Assessment of the ameliorative effect of Beta-Carotene against Gibberellic acid toxicity in kidneys of rats using comet assay

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ABSTRACT

This work was aimed to observe the protective role of β-carotene (β-CAR) against renal toxicity induced by gibberellic acid (GA3), a plant growth hormone commonly used in adult male rats. The studied rats were divided into six groups of eight each: group one served as a control, while Groups 2 and 3 received GA3 at concentrations of 50 ppm and 75 ppm in drinking water, respectively; Group 4 was orally administered β-carotene at the amount of 50 mg; Groups 5 and 6 were treated by both GA3 and β-CAR. All treatments were made daily for 90 days. Results revealed that exposure to GA3 significantly increased serum urea and creatinine with a concomitant reduction in serum albumin and total serum protein, indicating kidney dysfunction. Furthermore, GA3 significantly raised the values of ROS and LPO in the kidneys but lowered the activities of GPx, CAT, and SOD. These biochemical alterations were further confirmed by comet assay procedures representing photomicrographs of DNA damage and tail migration in kidney tissues. Histopathological examination showed marked pathological changes in the kidneys of all the GA3-treated groups. Importantly, co-administration with β-CAR reduced these toxic effects, restoring biochemical parameters to near normal compared to GA3-intoxicated rats. Histopathological findings further revealed reduced damage to kidneys in groups treated with both GA3 and β-CAR. This study therefore concludes that β-CAR has substantial kidney protective potential against GA3-induced kidney toxicity in rats.

Keywords: Gibberellic acid, Oxidative stress, Comet assay, Histopathology, kidneys and β-carotene.

1. INTRODUCTION:

Gibberellic acid (GA3) is a widely used plant growth regulator, particularly in Egypt, where it plays a crucial role in enhancing the growth and yield of fruits and vegetables. Based to studies by Çelik *et al*. (2002) and Kamel *et al*. (2009), GA3 is extensively applied in agricultural practices to stimulate cell elongation, improve fruit development, and increase crop productivity, making it an essential tool for boosting agricultural output. Even if it has a wide application in agriculture, little is known about harmful GA3 influences on many organisms' health despite its widespread use (Sakr *et al*., 2003). Relatively little is known concerning the cytogenetic and pathological effects of GA3 in animals. Morphological, immunohistochemical, and biochemical alterations in the kidneys of young mice treated with gibberellic acid were found to be longer compared to the period of treatment, in addition to oxidative stress, with partial signs of self-healing following withdrawal (Khalaf *et al*., 2019). Many studies on the kidney prove high oxygen consumption and a high lipid content make this organ highly susceptible to oxidative damage (Ozbek, 2012; Chae *et al*., 2023). It has been amply documented that GA3 has neurotoxic properties, proving it can induce kidney injury. In vitro and in vivo studies in mice showed that GA3 can increase the production of ROS while decreasing that of some

antioxidant enzymes like SOD and CAT, ultimately resulting in neuronal damage and apoptosis, with an impairment of cognitive function (El-Kenawy *et al*., 2017). In further support of these findings, studies on rats have revealed that GA3 can induce impairment in learning and memory, probably due to its neurotoxic actions (Ozbek, 2012)

It was indicated that rats exposed to GA3 suffered from oxidative stress situations where there is an imbalance between the body's capacity to detoxify active intermediates and the rate at which ROS are formed. According to Kalender *et al*. (2015), this causes DNA damage, protein, and lipid peroxidation.

One of the major biochemical pathways through which GA3 exerts its toxic effects is through oxidative stress. It is a disruption caused by an imbalance between the antioxidant defense of the body and the rate of ROS generation. High levels of ROS may cause damage to to protein, lipids, and DNA, leading to cellular impairment and death (Morsy *et al*., 2021). Being organs of high metabolic rate and rich blood vessels, the kidneys could easily suffer from damage *ia* oxidation. In this situation, the kidneys become one of the target organs for the toxicity of GA3 (Morsy *et al*., 2021

The GA3-induced effects on cytogenetic studies of the kidney cells are an added dimension to understanding. In principle, cytogenetic studies describe how chromosomes are isolated and scrutinized for possible changes resulting from exposure to toxicants. Chromosome changes could include chromosome aberrations, micronuclei formations, and other changes indicative of genetic damage (Zidan *et al.,* 2020). Such genetic damage is considered critical because it may lead to mutations, cancer, and other diseases.

In human lymphocyte culture, the cytogenetic effect of gibberellin A3 at concentrations of 0.1, 0.5, 1, and 2 mg was studied. Treatment of gibberellin resulted in sister chromatid exchange, chromosomal aberration, and DNA damage in the cultures. The comet assay also was used to confirm DNA damage (Sakr, *et al*., 2009).

Due to the high flow of blood through the kidney and its specialization for removing and concentrating injurious substances from the blood, it is the most sensitive organ to toxic injury. The first signs of renal function in the postnatal kidney are nephrogenic zones on the twelfth and peripheral cortical tubules on the twenty-eighth postnatal day. According to the researchers, postnatal morphogenesis is an undeniable feature of rodent kidneys (Munro *et al*., 2017).

Previous research demonstrated the effectiveness of beta-carotene in counteracting genetic damage caused by a wide variety of exogenous toxins (Zidan *et al*., 2020). Beta-carotene previously reduced the frequency of micronuclei formation and chromosome aberrations in different tissues, thus indicating that this compound protects at the genetic level (Kaufman *et al*., 2019). Beta-carotene is an active precursor of vitamin A, an efficient antioxidant that belongs to a wide range of fruits and vegetables. It is highly responsible for sweeping free radicals and thus saves cells from oxidative damage (Biesalski *et al*., 2018). Having shown beta-carotene to possess antioxidant action, many studies have been undertaken to establish its potential for reducing the toxicity of some environmental and chemical poisons. According to several literature sources, β-carotene was found to be useful against oxidative stress and improved cellular health in many tissues, such as the liver, lung, and kidney (Kaufman *et al*., 2019; Rajanandh *et al*., 2017).

Biochemical and cytogenetic evaluation for the ameliorative potential of beta-carotene against GA3-induced nephrotoxicity in rats is one of the most imperative aspects. The protective mechanisms of beta-carotene may give insights into strategies for developing therapeutic approaches against GA3 toxicity. The susceptibility of the kidney to GA3-induced oxidative stress and the potential protective role of beta-carotene underlie the

imperative need for an in-depth examination of changes in biochemical pathways and cytogenetic alterations upon exposure to these substances (Dennis, and Witting, 2017).

Further, it is known that beta-carotene's antioxidant activity might play a role in modulating the expression of genes involved in pathways related to oxidative stress and inflammation. Some previous studies have suggested that antioxidants may affect gene expression for the encoding of the primary antioxidant enzymes, such as SOD and CAT, which have a crucial role in defenses against oxidative stress (Rajanandh *et al*., 2017). In this regard, information on how beta-carotene could modulate these biochemical pathways may be relevant in gaining insights into the mechanisms underlying its nephroprotective actions against GA3-induced nephrotoxicity (Zidan *et al*., 2020).

