

The Effect of Exogenous Application of Chitosan in Beta Vulgaris (Beet) Drought Stressed Plants

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Abstract

Background: In recent years, chitosan has been used to reduce salt stress. Chitosan is a biodegradable, nontoxic molecule. It can boost stress tolerance, aid physiological processes, and prevent transpiration-related water loss. Beet cv. Rio Grande plants were treated with 0, 40, 80, or 100 mM salt. The plants' response to stress was mediated by chitosan at 0, 40, 80, or 100 mg L⁻¹.

Results: According to the findings, salt applications harmed almost all of the investigated criteria. Control beet were grown in salinity-free distilled water. Maximum plant height in centimeters, average number of compound leaves per plant, stem diameter in millimeters, number of fruits per plant, fruit firmness in kilograms per square centimeter, leaf chlorophyll content in micrograms per gram, fruit juice pH, yield per plant in kilograms, and minimum total soluble solids in degrees were recorded. Plants exposed to 100 mM salinity had the lowest plant height (in centimeters), lowest average number of compound leaves per plant, lowest stem diameter (in millimeters), lowest number of fruits per plant, lowest fruit firmness (in kilograms per square centimeter), lowest leaf chlorophyll content (in spades per acre), highest yield per plant (in kilograms), and highest TSS (in milligrams). Chitosan 150mgL⁻¹ Maximum plant height (cm), average number of compound leaves plant⁻¹, leaf area (cm²), stem diameter (mm), number of fruits plant⁻¹, fruit firmness (kg cm⁻²), leaf chlorophyll content (SPAD), total soluble solids (Brix[°]).conclusion plant⁻¹ yield (kg) with minimal juice pH. 100mg/L chitosan 100mg/L chitosan 80 mg L⁻¹ chitosan and 100 mM salt stress improve beet performance.

Keywords: Abiotic stress, Chitosan, Salinity, beet, Quality, Growth.

INTRODUCTION

Salt is a significant abiotic factor. One third of the world's irrigated land has been harmed, according to 1 Soil with high levels of salt inhibits plant growth. This alters the structure of the soil and the availability of nutrients.² As a result, plants have a hard time absorbing water and nutrients from the soil. The main soil issue in Pakistan is salt, which has a negative impact on plant growth and yield. Salinity reduces plant growth and yield by altering metabolism and increasing water use efficiency. There is a direct correlation between the amount of salinity in the soil and the rate at which plant growth is impeded by salinity.³ Drought stress is a major contributor to a decrease in plant growth, productivity, and yield. Drought resistance and water quality are improved when chitosan is consumed.

Chitosan is a biodegradable and inexpensive natural chemical.⁴ It can be used in a variety of agricultural settings. Its nutrients are beneficial to both animals and plants. Chitosan is a mixture of D-glucosamine and N-acetyl-D-glucosamine. Created when an amino group is substituted for chitin's acetyl group. As the most common biopolymer, it resembles cellulose structurally.⁵ In the post-harvest industry, chitosan is used as a coating to extend shelf life, and in agriculture, it is used to spur growth and production. ⁶The plant's defense mechanisms against biotic and abiotic stressors are bolstered as well). Crop quality and quantity are improved by encouraging the plants to take in more nutrients

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DOI:
10.47750/pnr.2022.13.03.106

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How to cite this article: Al-Sheikh M S A, Warqa, The effect of exogenous application of chitosan in Beta vulgaris (beet) drought stressed plants, J Pharm Negative Results 2022;13:703-708.

