cosmetics and as animal feed. Chlorella. Species shown a rich protein content (50–65%) and minimal lipid (5–20%), so it is widely consumed in human diet [21]. *Chlorella Vulgaris* (CV) is a group of unicellular green algae, autotrophic organisms that are most likely a photosynthetic. According to its high content of protein, carbohydrate, pigments such as carotenoids and multivitamins and minerals, it is exhibited as therapeutic agent (antioxidant effect) against toxins and toxicants [22]. Many of beneficial effects of CV revealed through animal models. In study on male rats using of 5 mg/100 g body weight of CV, presented a protectant effect on hepatic toxicity (Hernayanti and Simanjuntak, 2019). CV accelerated curative effect has been demonstrated via topical application of CV on wound in this study [23], a dose of 150 and 300 mg/kg of CV showed improvement and strengthen of muscle functions of young and old rats [24].
Antidepressant effect is conducted with dose 360 mg/kg on female rat suffered from unpredictable and chronic stress, but it was a short-term treatment [25]. CV extract has a significant suppressor effect on pancreatic oxidative stress caused imbalance blood sugars and act as hypoglycaemic agent on male mice [26]. Also it promoted testicular functions in study on male rats faced a lead-acetate toxicity through modulatory anti-apopotic and antioxidant effect of CV [14]. The objective of this study was to investigate the potentiality of Chlorella Vulgaris extract in reducing the deleterious effects of different doses of acrylamide on thyroid gland.

Material and Methods

Acrylamide

Acrylamide of ≥98.0% purity and high water solubility in a form of white solid crystal (CAS No.79-06-1) was obtained from Sigma–Aldrich Chemie Gmb (Schnelldorf, Germany).

![Figure 1. Chemical Structure of Acrylamide (prop-2-enamide) (Symyx Draw 4.0).](image)

Chlorella Vulgaris

*Chlorella Vulgaris* extract was supplied as powder from Jarrow Formulas (Los Angeles, California, US).

Animals

60 adult male Wistar albino rats weighing between 200 -220 g were used. Animals were obtained from the animal house of King Fahad of Medical Center (KFMC), Jeddah, KSA. The animals were housed in polycarbonate cage with steel cover, had free access to standard diet and tap water and acclimatized under laboratory condition for one week prior to the experiments. The rats were exposed to 12-hour dark/light cycles in a temperature-controlled room at 23±2°C and humidity 50±10% animals were treated according to [27] OECD guidelines 28 days repeated dose oral toxicity study in rodents.

Experimental Design

Animals were randomly divided into 6 groups (10 animals each). Groups were treated orally for 30 days as follow:
• Group (I): Control group: Rats were given distilled water gavaging orally with water. Served as –ve control.

• Group (II) (CV): Rats were gavaged orally with (300 mg/kg) of *Chlorella Vulgaris* for 28 days according to [24]. Served as (+ve) control.

• Group (III) (LACR): Rats were gavaged orally with low dose (5 mg/kg) of Acrylamide for 28 days according to [28].

• Group (IV) (HACR): Rats were gavaged orally with high dose (50 mg/kg) of Acrylamide for 28 days according to [12].

• Group (V) (CV+ LACR): Rats were gavaged simultaneously with (300 mg/kg) of CV and low dose (5 mg/kg) of Acrylamide for 28 days.

• Group (VI) (CV+ HACR): Rats were gavaged simultaneously with (300 mg/kg) of CV and high dose (50 mg/kg) of Acrylamide for 28 days.

**Sampling**

At the end of the experimental period, rats were anaesthetized by diethyl ether and blood samples were collected from retro-orbital venous plexus by heparinized capillary glass as characterized by [29]. Blood samples were centrifuged at 3500 rpm for 10 min in a refrigerated centrifuge to separate serum which were kept in deep-freezer at -20°C till the biochemical assays were done.