The present research work was, therefore, designed to determine the protective role of β-carotene treatment against gibberellic acid-induced oxidative stress and related damage in the kidneys of male albino rats. The study was an attempt to explore the potential therapeutic protective value of β-CAR in mitigating GA3-induced toxicity to such vital organs.

2. MATERIALS AND METHODS:

The materials used included 99% pure gibberellic acid (GA; C19 H22O6), specifically GA3, sourced from Nanjing Essence Fine-Chemical Co., Ltd., Jiangsu, China. The β-CAR was supplied by Sigma-Aldrich Chemical Company, St. Louis, MO. All chemicals used in the evaluations were of analytical grade.

2.1. Animals and study design:

2.1.1. Animals:

A total of 48 healthy male albino rats, weighing 180.93 ± 1.83 g, were acquired from the National Organization for Drug Control and Research (NODCAR) animal house in Egypt. The rats were divided into six groups, with each group of eight rats housed in plastic cages. They were given one week to acclimate to the laboratory environment before the experiment began.

A controlled environment consisting of 22±3ºC and a 12:12 h light-dark cycle was maintained for the rats. According to Dauchy *et al*., 2010.

Regarding the macronutrient composition and the diet structure, it is essential to highlight that the feed formulation aligns with the guidelines established by the National Research Council (NRC), as referenced in Table 1 of the NRC report: *Nutrient Requirements of Laboratory Animals.* This ensures that the diet meets the specific nutritional needs for maintenance, growth, and reproduction in laboratory animals.

In terms of micronutrients, the diet ensures sufficient vitamin intake through a combination of pellets, carrot/spinach powder, and premix. It includes 4,000 IU of vitamin A for vision and immune health, 1,000 IU of vitamin D for calcium absorption and bone health, 50 mg of vitamin E, and 1 mg of vitamin K. Additionally, B-complex vitamins, such as thiamin, riboflavin, and niacin, are supplied in adequate amounts from grains, soybean meal, and premix. Choline is provided at 1,000 mg, contributing to liver function and brain development.

For minerals, the diet includes 5 grams of calcium, primarily from calcium carbonate, pellets, and carrot/spinach powder, and 3 grams of phosphorus from grains, soybean meal, and fish meal, which are essential for skeletal health. Magnesium (500 mg), iron (35 mg), zinc (12 mg), and copper (3 mg) are included to support various metabolic functions and overall health. Finally, sodium and chloride are present at 500 mg, which helps maintain fluid balance and nerve function. This balanced nutrient breakdown ensures the health and well-being of rats across their life stages.

Every animal was handled under the guidelines set forth by the Scientific Research Ethics Committee (SREC) at the Genetic Engineering and Biotechnology

Research Institute. Proposal: SREC110324B20053.

2.1.2. Design Experiments:

Rats were randomly assigned to six groups, each containing eight rats (n = 8). The following labels applied to the groups: Group 1: Free drinking water and a basic diet (Control group). Group 2: The rats were given a basic diet and 50 ppm of gibberellic acid/ kg of body weight/ day (Soliman et al., 2022) in oral drinking water. Group 3: The rats were given drinking water containing 75 ppm gibberellic acid /kg /body weight /day (Celik, Tuluce, and Isik, 2007) and the basic diet was given to the rats. Group 4: Rats were given a basal diet and free access to water with 50 mg of beta-carotene per kilogram of body weight daily (Ma *et al*., 2021). Group 5: Rats were given a basal diet and free access to 50 ppm gibberellic acid and 50 mg of beta-carotene per kilogram of body weight daily in their drinking water. Group 6: The rats were fed a basic diet consisting of 50 mg of beta-carotene per kg of body weight each day, along with free access to drinking water with 75 ppm of gibberellic acid.

For ninety days, all treatments were administered orally. At the end of the study, each rat was sacrificed. Blood samples were collected, and serum was isolated to assess blood parameters and kidney function. The kidney tissues were immediately collected.

Tissue sections were stored at -80 ºC for comet test analysis. For the biochemical examination, the tissue sections were centrifuged, homogenized in ice-cold Tris–HCL buffer (150 mM, pH 7.4), and stored at -80 ºC. For a light microscope histological investigation, the remaining portions were placed in tightly sealed containers with 10% formalin saline solution for at least 10 hours.

2.2. Methods:

2.2.1. Body and kidney weights:

Body weight was evaluated at the beginning of the experiment, after the adaptation period, after thirty days, and at the end of the experiment, for all groups. After slaughtering the rats, the kidneys were separated and weighed directly using an automated balance.

2.2.2. Blood urea:

Blood urea levels were measured using kits manufactured by Diamond Diagnostics, Egypt, Using the methodology outlined by Fawcett and Scott (1960).

2.2.3. Blood creatinine:

According to Houot (1985), the colorimetric method was used to determine the serum blood creatinine using kits made by Diamond Diagnostics, Egypt .

2.2.4. Serum total Proteins:

Determination of serum total Proteins: A test reagent kit based on the Gornall *et al*., 1949. The method was used to quantify the total protein content in serum and kidney homogenate

2.2.5.Serum albumin:

Serum albumin levels were measured in serum using a test reagent kit that followed the protocol developed by Doumas *et al*., 1971.

2.2.6. Lipid peroxidation:

Lipid peroxidation (LPO) was measured by looking for the presence of thiobarbituric acid reactive substances (TBARS) in kidney homogenate. The absorbance of the resultant colorimetrically, typically at 532 nm according to Uchiyama and Mihara's methodology (1978). When kidney H_2O_2 is broken down, catalase absorption reduces with time. This drop can be used to calculate the activity of the enzyme, known as catalase (CAT) activity. The Aebi method (1984) employs a spectrophotometric approach where the decrease in H_2O_2 absorbance is monitored at 240 nm over time, as H_2O_2 is broken down by catalase in the sample. Measuring the activity SOD in kidney homogenate was measured. The SOD activity assay follows the kinetics of auto-oxidation of pyrogallol by monitoring the change in absorbance at 420 nm and determining the inhibition rate by SOD in the

sample according to Marklund and Marklund's methodology (1974). Evaluation of GPx activity is determined by coupling the reduction of hydroperoxide by GPx to the oxidation of NADPH, which is catalyzed by glutathione reductase in the presence of reduced glutathione (GSH). The decrease in NADPH absorbance at 340 nm provides a measure of GPx activity (Brigelius-Flohe, 1999).

Vrablic *et al.* Method (2001) for Measuring Reactive Oxygen Species (ROS) generation was applied using the intracellular conversion of Nitro Blue Tetrazolium (NBT) to Formazan by Superoxide Anion. This method quantifies ROS by measuring the reduction of NBT to formazan, which occurs in the presence of superoxide anions. The formation of formazan is followed by monitoring the increase in absorbance, typically at 560 nm, and is indicative of the level of superoxide production.

