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Chemical and Nutritional Studies of Neem Plant Leaves Planted in Kingdom of Saudi Arabia and Their Importance in Combating Oxidative Stress in Experimental Animals

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Oxidative stress is a condition that occurs when there is an imbalance between the levels of reactive oxygen species (ROS) in the body and its ability to detoxify these reactive molecules or repair the damage they cause. The objective of this study is to investigate the effects of neem tree (Azadirachta indica A.Juss) leaves collected in the Tehama al-Baha area of the Kingdom of Saudi Arabia in reducing oxidative stress induced by potassium bromate in rats. Materials and Methods: Thirty healthy adult male albino rats weighting 150±5g were used in the experiment, were used and divided into 5 groups, one was kept as a negative control group, while the other groups of rats (24 in total) were injected by a single intraperitoneal dose of potassium bromate at dose of 125 mg/kg body weight for induction of oxidative stress, the groups were divided into four groups fed on basal diet + neem leaves at different levels 5%, 10% and 15% and one group acting as a control(+) group that suffered from the disease but did not follow the experimental diet, also phenolic compounds have been extracted using the technique described. Results: BrO3 intoxication raised the AST (Aspartate Transaminase) / ALT (Alanine Transaminase) ratio, while feeding on plant diets lowered this ratio. Nevertheless, the best effect was recorded for G4 (10% neem leaves), with a non-significant difference from G3 (5% neem leaves). The non-significant difference between G3 and G4 could be attributed to the similar biochemical properties of the neem leaves at both concentrations. The revealed primary bioactive compounds in the neem leaves, such as pyrogallol and catechin, may reach a saturation point where increasing the concentration from 5% to 10% does not significantly enhance their impact on the AST (Aspartate Transaminase) / ALT (Alanine Transaminase) ratio. Catalase activity (Catalase) was reduced due to KBrO3 intoxication, while plant diets G 5(15% neem leaves) increased. Neem leaves led to the most significant enhancement of Catalase, Superoxide Dismutase, and Glutathione Peroxidase activities, indicating its superior protective effect against oxidative stress.

Keywords: Azadirachta indica; Functional Foods; Neem Leaves; Oxidative Stress; Potassium Bromate; Rats.

All living cells try to maintain a normal, diminished environment. This state is absent when reactive oxygen species, such as free radicals, are produced, resulting in significant damage to components of cells; such as proteins, lipids & DNA. Oxidative stress is being experienced by these cells. [1] Oxidative stress is a disorder that is distinguished by a disturbance in the systemic manifestations of ROS & the biological system's capability to efficiently detoxify reactive intermediates (antioxidant defenses) or restore the damage that results. [2]Alzheimer's illness, heart failure, myocardial infarction, Parkinson's disease, sickle cell illness, fragile X syndrome, schizophrenia, diabetes, chronic fatigue syndrome, cancer, & cardiovascular illness are all characterized by oxidative stress. [3] Potassium bromate (KBrO3) is a food additive that has been utilized as an oxidizing agent, primarily during the process of preparing bread. The production of free radicals & ROS was elevated by KBrO3.[4] Numerous toxicological investigations have indicated that KBrO3 induces carcinogenicity, nephrotoxicity, neurotoxicity, thyroid toxicity, & hepatotoxicity in experimental animals.[5] Numerous antioxidants have been demonstrated to minimize the toxicity that bromate causes to various organs, which is consistent with the participation of reactive oxygen species in its action. [6] It is essential to consume antioxidants in one's diet to protect the cellular system from oxidative stress, which is a risk factor for various chronic illnesses.[7] The neem tree *(Azadirachta indica)* is among the most extensively utilized medicinal plants globally.[8] illustrates how it has been utilized for several years. The US National Academy of Sciences has acknowledged the significance of neem tree. In 1992, it published a paper titled, neem—a tree for solving worldwide problems [9] have examined the pharmacological actions of neem extracts, clinical investigations, probable therapeutic applications of neem, and their safety assessment. The leaves of neem tree are conventionally utilized in pharmaceutical formulations for their antifungal, anti-inflammatory, immunomodulatory, antimalarial, antiulcer, antihyperglycemic, antimutagenic, anticarcinogenic, antiviral & antibacterial characteristics.[10] This investigation aims to assess the influence of the natural product neem against CDDP-induced hepatotoxicity. Current research indicates that the incorporation of plant-derived chemopreventive medicines with chemotherapy might augment the effectiveness of chemotherapeutic drugs while reducing their toxicity to normal tissues.[11] Neem is one of the candidate plants that possess a significant antioxidant potential and a chemoprotective effect.[12] The neem tree's components, including seed, leaf, bark, oil, gum, and fruit, contain compounds that have promising therapeutic applications.. An investigation has verified the non-toxic effects of extracts of neem leaves on rat liver & kidneys, even at elevated dosages above the effective dose.[13] One promising agent against a variety of toxicities related to peroxidative damage and oxidative stress is neem. Neem has demonstrated significant radical-scavenging, antiperoxidative and antioxidant characteristics. In the present investigation, the toxicity of CDDP was improved by MNLE (500 milligrams per kilogram) for five days. This was demonstrated by a significant decrease in the increased concentration of LPO and NO, as well as a normalization of the tissue GSH concentration. .[14]