**Biochemical Assay**

All assays in this study were measured in serum. All kits were obtained from MyBiosource (San Diego, California, US). Oxidative stress biomarkers were estimated as: Malondialdehyde (MDA) based on method of [30], 4-Hydroxynonenal (4-HNE) level was carried according to method of [31] and Nitric Oxide (NO) was assayed as prescribed colorimetric method by [32]. Detection of Glutathione (GSH) was carried out according to [33], and Glutathione peroxidase (GPx) was estimated according to [34]. Thyroid-Stimulating hormone (TSH), Thyroxine (T4) and Triiodothyronine (T3) were done by the described method of [35]. Pro-inflammatory cytokines were estimated as: C-reactive protein (CRP) were assayed by method of [36], Tumor Necrosis Factor alpha (TNF-α) were assayed as prescribed method based on [37], Interleukin 6 (IL-6) was done using method of [38] and Interleukin 1 beta (IL-1β) was carried out by method prescribed in [36].

**Statistical Analysis**

The collected data obtained from the biochemical analysis are represented in tables as Mean±Standard error (mean±SE). Significance difference between treated groups was calculated by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test to determine differences between the mean values of experimental groups at P < 0.05 using the SPSS-PC computer software package version 25.
Results

Changes in Oxidative Stress Biomarkers

Data expressed in Table 1 revealed that groups treated with low and high dose Acrylamide (LACR and HACR) induced significant elevation in levels of serum stress markers: MDA, 4-HNE and NO as compared to control and +ve control at (P<0.05). this elevation was dose dependent as expressed with % changes from control where MDA (365.63%, 540.63%), 4-HNE (327.91%, 774.42%) and NO (165.03%, 306.99%) respectively. On the other hand, Chlorella Vulgaris supplemented groups (CV+LACR and CV+HACR) exhibited remarkable improvement in levels of all stress markers versus non-supplemented groups at (P<0.05).

Changes in Antioxidant Biomarkers

Regarding GSH level and GPx activity, data in Table 1 declared that LACR and HACR induced significant reduction in GSH level concomitant with significant elevation in GPx activity. The percentage of GSH reduction was pronounced with (-60.83%, -86.09%) whilst the percentage of GPx elevation was (216.67%, 485.29%) versus control and +ve control groups at p<0.05. On the contrary, pre-supplementation with CV presented remarkable significant improvement in both markers as compared to non-supplemented groups at (P<0.05). The potentiality of CV to counteract the level of GSH & GPX was represented as % change from control (Table 1).

Changes in Thyroid Gland Biomarkers

Individual treatment with Acrylamide in LACR and HACR groups displayed significant reduction in serum TSH level as compared to control and CV groups at (P<0.05) with percentage (-85.97, -94.33%) from control. On the other hand, both doses of ACR induced remarkable significant elevation in serum T3 (62.92%, 101.16%) and T4 levels (92.21%, 197.14%) versus control and +ve control at (P<0.05). However, CV supplementation increased the level of TSH and reduced the levels of T3 and T4 in which remarkable improvement was obvious as compared to non-supplemented groups at (P<0.05). It should be noted that the above attenuating effect of CV was much more observable in CV+LACR groups in TSH (-20.00%), T3 (5.22%) and T4 (-7.53%) as expressed in (Table 2).

Changes in Pro Inflammatory Biomarkers

Table 3 illustrated the alteration on serum levels of different cytokines markers C reactive protein (CRP), tumour necrosis factor alpha(TNF-α), interleukin-6 (IL-6) and interleukin 1 β (IL-1β). Animals treated with LACR and HACR recorded dose dependant elevation in all cytokines markers. The increase in all pro-inflammatory cytokines was significant versus control and +ve control at (P<0.05). Whereas the chlorella vulgaris supplementation counteracted the elevation of cytokines levels and exhibited improvement as compared to LACR and HACR groups at (P<0.05). ameliorative effect of CV was more or less near the control and + ve control in CV+ LACR more than CV+ HACR (Table 3).
### Table 1

Effects of *Chlorella Vulgaris* on (MDA, 4-HNE, NO, GSH and GPx) on Serum of Albino Rats intoxicated with Different Doses of Acrylamide