2.2.7. Comets Assay:

Utilizing the comet assay (single cell gel electrophoresis method), DNA damage analysis and quantification were carried out to determine the comet assay for identifying DNA damage in renal tissue (Nandhakumar *et al.*, 2011). As described by Singh et al. (1988), the rats were sacrificed after 90 days of treatment, and then immediately slices of kidney tissues were taken out. Then the tissue was washed with phosphate buffered saline and kept at -80 degrees to analyze the levels of DNA damage. Damaged DNA migration was analyzed based on observation and measurement of nuclear DNA. A normal DNA spot was considered as a rounded DNA spot, while damaged DNA was identified as comet-shaped when it migrated towards the anode and its length became longer than the diameter of the basal nuclear DNA .

2.2.8. Histopathological examination:

The isolated kidney sections were first fixed in 10% formalin and transferred to ethanol. Following cryosectioning, the kidney sections were immediately viewed under a light microscope after being stained with hematoxylin and eosin (H&E). The statistical analysis was performed following Bancroft and Gamble *et al.*, 2002.

2.3. Analytical statistics:

Statistical analysis of the data was performed using SPSS version 20. An independent t-test was employed to compare the two groups. Values were expressed as mean ± standard error (SE) and were considered statistically significant if the p-value was less than 0.05 (Bailey, 1995).

3. RESULTS:

This work was undertaken to assess the protective role of β-carotene on the nephrotoxicity induced by GA_3 in rats. The results give insight into how β-CAR might reverse the toxic effect of GA_3 on kidney weights and body weight, thus having the potential to reduce GA3-induced nephrotoxicity.

3.1. Body and kidney weights:

Table 2 shows that the group treated at 50 ppm and 75 ppm GA3 increased in kidney weights while causing a decrease in body weight against the control. On the contrary, the groups treated by β-carotene alone or combined with GA3 showed values closer to control in kidneys and body weight while the group treated by β-CAR only did not exhibit significant differences relative to control. Comparing these β-CAR-treated groups with the GA3-only treated group, the data showed that when combined with GA3, β-CAR treatment lowered kidney weights and raised body weight, which suggested protection by β-CAR against the toxic effect of GA3. This effect was more pronounced in the 75 ppm GA3 group, where β-CAR significantly attenuated GA3-induced toxicity.

3.2. Ameliorative effects of β-carotene against gibberellic acid on urea and creatinine:

As shown in Table 3, the data indicates that the groups treated with gibberellic acid

at doses of 50 ppm and 75 ppm experienced a significant increase in urea and creatinine levels over time. This rise suggests severe kidney dysfunction compared to the control group.

The level of urea and creatinine were within normal range throughout the experimental period in the control group, and there was no significant difference noted in the β-carotene only group compared to that in the control group, which indicates that β-CAR doesn't hold any detrimental effects on such renal parameters. Comparing the β-CAR-pretreated groups against the GA3-only groups, the data showed that β-CAR pretreatment significantly reduced GA3-induced increase in the values of urea and creatinine. In greater detail, the increase in these levels was less prominent in the 50 ppm GA3 + β-CAR group compared with the 50 ppm GA3-only group. Likewise, using 75 ppm GA3 + β-CAR resulted in urea and creatinine values lower than what was noted with 75 ppm GA3 only, although such protective effects were lesser at this higher dose of GA3. These results indicate that β-CAR effectively mitigates GA3-induced kidney toxicity in rats.

3.3. Ameliorative effects of β-carotene against gibberellic acid on Albumin and total Protein:

The data obtained in Table 4, reveals a significant reduction over time in the values of albumin and total protein in GA3 groups at both 50 ppm and 75 ppm compared to the control group. In this period, the control group did not vary in the levels of albumin and total protein, and non-significant variation was observed with the β-carotene alone group in comparison with the control, which rules out any negative effect of β-CAR on these kidney parameters. In contrast to the GA3-only treated groups, data revealed that the β-CAR-treated groups indicated a significant attenuation of the reduction in albumin and total protein levels induced by GA3 upon β-CAR administration. For example, in the 50 ppm GA3 + β-CAR group, albumin and total protein levels were higher compared to the 50 ppm GA3-only group. Compared to the 75 ppm GA3-only group, the degrees of decrease in these levels were less in the 75 ppm GA3 + β-CAR group; however, this protective effect was not very prominent at this higher concentration of GA3. Therefore, these findings indicate that β-CAR significantly reduces the GA3-induced decline in albumin and total protein levels, pointing out its potential protective role against GA3-induced hepatic toxicity in rats.

3.4. Ameliorative effects of β-carotene against gibberellic acid on oxidative stress markers:

The data obtained upon study completion in Table 5 shows that groups treated with GA3 at both 50 ppm and 75 ppm significantly elevated the levels of ROS and LPO in kidneys tissue and lowered the activities of antioxidant enzymes like GPx, CAT, and SOD as compared to our control group. This, in turn, reflects increased oxidative stress and a worse defense system against antioxidants in the GA3-treated mice. In comparison to the GA3-only treated groups, it showed that β-CAR administration significantly reduced ROS and LPO levels and partially restored GPx, CAT, and SOD activities. More importantly, compared to the 50 ppm GA3 group, in the 50 ppm GA3 + β-CAR group, less of an increase in oxidative stress markers and better preservation of antioxidant enzyme activity were reflected. The combination of 75 ppm GA3 + β-CAR showed less oxidative stress and more expressed levels of antioxidant enzymes when compared to 75 ppm GA3 only. However, the protective effect was not as prominent at this higher concentration of GA3. It has been shown that β-CAR plays a protective role against GA3-induced oxidative stress by preserving antioxidant defense in kidney tissue.

3.5. Ameliorative effects of β-carotene against gibberellic acid on DNA using comet assay:

In our research, we used the comet assay to assess DNA damage in renal tissues by measuring several key markers: tail length, DNA percentage in the tail, tail moment, and Olive moment. These markers reflect the extent of DNA fragmentation and were used to study the protective effects of β-carotene against GA3-induced nephrotoxicity. The results, presented in Table 6, showed that β-carotene significantly reduced DNA damage across all parameters compared to the groups treated with GA3 alone.