Aim of study

The study aims to investigate the positive effects of neem plant leaves*(Azadirachta indica)* planted in KSA in combating oxidative stress caused by potassium 13 bromate in experimental animals.

MATERIALS AND METHODS

Materials

A- Source of neem leaves *(Azadirachta indica)*: Neem leaves obtained from the trees in the area of Tehama, al-Baha, KSA. Leaves were washed, dried and ground.

B-Experimental animals: Thirty male albino Sprague Dawley rats, ~~each~~ weighing 150±10grams, have been utilized.

C- Casein, choline chloride, cellulose, & DL Methionine: Cellulose, DL methionine powder, choline chloride powder and casein have been attained from Morgan Co. Cairo, Egypt.

D- Chemicals: Potassium bromate, ~~in the form of~~ a white powder, has been acquired from El-Gomhoria Company for Drugs & Medical Equipment, Cairo, Egypt.

Methods

Diets

Basal diet

The routine nutrition was composed of protein (ten percent), corn oil (ten percent), choline chloride (0.2 percent), cellulose (five percent), mixture of vitamins (one percent).[15] Salt mixture (four percent) and corn starch (up to one hundred percent).[16]

Experimental design

Thirty adult male white albino mice of the Sprague Dawley strain, aged ten weeks and weighing 140±10grams, have been utilized. All rats have been nourished with a routine diet .[15] For seven succeeding days. this adapting duration, mice were categorized into five groups, each including six mice as follows:

Group (1): Rats (number =six) have been nourished on routine nutrition only as a control negative.

Group (2): Rats (number = six) have been maintained without management and provided a baseline diet following a single intraperitoneal injection of KBrO3 (125 milligrams per kilogram body weight) as a positive control.

Group (3): Rats (number=6) received an intraperitoneal injection with KBrO3 (125 milligrams per kilogram body weight) and nourished on routine nutrition + 5% neem leaves powder.

Group (4): Rats (number =6) received an intraperitoneal injection with KBrO3 (125 milligrams per kilogram body weight) and nourished on basal diet + 10 % neem leaves powder.

Group (5): Rats (n =6) received an intraperitoneal injection with KBrO3 (125 milligrams per kilogram body weight) and nourished on basal diet + 15% neem leaves powder.

Body weight and food consumption have been assessed weekly throughout the trial. The overall behavior of the rats has been monitored. The experiment would continue for twenty-eight days when each rat would be weighed individually. Rats would be euthanized for blood sample collection after that. Blood samples were centrifuged at 4000 revolutions per minute for ten minutes to isolate the serum, and stored in a deep freezer until utilization. The kidney, heart, spleen and liver have been extracted for further pathological studies.