through the use of chitosan. 7Chitosan improves a plant's stress resistance and overall performance when applied topically or in the soil. Chitosan activates enzymes in plants' bodies to protect them from stress. Organic, environmentally friendly and biodegradable, chitosan is used as a growth stimulant. Stress-resilient plants are the result. Amaranthaceae and Betoideae flowering plants include *Beta vulgaris*, the common name for the beet. 8 Caryophyllales is the most important order in terms of economics. To make sugar, the sugar beet is available in the form of the beetroot or garden beet, as well as the chard or spinach beet and the mangelwurzel; the latter is a fodder crop. There are three distinct subspecies of this species. All cultivars of *Beta vulgaris* subsp. *vulgaris* are covered. 9Sea beets are the wild ancestors of domesticated beets (*Beta vulgaris* subsp. *maritima*). Biennial or perennial, the herbaceous *Beta vulgaris* can grow up to 120 cm (or, more rarely, 200 cm). The most common type is a biannual variety. Both cultivars and wild subspecies have roots that are moderately to severely swollen with fibrous and woody roots that are brown, fibrous, and sometimes even slightly swollen (subsp. *vulgaris*). In nature, stems can be either upright or procumbent. 10The surface of the upper stems is ribbed and striate, with simple or branched stems. Leaves with long petioles at the base (which may be thickened and red, white, or yellow in some cultivars). The length and shape of a wild plant's simple leaf blade ranges from 5 to 20 centimeters. Dark green to red, with a meaty flavor. There is usually a midrib on it (often much larger in cultivated plants). 11 Upper leaves are narrowly lanceolate to rhombic in shape. Around the base of the plant, the plant produces dense spike-like inflorescences. Tiny flowers cluster in glomerules of one to three (and occasionally up to eight) blooms per axill of short bracts or the upper portion of the inflorescence without bracts. Flowers of hermaphrodites can be either green or reddish in color. 11 Tepals measuring 3 to 5 mm in length, five stamens, and a subordinate ovary with 2 to 3 stigmas make up the five basally connate perianth segments (tepals). Neighboring perianths may form a single unit, depending on the species. Inserts or wind can pollinate flowers, but wind is more crucial. As a result of this study, we learned more about how beets grown in salty conditions respond to the addition of chitosan 12.

MATERIAL AND METHOD

Plant material

Seeds of beet cultivar were taken from Institute of Agricultural Engineering - Iraq.

Experimental procedures

Plastic pots were randomly arranged in a factorial fashion (CRD). Four kilograms of farmyard manure, clay, and sand were in each bag. Dirt was mixed with equal parts water (1:1). Saline solution concentration was varied among the four irrigation groups. NaCl concentrations ranged from 0 to

100 mM. A different concentration of foliar chitosan spray was applied to each of the four previous groups (0, 40, 80, and 100 mg L⁻¹ respectively). To ensure a fair test, the treatments were assigned at random to five identical replications of the experiment. There were 12 plants in each treatment. Two weeks after the seven-day period, each of the four solutions was irrigated (0, 40, 80 and 100 mM). In the course of the experiment, three applications of chitosan were made to the leaves of the plants after 14 days. Samples were taken for morphological and biochemical analysis after the experiment. Compound leaf count per plant (1 per 1), stem diameter in millimeters, number of fruits per plant (1 per), average weight in grams, kg/cm² firmness of fruit, and yield per plant were measured (1 per 1). (kg). Leaf chlorophyll, soluble solids (Brix 0), and fruit juice pH are all biochemical characteristics. A soil sample from the medium was analyzed by the Department of Soil and Environmental Sciences. After being shaken for 30 minutes with 50 ml of distiller's water, the soil sample was poured into a Whatman filter paper and filtered. Using a conductivity meter, we determined the water's salinity. Using the electrical conductivity meter's probe, the conductivity of the sample was found to be 0.67 dsm-1. In order to get an accurate pH reading, I used an electronic pH meter-1.

Table 1: Soil physical and chemical properties

<i>NO</i>	<i>Properties</i>	<i>Values</i>	<i>Method applied</i>
1	Sand	21%	Hydrometer
2	Slit	32%	Hydrometer
3	Clay	46%	Hydrometer
4	PH	7.01	PH meter
5	Electrical conductivity	0.77 dsm-1	5.1 (water.soil)

The tensile fruit (in kg cm²)

Beet fruit hardness is measured using a penetrometer (Model-Wagner FT-327, a fruit firmness analyzer) 13. Five fruits from each plant and treatment were randomly chosen to measure firmness.

Dimensions of a biochemical process

Chlorophyll in leaves Using a spade meter, chlorophyll was measured. The average of three leaves from each treatment was calculated by averaging random samples. Throw (Brix0). These findings were made possible by the use of a refractometer. The total soluble solids were measured using a hand refractometer after the juice had been extracted and strained to remove the seeds.

A statistical data

The data that were recorded during the experiment were put

through an analysis of variance (ANOVA) in order to determine the differences that exist between the various treatments and their interactions.

while the least significant difference, or LSD, was utilized to determine the mean difference at a significance threshold of 5% 14. STATISTIX is a statistical computer program. The ANOVA and LSD calculations were done with 8.1 as the factor.