To ensure accurate interpretation, we classified DNA damage based on the following criteria: Tail length (an indicator of the distance DNA fragments migrate under electrophoresis) was measured in microns, with longer tails indicating higher levels of DNA strand breaks. DNA in the tail percentage indicates the proportion of fragmented DNA, while tail moment and Olive moment are calculated to provide a more integrated view of the damage. Specifically, the tail moment is calculated by multiplying the length of the tail by the proportion of DNA located in the tail while Olive moment takes into account the distribution of the DNA fragments. As for controls, we employed a standard negative control (untreated rats), which exhibited minimal DNA damage, as shown by tail lengths and tail moments similar to the β-carotene group. In our study, we used gibberellic acid (GA3) at both low (50 ppm) and high (75 ppm) doses as positive controls to induce nephrotoxicity and assess the extent of DNA damage in renal tissues. These doses were selected based on previous studies demonstrating their ability to induce oxidative stress and DNA fragmentation in kidney tissues (Singh et al., 2017). As shown in Table 6, the high dose of GA3 (75 ppm) significantly increased all comet assay parameters, like tail length, DNA in tail, tail moment, and Olive moment. For example, the tail length in the GA3 (75 ppm) group increased to 9.90 ± 0.64 µm compared to the control group's 6.20 ± 0.39 µm, reflecting significant DNA damage. The Olive moment in the GA3 (75 ppm) group was also elevated at 1.64 ± 0.18, confirming increased DNA fragmentation. The low dose of GA3 (50 ppm) similarly induced DNA damage, though to a lesser extent, as indicated by tail length (7.48 ± 0.36 µm) and Olive moment (1.08 ± 0.27). These findings confirm that both GA3 doses acted as effective positive controls, demonstrating a dose-dependent increase in DNA damage in renal tissues. Importantly, the co-administration of β-carotene with GA3 (at both doses) significantly mitigated these effects, as reflected in the reduced tail length, DNA in tail percentage, and Olive moment in the β-CAR+GA3 groups compared to the GA3 groups alone.

3.6.Histopathological examination:

Figure (2-A) displays a photomicrograph of a kidney segment taken from a control group demonstrating the normal histological structure of the glomeruli and tubules at the cortex and no histopathological abnormalities were detected. (H&E. 400X). Figure (2-B): is a photomicrograph of a kidney slice from rats given 50 ppm gibberellic acid reveals Sclerotic blood arteries in the kidneys showing signs of congestion. (H&E. 400X). In Figure (2-C), the kidneys section photomicrograph, obtained from rats exposed to 75 ppm gibberellic acid, revealed degeneration and enlargement of the tubular epithelium bordering the cortex. (H&E. 400X). Figure (2-D): reveals that histopathological changes were not noted in the kidney section photomicrograph (H&E, 400X) of rats treated with 50 mg β-carotene and 50 ppm gibberellic acid. The kidneys section photomicrograph (2-E) reveals vacuolization in the endothelial cells lining the tufts of the glomeruli and deterioration in the lining tubular epithelium at the cortex in animals treated with 75 ppm gibberellic acid and 50 mg β-carotene. Finally, Figure (2-F) shows a photomicrograph of the kidney segment

from animals given 50 mg of β-carotene; no histological changes were noted.

4. DISCUSSION:

Gibberellic acid is a naturally occurring plant growth regulator used in large quantities in agriculture to promote crop growth and increase yields. However, massive doses have raised concerns about their possible toxic effects on non-target organisms, particularly mammals (Zhu *et al*., 2018). Kidneys are significantly sensitive to such toxicants as GA3, being an important organ responsible for the filtration and detoxification of waste products from the blood (El-Gawish *et al*., 2016).

Our study attempted to elucidate the mechanism of these possible toxic effects; where GA3 increased kidney weight, reduces body weight, and alters various biochemical markers such as albumin, total protein, urea, creatinine, ROS, LPO, and antioxidant enzymes like GPx, CAT, and SOD in rats

GA3 has already been found to induce inflammation and oxidative stress, which involves increases in kidney weight in treated rats (Al-Malki & Moselhy, 2013). Inflammation is a critical response to cellular injury and is mediated by the release of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) (El-Borm, 2021). These cytokines further facilitate the infiltration of immune cells into the kidney, leading to tissue edema, which contributes to increased kidney weight (El-Borm, 2021). Moreover, the induction of GA3 causes pronounced oxidative stress that plays an important role in GA3-induced nephrotoxicity (Kamel & Abdelkhalek, 2016). Increased ROS production within the kidney tissues activates nuclear factor-kappa B (NF-κB), a transcription factor controlling the expression of genes related to inflammation and apoptosis (El-Shafei et al., 2020). Activation of NF-κB aggravates the inflammatory response, consequently resulting in kidney hypertrophy and increased organ weight (El-Shafei *et al*., 2020). Additionally, high ROS levels lead to lipid peroxidation, manifesting in cell membrane disruption, contributing to the total increase in kidney weight (Owumi *et al*., 2021).

The body weight decrease recorded for GA3-treated rats may be due to reduced food intake, increased energy expenditure, or even muscle wasting (Nassar *et al*., 2017). GA3 decreases appetite and reduces food intake by acting on the central nervous system, likely affecting hunger-regulating hormones (Nassar *et al*., 2017). The increase in GA3-induced oxidative stress and inflammation may also increase energy expenditure as the body attempts to counteract the damage caused by these processes (Kamel & Abdelkhalek, 2016). This negative energy balance, associated with increased energy expenditure and decreased food intake, results in weight loss (El-Shafei *et al*., 2020).

GA3-induced inflammation and oxidative stress can lead to muscle cachexia, characterized by the breakdown of muscle proteins and a loss of muscle mass (Kamel & Abdelkhalek, 2016). The increase in ROS and TNF-α, a pro-inflammatory cytokine, activates the proteolytic ubiquitin-proteasome system, degrading muscle proteins and contributing to body weight decrease (El-Borm, 2021).

Urea and creatinine are key markers of renal function; high levels indicate renal malfunction. The increase in urea and creatinine in rats treated with GA3 thus demonstrates that GA3 induces nephrotoxicity, causing elevated levels of waste products in the blood (Zhu *et al*., 2018). The mechanisms underlying this increase are closely related to inflammation and oxidative stress induced by GA3 (Owumi *et al*., 2021).

Kidney tissue increased ROS generation leads to renal cell oxidation, decreasing glomerular filtration and reducing urea and creatinine clearance in the kidneys (Zhu *et al*., 2018). Furthermore, GA3-induced inflammation, demonstrated by an elevated expression of pro-inflammatory cytokines, may cause damage to both glomeruli and tubules, further deteriorating kidney function

and resulting in the accumulation of both urea and creatinine in blood circulation (Rapa *et al*., 2019).

ROS consists of highly active molecules that induce severe damage to cellular elements, including lipids, proteins, and DNA. The increased ROS levels in the kidneys of GA3-treated rats reflect the status of oxidative stress, which is subsequently linked with LPO and the formation of a marker of LPO, MDA (Hussein *et al*., 2015). LPO produces the formation of lipid radicals and disrupts the integrity of cell membranes, making it highly dangerous (Rapa *et al*., 2019).

The increase in ROS and LPO in GA3-treated rats might result from the interruption of the mitochondrial electron transfer chain, which may overproduce superoxide radicals. These superoxide radicals are further converted to hydrogen peroxide and hydroxyl radicals, finally contributing to the observed oxidative damage in kidney tissues (Zhu *et al*., 2018).