Blood sampling

After fasting for 12 hours, blood samples in initial times were obtained from retro orbital vein, while it obtained from hepatic portal vein at the end of each experiment. Blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath (37°C) for 28minutes, then centrifuged for 10 minutes at 4000 rpm to separate the serum, which were carefully aspirated and transferred into clean Eppendorf tube and stored frozen at -20°C till analysis. [17]

Organ's weight

The various organs of mice (kidney, liver, heart and spleen) have been carefully removed, rinsed in saline solution, dried on two filter papers and rapidly weighed before being conserved in buffered formalin solution (ten percent) for histological examination.

Biological evaluation

The biological assessment of several diets has been conducted by measuring body weight gain % (BWG) and food efficiency ratio (FIR) as per.[18] utilizing the specified formulas:

BWF = (Final weight - initial weight) / Initial weight

Biochemical analysis

Lipids profile

Estimation of serum total cholesterol: The total cholesterol in serum has been measured using the colorimetric technique reported by.[19]

Estimation of serum triglycerides: Serum triglycerides have been measured utilizing an enzymatic approach utilizing kits, based on the protocols established by.[20-21]

Estimation of high-density lipoprotein (HDL-c): High-density lipoprotein has been estimated utilizing the techniques defined by. [22]

Estimation of very low-density lipoprotein cholesterol (VLDL-c): VLDL-c has been measured in milligrams per deciliter with regard to .[23]

Estimation of low-density lipoprotein cholesterol (LDL-c): LDL-c has been measured in milligrams per deciliters with regard to**.**[23]

Determination - Estimation of atherogenic index (AI): Calculation of atherogenic index = (very low-density lipoprotein cholesterol

+ low-density lipoprotein cholesterol) is based on .[23]

Functions of liver

Determination of ALT: - conducted according to the method.[24]

Determination of aminotransferase (AST): Determination of serum aminotransferase has been conducted ~~according to~~. [25]

Estimation of serum Globulin: Serum globulin has been determined with regard to the method defined by. [26]

Serum albumin (SAlb): SAlb has been measured utilizing a technique defined by. [27]

Kidney functions

Determination of serum urea: Urea has been measured via enzymatic method by.[28]

Estimation of serum creatinine: Serum creatinine has been measured with regard to the technique defined by.[26]

Estimation of serum uric a': Serum uric a' has been measured calorimetrically with regard to the technique. [29]

Blood glucose

Enzymatic estimation of serum glucose has been performed calorimetrically with regard to the technique.[30]

Determination of antioxidant enzymes

Assay of superoxide dismutase (SOD) activity (U/L): superoxide dismutase has been estimated with regard to the technique.[31]

Assay of glutathione peroxidase (GPX) activity (ng/ml): Determination of GPX followed the technique. [32]

Assay of catalase (CAT) activity (mmol/L): Catalase activity has been evaluated following the technique. [33]

HPLC Identification of phenolic compounds

Phenolic compounds have been extracted using the technique described. [34] A specified mass of dried powdered sample has been soaked in twenty-five milliliters of sterilized water and stirred on a rotary shaker for twenty-four hours at 200 revolutions per minute (rpm). Filtering was done using Whatman filter paper under vacuum, and subsequently centrifuged at 12,500 g for thirty minutes at eighty degrees Celsius. The aqueous extract has been acidified to a pH of 2.5 with diluted phosphoric acid. Each sample has been partitioned thrice with an equivalent amount of diethyl ether. The mixed diethyl ether layer has been evaporated to dryness under decreased pressure at thirty degrees Celsius. The resultant residue has been re-dissolved in three milliliters of spectral grade methanol and filtered through a 0.2-millimeter filter-sterilized membrane before HPLC analysis.

Statistical analysis

Student-Newman-Keuls Test was utilized to separate the means after a significant main effect has been identified. The data was examined utilizing an entirely randomized factorial design [35] . Using the Costat Program, significant distinctions among therapies have been defined as ($P<0.05$). Analyses of biological outcomes were conducted using One-Way ANOVA. [35]

Approval of ethics

The Science Research Ethics Committee of the Faculty of Home Economics has approved the research protocol #04-SREC-10-2021.