<table>
<thead>
<tr>
<th>Groups and Parameters</th>
<th>MDA (mIU/L)</th>
<th>4-HNE (pg/ml)</th>
<th>NO (µ/L)</th>
<th>GSH (ng/ml)</th>
<th>GPx (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.32±0.02</td>
<td>0.86±0.09</td>
<td>28.60±1.21</td>
<td>13.66±0.74</td>
<td>20.40±1.19</td>
</tr>
<tr>
<td>CV</td>
<td>0.43±0.07</td>
<td>0.89±0.05</td>
<td>21.40±2.23</td>
<td>15.6±1.12</td>
<td>18.54±1.08</td>
</tr>
<tr>
<td>LACR</td>
<td>1.49±0.07ab</td>
<td>3.68±0.23ab</td>
<td>75.80±2.25</td>
<td>5.38±0.71ab</td>
<td>64.60±4.48ab</td>
</tr>
<tr>
<td>HACR</td>
<td>2.05±0.06abc</td>
<td>7.52±0.59abc</td>
<td>11640±1.91</td>
<td>1.90±0.21abc</td>
<td>119.40±2.11abc</td>
</tr>
<tr>
<td>CV+ LACR</td>
<td>1.31±0.21abcd</td>
<td>1.02±0.43abcd</td>
<td>27.80±2.22</td>
<td>16.86±0.39abcd</td>
<td>19.40±1.63ed</td>
</tr>
<tr>
<td>CV+ HACR</td>
<td>0.47±0.08cde</td>
<td>0.88±0.11cde</td>
<td>38.20±3.77</td>
<td>11.68±0.69bcde</td>
<td>27.00±2.51bcde</td>
</tr>
</tbody>
</table>

All groups were expressed as mean ± SE 10 rats; % changes from control; a Significant difference versus control at p < 0.05; b Significant difference versus CV Group at p < 0.05; c Significant difference versus LACR Group at p < 0.05; d Significant difference versus HACR Group at p < 0.05; e Significant difference versus CV + LACR Group at p < 0.05; f Significant difference versus CV + HACR Group at p < 0.05.

### Table 2

Effects of *Chlorella Vulgaris* on (TSH, T3 and T4) on Serum of Albino Rats intoxicated with Different Doses of Acrylamide

<table>
<thead>
<tr>
<th>Groups and Parameters</th>
<th>TSH (mIU/L)</th>
<th>T3 (ng/dL)</th>
<th>T4 (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.35±0.22</td>
<td>172.40±3.83</td>
<td>3.85±0.29</td>
</tr>
<tr>
<td>CV</td>
<td>3.34±0.23</td>
<td>189.80±1.63</td>
<td>3.44±0.38</td>
</tr>
<tr>
<td>LACR</td>
<td>0.47±0.09ab</td>
<td>279.80±7.14ab</td>
<td>7.40±0.33ab</td>
</tr>
<tr>
<td>HACR</td>
<td>0.19±0.04ab</td>
<td>346.80±18.87abc</td>
<td>11.44±0.66abc</td>
</tr>
<tr>
<td>CV+ LACR</td>
<td>2.68±0.11abcd</td>
<td>181.40±4.53cd</td>
<td>3.56±0.21cd</td>
</tr>
<tr>
<td>CV+ HACR</td>
<td>1.01±0.17abcde</td>
<td>224.00±15.43abcde</td>
<td>7.78±0.76abcde</td>
</tr>
</tbody>
</table>

All groups were expressed as mean ± SE 10 rats; % changes from control; a Significant difference versus control at p < 0.05; b Significant difference versus CV.
Group at p < 0.05; c Significant difference versus LACR Group at p < 0.05; d significant difference versus HACR Group at p < 0.05; e Significant difference versus CV + LACR Group at p < 0.05; f Significant difference versus CV + HACR Group at p < 0.05

Table 3
Effects of Chlorella Vulgaris on (CRP, TNF-α, IL-6 and IL-1β) on Serum of Albino Rats intoxicated with Different Doses of Acrylamide