GPx, CAT, and SOD are key antioxidant enzymes that play an important role in protecting the kidney against oxidative stress. The reduced activity of these enzymes in GA3-treated rats reflects an impaired antioxidant defense system, allowing the accumulation of ROS and leading to cellular damage (Hussein *et al*., 2015).

Glutathione peroxidase (GPx) reduces hydrogen peroxide to water, preventing the generation of hydroxyl radicals, which are highly reactive and harmful to cells. The decrease in GPx activity in GA3-treated rats suggests impairment in the kidneys' ability to detoxify hydrogen peroxide, thereby increasing oxidative stress (Hussein *et al*., 2015). Similarly, catalase catalyzes the degradation of H_2O_2 into H_2O and oxygen; decreased CAT activity in GA3-treated rats promotes the accumulation of hydrogen peroxide and related oxidative damage (Zhu *et al*., 2018). SOD catalyzes the dismutation of superoxide radicals, converting them into oxygen and hydrogen peroxide. The lowering activities of SOD in GA3-treated rats point out that the efficiency of removal of superoxide radicals is reduced in the kidneys of such animals. The collectively lowered activities of SOD, GPx, and CAT might be responsible for the general increase in oxidative stress and cell injury noted in rats pre-treated with GA3.

From our study, it can be concluded that GA3 exhibits cytotoxic activity. Animals exposed to acute treatment with GA3 have more pronounced symptoms than the control and B-CAR groups, in that order. The aforementioned genotoxic activity is accompanied by cytotoxic activity as well.

Nassar *et al*. (2012) stated that a higher genotoxic effect is associated with reduced mitotic activity of bone marrow cells. Aside from hepatic and nephrotoxicity, which manifests as liver fibrosis, fatty transformation, and necrosis, kidney interstitial fibrosis manifests as tubulointerstitial damage and segmental and diffuse glomerular sclerosis.

Troudi *et al*. (2011) noted that nephrotoxicity, manifested as a significant reduction in antioxidant enzyme activity in the kidneys of suckling puppies and their dams during the late pregnancy and early postpartum periods, was another adverse effect.

The comet assay, a sensitive and reliable method for detecting DNA strand breaks, offers the advantages of being rapid, straightforward, and applicable to all eukaryotic cell types both in vivo and in vitro (Kumaravel and Jha, 2006; Speit and Hartmann, 2006). The percentage of DNA migrated from the nucleus into the tail is a particularly informative parameter, directly correlating with the frequency of DNA breaks. Damaged sites of DNA were seen as strong spots in both treated groups, as detected by DNA detection via comet assay. Results on DNA damage through the comet assay were recorded in our study and showed high damage to DNA, as detected by the significant increments in tail length and tail DNA percentage in treated groups with GA3. These outcomes agree with those reported by Abou-Eisha, (2001) who indicated that the GA3 caused a marked increase in a dose-

dependent manner in the DNA breakage level in human blood cells. Analogously, Hassab-Elnabi and Sallam, (2002) and Sakr *et al*, (2009) obtained an increase -in human lymphocyte culture-damaged cells with DNA spots that were damaged in high concentrations of GA3. The observed DNA damage may be related to the direct alkylation of DNA bases by gibberellic acid, leading to alkali-labile and single-strand breaks and ultimately, total genomic damage. Alternatively, as suggested by Fath *et al*. (1999), the accumulation of nucleases might contribute to DNA damage. Furthermore, gibberellic acid could induce DNA damage by elevating oxidative stress indicators (Abou-Eisha 2001). Despite these DNA strand breaks; the cells may be able to repair them without incurring persistent lethal effects or fixing them into mutations that result in permanent viable changes. Our study revealed that the kidney tissue from animals treated with gibberellic acid exhibited tubular epithelium surrounded by cortical degeneration and edema, indicating that GA3 treatment in rats led to necrosis.

There may be a correlation between the histological changes in the kidneys of GA3-treated rats and the biochemical changes observed in the treated rats. In agreement with Khalaf *et al*., (2019) who observed large tubular dilatation in male albino rats given the same dose of GA3 in water, along with wide lumen, thin epithelium, desquamated cells, and hyaline casts in the lumen of some tubules. According to Abdel-Aziz and Mohamed (2013), there may be a correlation between the histological variations in the kidneys of rats treated with GA3 and the biochemical alterations observed in the siblings of those mothers. Immature configurations of renal glomeruli were more commonly observed in GA3-treated newborn and 7-day-old rats, indicating delayed development of the glomeruli. This study examined the influence of maternal therapy of GA3 on the architecture of the developing renal cortex in the offspring. Additionally, in rats given GA3, homogeneous dense masses of cells showed up as additional proof of impaired kidney development. In the current study, renal tubule lining cells in the treated rats exhibited vacuolations and pyknotic nuclei in the age groups under investigation.

These findings were consistent with the results of previous studies, Abdel-Rahman *et al*. (2017). Cytoplasmic vacuolization was one of the major early responses to various types of cellular injury. This can occur by increased cell membrane permeability, thereby allowing an increase in intracellular water leading to vacuolization (Filiopoulos and Vlassopoulos 2009) Beta-carotene is a known vitamin A precursor and one of the strongest antioxidants. It actively participates in scavenging free radicals, thereby decreasing oxidative stress to protect cells from damage. This protective mechanism makes beta-carotene a potential candidate for ameliorating GA3's toxic effects on various physiological and biochemical parameters in rats, especially in the kidneys, body weight, and levels of albumin, total protein, urea, creatinine, ROS, LPO, and key antioxidant enzymes like GPx, CAT, and SOD (Giordano *et al*., 2017). The GA3-induced increase in kidney weight is mainly due to inflammation and oxidative stress, resulting in cellular hypertrophy and edema. Beta-carotene reduces this effect by inhibiting both oxidative stress and inflammation. Giordano *et al*., (2017) reported that; its antioxidant activity protects against ROS, lowering the activation of pro-inflammatory cytokines like TNF-α and IL-6. By reducing the levels of these cytokines, beta-carotene inhibits the infiltration of immune cells into the kidney, reducing inflammation and edema, and preventing an increase in kidney weight.

Beta-carotene also modulates gene expression related to inflammatory and redox status. It attenuates the inflammatory response in the kidneys by inhibiting the increased activation of nuclear factor-kappa B (NF-κB), a transcription factor controlling genes encoding pro-inflammatory molecules. This decrease in inflammation, along with the antioxidant effects of beta-

carotene, has been shown to recover kidney weight in GA3-treated rats (Giordano et al., 2017).

The body weight reduction of GA3-treated rats is related to increased oxidative stress, inflammation, and muscle wasting. Beta-carotene, by inhibiting both oxidative stress and inflammation, restores normal body weight in these animals .

The antioxidants in beta-carotene reduce ROS generation, offering protection against the oxidative component of muscle damage. These antioxidative properties are important for preventing muscle wasting or destruction, which is a hallmark of continued body mass loss (Giordano *et al*., 2017).