RESULTS

Plant height (cm)

a person's height (cm) Figures 1 and 2 show the heights of the plants. Salt and chitosan both had an impact on the height of beet plants, and the interaction between the two was also significant. Saline content reduced the height of beet plants more than it did for control plants. Salinity-treated plants were shorter than control plants, with a mean height of only 64.81 cm (55.750 cm). The tallest control plants were found (100 mM). One hundred milligrams-per-liter chitosan resulted in the highest plant height (63.75 centimeters), while another treatment resulted in the lowest (57.10 centimeters). Chitosan at any concentration can reduce the harmful effects of salt (Table 1). When compared to salt-stressed plants, chitosan at 100 mg L-1 produced the best results.

This was true, regardless of how salient it was. Leaves' standard deviation Beet leaves were affected by salt levels and foliar chitosan treatments. Salinity stress and chitosan interacted in a way that had a big impact (p 0.05). Sulfuric acid (100 mM saline) reduced the number of leaves on the average plant from 21.99 to 11.99. Compared to control plants, which had an average of 11.91 leaves, plants treated with 100 mg L-1 chitosan had the most leaves on average. (Figs.1) When compared to salt-stressed plants, foliar spraying with 100 mg L-1 of chitosan increased plant leaf count (Table 1). Leaves on the ground (cm²) Beet leaf area was significantly reduced as a result of the combination of salinity and foliar chitosan spraying (p 0.05). Leaf area decreased significantly as salt concentrations increased, with 100 mM showing the lowest value (15.96 cm²) compared to control plants, which had the highest value (19.19 cm²). Plants treated with chitosan foliar spray received 18.53 cm² more leaf area than control plants (16.87 cm²) (Table 1 and Figs. 1). Chitosan-treated plants grew more leaves than salt-stressed plants (Table 1).

Rigidity (mm)

Stem diameter (mm)

The stem diameter of the control plants was 10.37 mm, whereas the stem diameter of the 100 mM salt-treated plants was smaller (9.09 mm). The stems of the control plants were the thickest. In comparison to salt-stressed plants, stem diameter increased when chitosan was added to plant leaves.

The best growth was seen in plants fed 100 mg L-1 chitosan (Figs. 1). Average weight and number of fruits per plant (g) Plant-1 of the salinity-stressed plants produced the fewest (15.83) and heaviest (55.43 g) fruits, while plant-1 of the control plants produced the most (23) and heaviest (64.31) (150 mM). In 100 mg L-1 of chitosan foliar spray, the highest number of fruits plant-1 (23.58) and fruit weight (64.35 g) were observed, while the lowest numbers (15.66) and weights (57.26 g) were observed in the control treatment (Figs.1). Fruit production and weight were increased by all concentrations of chitosan when compared to salt-stressed plants (Table 1). Hardness and pH of the fruit The firmness of beet fruit was affected by salinity, chitosan, or the combination of the two, depending on the stage of development.

Number of fruits plant-1 and average fruit weight (g)

Fruit firmness (3.73 kg cm²) and fruit juice pH (5.66) were highest in the untreated plants, while fruit firmness (2.78 kg cm²) was lowest in the 100 mM salinity-treated plants (4.93). (Figs.1) When chitosan was applied at 100 mg L-1, fruit firmness and pH were increased compared to untreated and salt-stressed plants (Tables 1). The fruit juice's pH was drastically altered by the salt and chitosan treatments. Fruit juice with a pH of 5.84 was produced by plants given salt and chitosan. Excess salt and chitosan did not affect the pH of the fruit juice. a single tally (kg) For example, the yield of plant-1 is shown in Tables 2 and 3. The yield is also affected by the interaction between these two factors. At 100 mM salinity, plants yielded the least (1.41 kg) per plant (1.16 kg). It was found that foliar application of 100 mg L-1 chitosan per plant resulted in the highest yield per plant (1.38 kg) compared to untreated plants (1.22 kg) (Figs.1). Compared to salt-stressed plants, chitosan increased yield per square meter at all concentrations. TSI (Brix °) There is total soluble solids information in tables 1. Beet total soluble solids were affected by salt and foliar chitosan treatments, as well as their relationship. The total soluble solids in control plants were the lowest (3.34 Brix°), while those in 100 mM salinity levels were the highest (5.05 Brix°). The total soluble solids (4.56 Brix°) of 100 mg L-1 chitosan were higher than those of the control plants (3.80 Brix°) (Figs. 1). Chlorophyll is found in the leaves (SPAD) Beet chlorophyll content was affected by salinity and chitosan concentrations either alone or in combination. When plants were exposed to high levels of salinity, they lacked chlorophyll. The chlorophyll content (SPAD) was highest (65.29) in the control salt treatment and lowest (100 mM) in the 100 mM treatment (51.45). There was a significant difference in chlorophyll content between plants fed 100 mg L-1 chitosan and those fed the control treatment (48.80 mg L-1). As shown in Figures 1 and 2. Chitosan foliar spray reduces plant salt stress by increasing chlorophyll concentration.