<table>
<thead>
<tr>
<th>Groups and Parameters</th>
<th>CRP (mg/L)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>IL-1β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.50±0.46</td>
<td>13.40±0.56</td>
<td>4.57±0.23</td>
<td>13.62±0.33</td>
</tr>
<tr>
<td>CV</td>
<td>5.94±0.29</td>
<td>14.66±0.82</td>
<td>5.74±0.39</td>
<td>14.76±0.54</td>
</tr>
<tr>
<td>(8.00%)</td>
<td>(9.40%)</td>
<td>(25.60%)</td>
<td>(8.37%)</td>
<td></td>
</tr>
<tr>
<td>LACR</td>
<td>15.60±0.43a</td>
<td>23.40±1.03ab</td>
<td>9.28±0.19ab</td>
<td>26.02±1.51ab</td>
</tr>
<tr>
<td>(183.64%)</td>
<td>(74.63%)</td>
<td>(103.06%)</td>
<td>(91.04%)</td>
<td></td>
</tr>
<tr>
<td>HACR</td>
<td>30.99±2.43abc</td>
<td>38.74±1.76abc</td>
<td>14.68±0.34abc</td>
<td>37.58±1.69abc</td>
</tr>
<tr>
<td>(463.45%)</td>
<td>(189.10%)</td>
<td>(226.22%)</td>
<td>(175.92%)</td>
<td></td>
</tr>
<tr>
<td>CV+ LACR</td>
<td>5.82±0.60cd</td>
<td>14.40±1.16cd</td>
<td>5.74±0.52cd</td>
<td>14.78±1.32cd</td>
</tr>
<tr>
<td>(5.82%)</td>
<td>(7.46%)</td>
<td>(25.60%)</td>
<td>(8.52%)</td>
<td></td>
</tr>
<tr>
<td>CV+ HACR</td>
<td>7.86±1.04cd</td>
<td>17.74±0.93acde</td>
<td>9.26±0.54abde</td>
<td>19.39±0.68acde</td>
</tr>
<tr>
<td>(42.91%)</td>
<td>(32.39%)</td>
<td>(102.63%)</td>
<td>(42.36%)</td>
<td></td>
</tr>
</tbody>
</table>

All groups were expressed as mean ± SE 10 rats; % changes from control; a Significant difference versus control at p < 0.05; b Significant difference versus CV Group at p < 0.05; c Significant difference versus LACR Group at p < 0.05; d Significant difference versus HACR Group at p < 0.05; e Significant difference versus CV + LACR Group at p < 0.05; f Significant difference versus CV + HACR Group at p < 0.05

Discussion

In the current study we explored the beneficial effects of Chlorella Vulgaris on thyroid gland as presupplementation against Acrylamide exposure to low and high doses for 28 days. Acrylamide induced excessive increase of reactive oxygen species through disruption of cellular redox state either through formation of ACR a conjugates with reduced glutathione (GSH), p N-acetyl-S- (3-amino-3-oxopropyl) cysteine, or via production of the metabolite glycidamide when ACR reacts with cytochrome P450 [39]. Additionally, Glycidamide forms conjugates with GSH [40]. In state of higher intake or exposure to ACR, more GSH is utilized in ACR conjugation leading to depletion of GSH levels that is associated with numerous of diseases and health disorder [41]. The present results are consistent with [17, 42] who all indicated that adult male rats treated with 20 mg/kg b.wt. of ACR increased levels of oxidative stress markers (MDA, NO), Pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β), the intense effects on oxidative stress markers also obviously reflected due to decreased level of GSH [43].
On the other hand, GPX recorded dose dependent significant elevation in ACR treated groups. These results are in consistent with [44] an increase in GPx activities during ACR exposure. moreover, brain and liver GPx recorded a remarkable elevation after ACR administration with concentration of 0.03% for 40 days. GSH served as cofactor for GPx that helps in hydrogen peroxide removal, the increase in GPx activities may be due to the generation of free radicals during ACR toxicity [45]. In relation to changes in 4-hydroxynonenal (HNE) levels, 4-HNE and Acrylamide have drawn a lot of attention due to their extraordinarily high toxicity as well as their possibility to be produced at concentrations that could be harmful to human health [46]. According to [47] study, on astrocytes/microglial co-culture model showed that ACR treatment led to dose-dependent toxicity. Oxidative stress was induced as indicated by an increase of ROS, a decrease of GSH levels, and an increase in the formation of 4-hydroxynonenal-adduct. The accumulation of free radicals and NO are contributed to ACR which react together to produce a chemical known as peroxynitrite that can damage cell membranes. And elicit pro-inflammatory markers.