The anti-inflammatory characteristics of beta-carotene also reduce the concentration of pro-inflammatory cytokines that play a role in muscle catabolism. By preventing the activation of the ubiquitin-proteasome system, which is responsible for degrading proteins in muscle cells, beta-carotene helps maintain protein levels in muscles and prevents the loss of body weight (Giordano *et al*., 2017). Additionally, beta-carotene could increase appetite and food consumption by blunting oxidative stress and inflammation, which may lead to a decrease in appetite hormones, thus contributing to maintaining the required body weight in rats' post-GA3 treatment (Hussein *et al*., 2015). Serum creatinine and urea are renal function markers, where increasing levels may indicate impaired renal function. The increased levels of these markers in GA3-treated rats suggest nephrotoxicity and impaired kidney function. The ameliorative effects of beta-carotene could be due to its ability to decrease oxidative stress and inflammation, thereby protecting renal function and preventing the accumulation of urea and creatinine in the blood (Hussein *et al*., 2015).

The antioxidant effect of beta-carotene diminishes ROS within the kidneys, preventing renal cells from oxidative damage and consequently maintaining the glomerular filtration rate. This downregulation of oxidative stress helps preserve renal functions and avoid instances of acute kidney injuries where urea and creatinine levels are raised. This protective effect is evident by the inhibition of the kidneys' inflammatory response; hence, beta-carotene preserves the glomeruli and renal tubules from injuries, leading to the normalization of urea and creatinine levels (Giordano *et al*., 2017).

Beta-carotene exerts its nephroprotective effects through several complex and interrelated mechanisms, primarily centered on its ability to modulate oxidative stress, inflammation, and apoptosis. One of the primary mechanisms involves beta-carotene's antioxidant properties, where it effectively scavenges free radicals and reduces oxidative stress by neutralizing ROS. In toxic environments, such as exposure to gibberellic acid (GA), The accumulation of ROS can lead to oxidative damage of lipids, proteins, and DNA, all of which contribute to nephrotoxicity (Arulselvan *et al*., 2016). Beta-carotene enhances the activity of endogenous antioxidant enzymes such as SOD, catalase, and GPx, which detoxify ROS and reduce oxidative damage (Kawata *et al*., 2018). Additionally, beta-carotene activates the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, a pivotal mediator of cellular antioxidant defense. Nrf2 activation leads to the transcription of detoxifying and antioxidant enzymes, providing a strong defense against oxidative injury in renal tissues (Ma, 2013).

Beyond its antioxidant capabilities, beta-carotene also modulates inflammatory pathways. One critical pathway involves the inhibition of nuclear factor kappa B (NF-κB), a transcription factor that is activated in response to oxidative stress and plays a important role in the regulation of inflammation. Upon activation, NF-κB promotes the expression of pro-inflammatory cytokines like TNF-α and interleukins, contributing to inflammation and tissue damage in the kidneys (Arulselvan *et al*., 2016). Beta-carotene suppresses NF-κB activation, thereby reducing the inflammatory response and limiting

cytokine-induced renal injury (Cho *et al*., 2018). Furthermore, beta-carotene has been shown to influence apoptotic pathways. It can inhibit the mitochondrial-dependent apoptosis pathway by reducing the release of cytochrome c, which in turn inhibits the activation of caspase-3, a critical enzyme in the execution phase of apoptosis (Bossy-Wetzel *et al*., 1998). This reduction in apoptotic cell death contributes to its cytoprotective effects in the kidneys. Collectively, these mechanisms-antioxidant defense, anti-inflammatory regulation, and inhibition of apoptosis-underscore the multifaceted role of beta-carotene in protecting renal tissues from GA-induced nephrotoxicity.

ROS are highly reactive and can cause significant damage to various cellular structures, including lipids, proteins, and DNA. The elevation of ROS levels in GA3-treated rats is a clear indication of oxidative stress, leading to an increase in lipid LPO and malondialdehyde, a marker of LPO (Fujii *et al*., 2022). Beta-carotene, being a potent antioxidant, effectively scavenges ROS and lowers LPO, thereby protecting cellular membranes and other structures from oxidative attacks (Zhu *et al*., 2018).

By decreasing ROS levels, beta-carotene prevents the initiation of LPO and, as a result, prevents cell membrane damage. This protection supports cellular structures, preventing oxidative damage responsible for various pathologies, including nephrotoxicity. Moreover, Hussein *et al*., (2015) revealed that the reduced levels of LPO restrict the release of pro-inflammatory mediators, thus reducing inflammation and further injury to the kidneys.

GPx, CAT, and SOD are key antioxidant enzymes that offer protection against oxidative stress. The reduced enzymes activities observed in GA3-treated rats reflects a compromised antioxidant defense system, where ROS accumulate and cause cellular damage. Beta-carotene helps restore the activity of these enzymes by reducing oxidative stress and preventing the depletion of antioxidant defenses (Kaufman *et al*., 2019). It increases the expression and activity of GPx, which is responsible for reducing hydrogen peroxide to water, thereby hindering the formation of hydroxyl radicals and reducing oxidative damage. Similarly, beta-carotene helps maintain normal levels of catalase, that breaks down enzymatically hydrogen peroxide into water and oxygen.", further reducing oxidative stress. Additionally, beta-carotene supports the activity of SOD, which mediates the disproportionation of superoxide radicals, yielding hydrogen peroxide and oxygen. This prevents the accumulation of superoxide radicals, thus protecting cells against oxidative damage (Fujii *et al*., 2022). So, it can be concluded that by restoring the activities of these antioxidant enzymes, beta-carotene helps maintain the delicate balance between ROS generation and antioxidant defenses, thereby preventing oxidative damage that underlies nephrotoxicity and other pathologies in GA3-treated rats.

5. CONCLUSION:

Following the findings, administering gibberellic acid (GA3) at various doses results in variable degrees of nephrotoxicity, increased oxidative stress in addition to damage to DNA. Crucially, our research indicates that if taken regularly, beta-carotene (β-CAR) exhibits potential effects as a kidney protective medication. By restoring normal kidney function and lowering ROS levels, β-carotene has the ability to attenuate GA3-induced nephrotoxicity and strengthen the body's defenses against oxidative stress.

6. AUTHOR CONTRIBUTIONS:

The conception and design of this study were collaboratively developed by all authors. A.M.A. was responsible for the collection of samples, while material preparation and data analysis were conducted by A.M.A., K.M.E., and R.M.A. The manuscript drafting and revisions were equally shared among A.M.A., K.M.E., and R.M.A. All authors provided valuable input and feedback during the manuscript's

evolution, and they collectively approved the final version of the manuscript.

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Table 1: Composition of Nutrient Feed Ingredients for Adult Male Rat Diet according to NRC requirements .