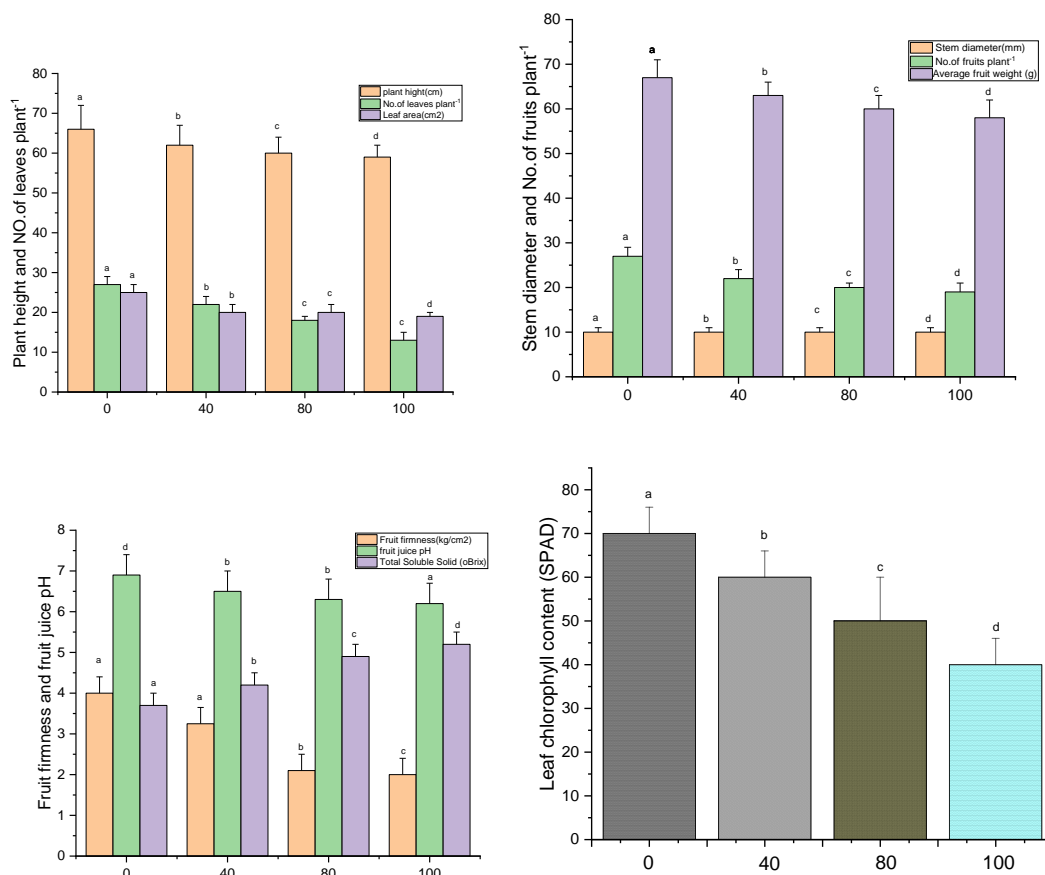


Fig. 1 Beet's morphological, yield, and quality characteristics as influenced by salinity levels vary widely. The different letters in each bar indicate a significant difference (LSD $p \leq 0.05$) between the means (\pm SE) of three replications.