Moreover, when ROS generation exceeds the capacity of the antioxidant defense system of the cell, many pathological conditions may occurred due to oxidative stress [48]. The present results recorded a significant depletion in TSH hormone levels in both ACR treated groups whereas T3 and T4 are elevated, these results are in accordance with [49] study on male rats that treated orally with 50 mg/kg b.wt. of ACR. The reduction in TSH in may be attributed to ACR-induced massive stress on endocrine system involving, thyroid, adrenal cortex, adrenal medulla, ovaries and pituitary. ACR administration triggered a significant drop in serum corticosterone recommending that the adrenal cortex faces acute effects of ACR and/or stimulates disturbances in the hypothalamic–pituitary adrenal relationships. As regards to pro-inflammatory cytokines, they have increased along both doses of ACR groups. These results are in line with [50] study, pregnant Wistar rat orally administrates ACR at doses of 2, 5, 10 mg/kg during gestational period. The levels of C-reactive protein (CRP) and MDA were increased along with reduction of GSH content. CRP is produced by liver in response to early state of inflammation, infection and tissue damage [51]. The increased level of CRP in the ACR-treated mother rats and their litters is an inflammation indicator. One of the site of ACR accumulation is kidney [52].

Detoxification of ACR and its metabolite glycidamide via conjugation with GSH, further both metabolized to mercapturic acids and excreted in urine [53]. As regard to result of accumulation of ACR and its metabolite glycidamide in the kidney, which subsequently resulted in inflammation as shown by the elevation of CRP in the blood. Recent report suggests that the level of CRP and TNF-α are significantly elevated after ACR treatment with dose of 35 mg/kg for 2 weeks. The increase could be attributed to cytokines and adipokines dysregulation due to ACR-induced oxidative stress [54]. Ameliorative effects of presupplementation with chlorella vulgaris (CV) on most studied parameters recorded in the present study. CV has High content of number of nutrients including pigments, minerals and vitamins, it has capacity to act as therapeutic agent and counteract the oxidative damage [21]. In the present study pre-administration of CV with low and high dose of ACR, significantly decreased levels of oxidative stress markers. The
results of present work at 300 mg/kg of CV are agree with the findings presented by [24]; they reported that there were observed decrease in MDA and 4-HNE levels in both the young and old rats compared to the untreated controls for 3 months.

This decrease could be due to antioxidant properties of *C. vulgaris*, it reduced fat mass that CV can enhance muscular strength by regulating the lean bone mineral contents (BMC) and body fat mass. *Spirulina* and *Chlorella* are the two of the most well-known microalgae genus. Both microalgae genus have a significant content of proteins, vitamins, pigments, fatty acids, sterols, among others which make their production/application. In the study of [55], pre-administration of *Spirulina Platensis* (SP) ameliorated the cisplatin-induced oxidative stress and decreased the levels of MDA, NO as well as decreased the gene expression of TNF-α, IL-6 and IL-1β genes along with elevated GSH contents, these findings are in consistent with our results. On the other hands, antioxidant marker GPx enzyme exhibited near control level, whereas levels of MDA were inhibited when a male rats treated with CV for 30 days. The elevation may be attributed to existence of Glutathione content as a component of CV which act to reduce ROS generation via GPx utilization of Glutathione as electron donor [56].

In this study, CV treatment with 300 mg/kg for 30 days revealed a rebalance through TSH elevation besides decreased level of T3 and T4 in ACR-induced toxicity groups. Our results are clearly in line with previous study of [57-59] who all indicated similar results after treatment with different doses of Spirulina. A considerable imbalance between the activity of peroxidation processes and the antioxidant defense system was obvious in thyroid tissue [60]. This leads to promote a proper conditions for damaging processes as result of peroxidation on thyroid as well as how ROS affect pro- and anti-apoptotic targets and other mechanisms that are either directly or indirectly controlled by the intracellular redox dependent [61] has its own ability of free radicals scavenging as well as potent antioxidant properties [62].

**Conclusion**

*Chlorella Vulgaris* supplementation can counteract level of oxidative stress and induction of inflammation by enhancing defense system. Which attenuate the adverse effects of ACR-induced oxidation on thyroid gland. CV supplemented groups showed more effective improvement as antioxidant with low dose ACR treated groups.

**References**