Components	Percentage
Alfalfa Meal	5.90%
Wheat Bran	5.87%
Soybean Meal	8.50%
Oats	10.94%
Corn	11.65%
Sunflower Meal	7.69%
Barley	9.14%
Rice Bran	10.29%
Beet Pulp	6.82%
Clover	8.96%
Cottonseed Meal	8.5%
Rice Straw	5.55%
Molasses	0.07%
Salt	0.01%
Vitamin/Mineral Premix	0.07%
Methionine	0.02%
Lysine	0.02%

Table (2): Effect of gibberellic acid and β-carotene on body and kidney weights in male albino rats.

Groups	Kidneys weight (g)	Body weight (g)
Control	1.58 ± 0.66^{d}	261 ± 1.65^{a}
GA_3 (50ppm)	1.72 ± 0.41^{b}	246 ± 2.17^{c}
GA_3 (75ppm)	1.91 ± 0.63^{a}	198 ± 1.58^{e}
β-CAR	1.55 ± 0.32^{d}	265 ± 1.45^{a}
β-CAR + GA_3 (50ppm)	1.55 ± 0.29^{e}	255 ± 1.27^{b}
β-CAR + GA_3 (75ppm)	1.68 ± 0.42^{c}	241 ± 1.55^{d}

Values are mean±SE. Values with the same letters are not significant at (P≤0.05). GA3= gibberellic acid, β-CAR= β-carotene. Values on the same column not sharing the same superscript letters were significantly different (P<0.05).

Table (3): Ameliorative effects of β-carotene against gibberellic acid on urea and creatinine in male albino rats.

Groups	Urea (mg /dl)			Creatinine (mg /dl)		
	Zero time	**Half time**	**End time**	**Zero time**	**Half time**	**End time**
Control	28.2 ± 0.34^{a}	30.0 ± 0.41^{e}	31.6 ± 0.80^{d}	0.63 ± 0.03^{a}	0.63 ± 0.05^{e}	0.64 ± 0.03^{e}
GA_3 (50ppm)	28.5 ± 0.18^{a}	35.00 ± 0.5^{c}	40.8 ± 0.51^{d}	0.64 ± 0.04^{a}	0.80 ± 0.41^{b}	1.0 ± 0.15^{b}
GA_3 (75ppm)	28.0 ± 0.20^{a}	58.50 ± 0.27^{a}	64.4 ± 0.86^{a}	0.62 ± 0.06^{a}	1.35 ± 0.09^{a}	1.55 ± 0.03^{a}
β-CAR	28.3 ± 0.40^{a}	30.08 ± 0.32^{e}	28.9 ± 0.15^{d}	0.64 ± 0.06^{a}	0.64 ± 0.23^{e}	0.61 ± 0.05^{e}
β-CAR + GA_3 (50ppm)	27.9 ± 0.14^{a}	32.06 ± 0.49^{d}	35.40 ± 0.19^{c}	0.65 ± 0.08^{a}	0.68 ± 0.023^{d}	0.71 ± 0.08^{d}
β-CAR + GA_3 (75ppm)	28.5 ± 0.27^{a}	44.05 ± 0.31^{b}	47.00 ± 0.44^{b}	0.66 ± 0.04^{a}	0.70 ± 0.023^{c}	0.88 ± 0.05^{c}

Values are Mean±SE. Values with the same letters are not significant at (P≤0.05). GA3= gibberellic acid, β-CAR= β-carotene. Values on the same column not sharing the same superscript letters were significantly different (P<0.05).

Table (4): Ameliorative effects of β-carotene against gibberellic acid-induced on albumin and total protein in male albino rats.

Groups	Albumin (g/L)			Total Protein (g/L)		
	Zero time	**Half time**	**End time**	**Zero time**	**Half time**	**End time**
Control	5.38 ± 0.16^{a}	5.51 ± 0.13^{a}	5.56 ± 0.14^{a}	8.42 ± 0.26^{a}	8.40 ± 0.61^{a}	8.42 ± 0.22^{a}
GA_3 (50ppm)	5.21 ± 0.18^{a}	5.16 ± 0.16^{b}	4.80 ± 0.19^{b}	8.38 ± 0.30^{a}	6.91 ± 0.32^{c}	6.00 ± 0.18^{c}
GA_3 (75ppm)	5.17 ± 0.14^{a}	4.30 ± 0.21^{e}	3.61 ± 0.25^{e}	8.47 ± 0.18^{a}	5.12 ± 0.19^{e}	4.00 ± 0.15^{e}
β-CAR	5.41 ± 0.10^{a}	5.55 ± 0.16^{a}	5.60 ± 0.12^{a}	8.50 ± 0.16^{a}	8.40 ± 0.38^{a}	8.54 ± 0.27^{a}
β-CAR + GA_3 (50ppm)	5.42 ± 0.15^{a}	5.00 ± 0.13^{c}	4.89 ± 0.17^{c}	8.48 ± 0.19^{a}	7.00 ± 0.16^{b}	6.54 ± 0.40^{b}
β-CAR + GA_3 (75ppm)	5.27 ± 0.18^{a}	4.82 ± 0.11^{d}	4.69 ± 0.10^{d}	8.44 ± 0.25^{a}	5.60 ± 0.14^{d}	5.04 ± 0.31^{d}

Values are Mean±SE. Values with the same letters are not significant at (P≤0.05). GA3= gibberellic acid, β-CAR= β-carotene. Values on the same column not sharing the same superscript letters were significantly different (P<0.05).

Table (5): Ameliorative effects of β-carotene against gibberellic acid -induced oxidative stress markers in male albino rat's kidney.

Groups	Oxidative stress in Kidney tissue				
	ROS	**LPO**	**GPx**	**CAT**	**SOD**
Control	10.61 ± 0.41e	0.28 ± 0.08e	18.04 ± 0.33a	19.23 ± 0.25a	102.17 ± 0.81a
$GA_{3\ (50ppm)}$	13.51 ± 0.18c	0.40 ± 0.26d	14.61± 0.28c	14.10±0.41d	86.00± 1.09c
$GA_{3\ (75ppm)}$	16.44 ± 0.29a	1.14 ± 0.16a	10.05 ± 0.40e	10.21±0.20e	65.18±0.83e
β-CAR	10.20 ± 0.32e	0.27 ± 0.06e	18.00 ± 0.15a	19.19 ± 0.30a	103.00 ± 0.99a
β-CAR+$GA_{3(50ppm)}$	11.60 ± 0.38d	0.55 ± 0.16c	16.55± 0.15b	16.32 ± 0.27b	91.44 ± 1.22b
β-CAR+$GA_{3(75ppm)}$	14.25 ± 0.25b	0.92 ± 0.11b	13.09 ± 0.10d	14.55 ± 0.18c	79.44 ± 0.68d

Values are mean±SE. Values with the same letters are not significant at (P≤0.05). GA3= gibberellic acid, β-CAR= β-carotene. Values on the same column not sharing the same superscript letters were significantly different (P<0.05).

Table (6): Ameliorative effects of β-carotene against gibberellic acid-induced DNA damage in male albino rat's kidneys.