DISCUSSION

Increased salinity lowered beet growth. If salinity inhibited root growth, altering the shape and physiology of plants, decreasing the uptake of water and ion, and reducing the plant's ability to absorb water and metabolic activities, then this may have been the cause of this. According to Mohamed *et al.*, salt stress inhibited wheat plant growth 15. Akladious and Mohamed (2018) found that pepper plant morphology is negatively affected by salt stress. 16 Chitosan boosted the height of beet plants. The amino group in chitosan may increase the area of photosynthesis in plants. As a result, the plants grow taller (Sofy *et al.* 2020a). The increase in plant growth after treatment may have been caused by the chitosan's effect on mineral uptake and transport. Chitosan aids in the heightening of peppers, cucumbers, and radishes through the application of chelating agents (Farouk *et al.* 2008). Chicory plant height is affected by Chitosan content, according to a study by Guan *et al.* (2009). They found that it was beneficial. Fewer complex leaves may be a result of increased salinity. This causes osmosis, which reduces the amount of water and nutrients available to the plant's roots. This interferes with plant tissue, reducing cell growth and meristematic activity. Sodium chloride loss in Brassica species was observed by 17 when the concentration of sodium

chloride was elevated. The growth of peeper plants is impeded by exposure to salt (Akladious and Mohamed 2018). Plant-1 produced more complex leaves when exposed to chitosan, possibly as a result of the increased phosphate and potash content, which in turn boosts cell growth, cell size, chloroplast development, and chlorophyll synthesis in the plants 17. Using chitosan, Jian *et al.* (2002) found that the number of branches, height, and leaves on rice plants increased. By spraying soybeans and maize with chitosan, 18 confirmed the findings. The presence of chitosan in soy beans and maize leaves led to an increase in the number of leaves. Supporting evidence has been found. More leaves were found in beets treated with chitosan, according to 18. Beet leaf area is significantly reduced by salinity stress at higher concentrations as nitrogen levels are reduced and photosynthesis slows 19. A variety of chitosan concentrations increased the leaf area of the beet, which may be due to an increase in the availability and consumption of water and essential nutrients, as well as an increase in key nitrogen metabolism enzyme activities and improved nitrogen transport 20. Many studies have shown that chitosan can help cucumber plants grow taller and more quickly. Because of the increased plant growth and development brought on by the consumption of nitrogen and potassium, stem thickness increases 21. Salt concentrations affect the

availability of essential nutrients for plants in varying ways. Because of this, fewer fruits are produced. Salt, osmotic stress, and ion imbalance all had an impact on the metabolic processes of plants. Beet fruit production may have increased because of chitosan's ability to boost photosynthetic pigments and biochemical activity in plants.²² These improvements resulted in more fruits per plant as a result of an increase in the amount of photosynthates sent to the fruits. Chitosan increased the yield of beet fruits ²³. According to²⁴, 75 mg L⁻¹ of foliar chitosan increased beet fruit production, and our results are similar. Chitosan-treated rice and soybeans increased the number of soybean pods in the early stages of growth, according to²⁵. Chibu *et al* findings 's are in agreement with ours (2002). The fruit's weight decreased as salt concentration increased because saltwater affects chlorophyll pigments and reduces photosynthesis. Results that are similar²⁶. Chitosan increased the yield of beet fruits. Regulating cell osmotic pressure improves water supply and food absorption, which is why chitosan is beneficial. It has been shown that chitosan enhances the enzyme and antioxidant activity of fruit plants. Chitosan enhanced strawberry fruit weight in our study, confirming the findings of²⁷. The firmness of beets is weakened by salt. In tissues, salt reduces the deposition of calcium pectate and calcium phosphate and thus limits the development of cell walls and the firmness of fruits (Islam *et al.* 2018). Beet fruit firmness may have been improved by chitosan's ability to thicken the cell walls of the beet root. Chitosan enhances the transport of calcium to the tissues of the fruit, resulting in a firmer fruit. Our findings corroborate those of Ali *et al.* (2015), who found that adding chitosan to papaya increased its firmness. Our findings are in agreement with those of²⁸. According to ²⁹, peach fruit treated with chitosan reduced the early onset of edema. There was less loss of firmness, freshness, and weight from the fruit's firm structure.