RESULTS

The currents study investigates the positive effects of neem plant leaves planted in KSA in combating oxidative stress caused by potassium bromate in experimental animals.

Chemical composition of laurel leaves powder

The chemical composition of laurel leaves powder is illustrated in Table 1. The findings have revealed that the powder of laurel leaves contained protein, moisture, lipids, fiber, carbohydrates, ash, and energy value. The dry weight (D/W) was 3.43, 18.95, 3.67, 6.87, 16.51, 50.57, and 390.23 kilocalories per one hundred grams.

Total phenolic compounds of neem leaves

Data in Table (2) illustrate that the total number of 20 distinct phenolic compounds have been evaluated in neem leaves, 12 of them existed, while eight were absent. Total phenolic compounds recorded (1267.666 ppm). By focusing on the major phenolic compound, it was found that the highest content was recorded for Catechein (346.295ppm), Pyrogallol (344.863ppm) & Salycillic (85.896ppm).

Biological results

Data demonstrated in Table (3) illustrate the influence of different levels of neem leaves (5%,10% and 15%) powder " for $KBrO_3$-intoxicated rats on biological changes (BWG, FI and FER)

Body weight gain (BWG (g/d))

Data represented in Table (3) illustrate

the influence of feeding tested plants on the BWG (g/d) of experimental rats. The attained outcomes illustrate that control (+) have significantly decreased from 0.99 to 0.219 g/day compared to control (-) group. In the same time, G4 (10% neem leaves) has significant decrease (-68.18%) in comparison with control (+) group, and G5 (15% neem leaves) has a non-significant decrease (-18.18%) compared to control (+) group. (G3) have significant variances in comparison with control (+) group.

Feed intake (FI)

Results of FI of experimental mice are reported in Table (3). It illustrates that KBrO3-intoxication lowered considerably the appetite and the FI of rats from 38.93 to 24.34 g showing significant reduction in comparison with control (-) group. Nevertheless, tested plants raised the FI which reached maximum increase (+40.03%) compared to control (+) group in case of G4 (10% neem leaves). Also, all experimental group rats have significant increases compared to control (+).

Feed efficiency ratio

Table 3 data illustrate the FER of KBrO3-intoxicated mice as affected by nourishing on tested diets. It is clear that control (-) group revealed +223.111% increase in FER in comparison with that of the control (+). In contrast, G3 (5% neem leaves), G4 (10% neem leaves), G5 (15% neem leaves) decrease in FER in comparison with control (+) group.

Biochemical changes

Liver enzymes

The data in Table (4) illustrates the influence of varying concentrations of neem leaf powder (five percent, ten percent, and fifteen percent) on hepatic enzymes (ALT, ALP & AST) in KBrO3-intoxicated rats.

AST enzyme (GOT)

Data of Table (4) demonstrates the AST activity of experimental rats. It might It might be noted that control (-) group revealed -79.97 less than that noted for control (+) group, with a significant distinction among them. Meanwhile, antioxidant effect of phenols in tested plants reversed this change, leading to decrease of AST activity. Consequently, G3 (five percent neem leaves) was the most efficient therapy, as evidenced by the highest numerical decreases in AST activity.

ALT enzyme (GPT)

The outcomes of Table (4) demonstrate the ALT activity of experimental rats. It was seen that ALT activity was elevated due to KBrO3 intoxication (from 33 to 42 (U/L)) compared to control (-) rats with significant difference between them. All experimental groups had significant decrease in ALT activity (U/L) ranging from -49.39% to -60.24 % of control (+) group. The best group was G5 (15% neem leaves).