Groups	DNA marker in kidney				
	%	**Tail Length**	**DNA in Tail**	**Tail moment**	**Olive moment**
Control	10.70 ± 0.52 a	6.20 ± 0.39 a	7.00 ± 0.82 a	0.56 ± 0.04 a	0.90 ± 0.07 a
$GA_{3\ (50ppm)}$	11.70 ± 0.41 d	7.48 ± 0.36 d	7.70 ± 0.49 d	0.71 ±0.03 d	211.30 ± 0.21 d
$GA_{3\ (75ppm)}$	13.80 ± 1.09 e	9.90 ± 0.64 e	10.05 ± 0.62 e	0.82 ± 0.08 e	1.64 ± 0.18 e
β-CAR	11.06 ± 0.28 a	6.23 ± 0.19 a	7.12 ± 0.32 a	0.53 ± 0.08 a	0.88 ± 0.10 a
β-CAR+$GA_{3\ (50ppm)}$	11.10 ± 0.88 b	7.20 ± 0.50 b	7.41 ± 0.63 b	0.63 ± 0.15 b	1.08 ± 0.27 b
β-CAR+$GA_{3\ (75ppm)}$	11.50 ± 0.69 c	8.42 ± 0.74 c	8.98 ± 1.14 c	0.75 ± 0.17 c	1.35 ± 0.42 c

Values are mean±SE. Values with the same letters are not significant at (P≤0.05). GA3= gibberellicacid, β-CAR= β-carotene. Values on the same column not sharing the same superscript letters were significantly different (P<0.05).

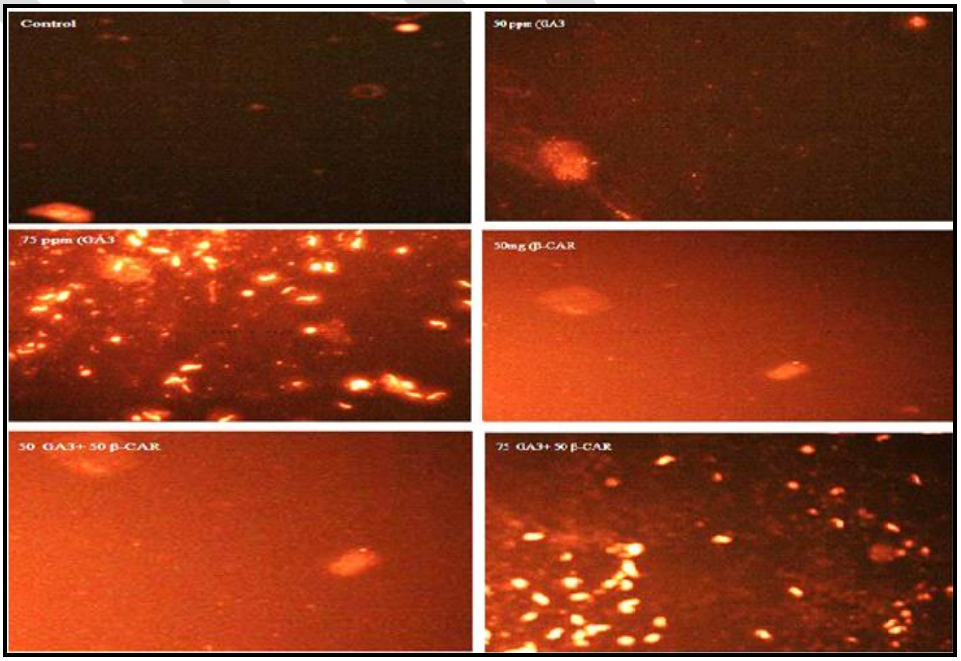

Fig.(1): Comet assay photos showing DNA damage in kidney tissues .**G1**: control. **G2**: 50 ppm (GA3). **G3**: 75 ppm (GA3). **G4**: 50 mg (β-CAR). **G5**: 50 GA3+ 50 β-CAR). **G6**: 75 GA3+ 50 β-CAR

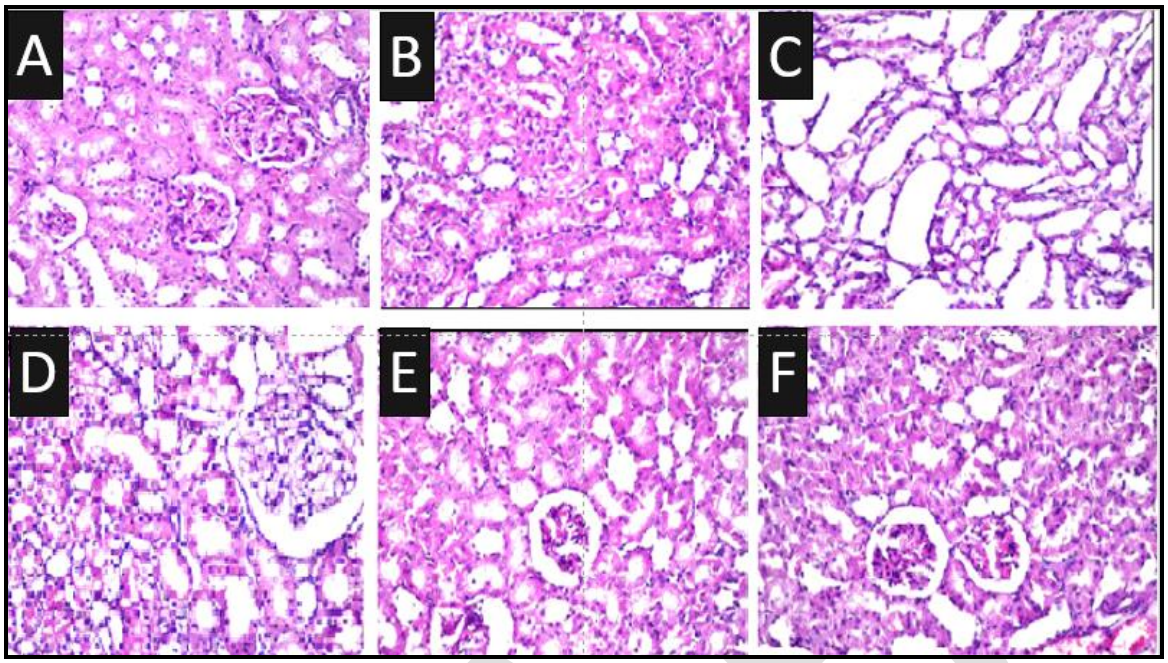

Fig. (2): (A) kidney segment from control group. (B): kidney slice from rats given 50 ppm gibberellic acid (C): kidney section from rats given 75 ppm gibberellic acid, (D): kidney section from rats given 50 mg β-carotene and 50 ppm gibberellic acid. (E) From rats given 75 ppm gibberellic acid and 50 mg β-carotene. (F) Kidneys segment from rats given 50 mg of β-carotene.