Acidity decreased CO₂ incorporation, stomatal conductance, photosynthetic activity and the pH of beet juice. A decrease in carbohydrate synthesis results in a decrease in pH. Fruit juice pH is lowered by increasing salinity, which raises respiration rate and ethylene production. It is possible that increased water availability to the plant and within the cells through osmotic pressure and enzymatic activity may have an impact on the pH of beet fruit juice. Chitosan foliar spray enhances the antioxidant properties of beet juice³⁰. Chitosan, according to³¹, lowers the pH of beet juice.

Several physiological aspects of the plant are altered by high salt levels, which may result in decreased fruit production and a smaller fruit size According to ³² high concentrations of Na and Cl in the plant's growing medium cause physiological and biochemical changes that limit the plant's growth and development, which in turn reduces yield under salinity stress. According to³³, a combination of the ion's unique action and poor water and nutrient absorption may be to blame for the drop in yield. Due to physiological mechanisms such as enhanced nitrogen uptake, the chitosan concentration increased beet yield, which in turn increased plant growth and development, which in turn increased yield

and its contributing factors³⁴. Chitosan was found to increase the yield of mung beans, according to³⁵. When pepper plants were exposed to foliar chitosan, it reduced transpiration and water use while maintaining biomass output and yield, as reported by³⁵. The increase in sodium (Na⁺), potassium (K⁺), and chloride (Cl) ions in the fruit may be the cause of the rising total soluble solids due to rising salinity. A few examples of these ions are Na⁺, K⁺, and Cl. TSS in beets increased with saline concentrations, according to ²⁵. This was found out about. Beet TSS is raised by foliar application of chitosan due to an increase in metabolite concentrations and an immediate starch conversion in response to growth promoters. Sugar beet TSS is boosted by the addition of chitosan. Sugar beet leaves that have been treated with chitosan have a higher sugar content. Chitosan at 80 to 100 mg L⁻¹ increased the total soluble solids (TSS) in beet fruits, according to our findings. Chitosan increased total soluble solids, according to ²⁸. (TSS). Similar results were found by ³¹. Beet plants were sprayed with various concentrations of chitosan, and the results showed that they grew better than untreated plants in every way. In spite of not being given any attention, these plants succumbed to the elements . Chitosan was found to promote the growth of beets and eggplants by ¹⁸. A greater amount of nitrogen was transferred to leaves as a result of these increases, which improved growth¹⁷. The loss of chlorophyll pigments in beet leaves may have been caused by salt stress at higher concentrations, which induces early leaf maturation and diminishes chlorophyll pigments. chlorophyll concentration is reduced by early leaf development and lower chlorophyll pigments. In¹⁷ found that high salt levels reduced chlorophyll synthesis by causing an ion imbalance. These findings were published in *Plant Physiology*. Nitrogen, phosphorus, and potassium uptake was enhanced by increasing the chitosan concentration. An increase in chlorophyll pigment and chlorophyll content can be achieved by increasing nitrogen transport to the leaves through chitosan²¹. Chitosan increased leaf chlorophyll and cucumber and radish starch, according to²¹. This lends credence to the arguments put forth by¹⁹. Chitosan spray was found to increase the amount of chlorophyll in cucumber plants, according to²². The results of this study are also consistent. The effects of salt stress and chitosan on the chlorophyll content of beets are clearly visible. It doesn't matter if the plants are healthy or stressed. Plants treated with chitosan concentrations (50 mg L⁻¹) below the salinity level had the most growth and photosynthetic pigments, according to²². (4000 mg L⁻¹). Similar findings were made by Bakhroum *et al* (2020).

CONCLUSION

This research underlines the significance of using foliar sprays containing natural compounds of chitosan, particularly in soils that are impacted by salt. It is possible that the application of the appropriate concentrations of chitosan could effectively reduce the dangerous effect of higher salinity levels and improve the growth and yield of

beet plants by improving the various morphological aspects as well as the quality of the chlorophyll. According to the findings of this study, a dosage of chitosan equal to 100 mg L⁻¹ is suggested for improving the growth and yield of beets that are cultivated in salty environments.

Compliance with Ethical Standards statements

Ethical approval: The University of Al-Qadisiyah / Iraq / certifies the ethical approval, Funding details (In case of Funding): I am responsible for paying the financing, Conflict of interest: There is no conflict of interest, Informed Consent: Department of Research and Studies, University of Al-Qadisiyah \ Agreed

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