ALP activity

Data of Table (4) demonstrates the ALP activity of experimental rats. It was found that ALP activity was raised due to oxidative stress. It might be noticed that control (-) group indicated

Table 1. Chemical composition of neem leaves powder

Constitutes (%)	Value D/W
Ash	6.87±0.24
Carbohydrates	50.57±0.50
Fat	3.67±0.11
Moisture	3.43±0.11
Fiber	16.51±0.31
Protein	18.95±0.23
Energy value (Kcal/lOOg)	390.23±0.63

DW= Dry weight

Table 2. Total phenolic compounds of neem leaves

N.	Component	Content (ppm)
1	Pyrogallol	344.863
2	Gallic	21.091
3	3-Hydroxy Tyrosol	83.401
4	Catechol	6.167
5	4-Aminoben/oic	7.001
6	Catechein	346.295
7	Chlorogenic	59.709
8	Benzoic	74.194
9	P-OH-benzoic	21.091
10	Vanillic	82.454
11	Caffeic	24.596
12	Caffeine	49.594
13	Ferulic	19.511
14	Salycillic	85.896
15	Ellagic	28.430
16	Coumarin	13.373
17	Total	1267.666

Table 3. The influence of various levels of neem leaves powder (five percent, ten percent and fifteen percent) for KBrO3-intoxicated rats on biological changes (BWG, FI & FER)

Groups Parameters	BWG (g/d)Mean ± SD	%Change of C(+)ve	FI (g) Mean ± SD	%Change of C(+)ve	FER Mean ± SD	%Change of C(+)ve
G1 (-ve)	0.99^{a}±0.02	+360.11	36.01^{a}±0.13	+39.99.79	0.030^{a}±6.5	+223.111
G2 (+ ve)	0.219^{e}±0.01	———	19.99^{h}±0.05		0.009^{c}±4.1	———
G3Neem leaves 5%	0.33^{cd}±0.04	+50	32.87^{f}± 0.02	+38.93	0.006^{d}±0.0015	-33.33
G4Neem leaves 10%	0.07^{f}±0.03	-68.18	33.13^{de}±0.02	+40.03	$0.001^{e}\pm2\times10^{-4}$	-88.89
G5Neem leaves 15%	0.18^{e}±0.05	-18.18	29.42^{g}±0.07	+24.34	$0.002^{e}\pm1\times10^{-4}$	-77.78

All outcomes are stated as mean ± SD (standard deviation of the mean).
* Values in each column with various letters are significantly various (P-value less than 0.05).
* One-way ANOVA test used.

Table 4. illustrates the influence of various concentrations of neem leaves powder (5%,10% & 15%) for KBrO3-intoxicated mice on hepatic enzymes (AST, ALT, & ALP)

Groups Parameters	AST (U/L) Mean ± SD	%Change of C(+)ve	ALT (U/L) Mean ± SD	%Change of C(+)ve	ALP (U/L) Mean ± SD	%Change of C(+)ve
G1 (-ve)	39.99^{g}±2.00	-79.97	26.9^{e}±1.00	-68.39	86.9^{f}±0.49	-50.11
G2 (+ ve)	202^{a}±3.00		82^{a}±1.00		169^{a}±3.00	
G3Neem leaves 5%	65^{d}±1.00	-67.82	36^{c}±4.1	-56.63	92.67^{de}±1.23	-46.43
G4Neem leaves 10%	72^{b}±3.00	-64.36	42^{b}±0.1	-49.39	98.33^{c}±1.53	-43.16
G5Neem leaves 15%	60^{ef}±2.00	-70.29	33^{cd}±0.9	-60.24	89^{ef}±1.00	-48.55

Table 5. illustrates the influence of various concentrations of neem leaves powder (5%,10% and 15%) for KBrO3-intoxicated rats on serum protein fractions (Total protein, Albumin, and Globulin)

Groups Parameters	Total protein (g/dl) Mean ± SD	%Change of C(+)ve	Albumin (g/dl) Mean ± SD	%Change of C(+)ve	Globulin (g/dl) Mean ± SD	%Change of C(+)ve
G1 (-ve)	6.99^{a}±0.05	+49.99	3.77^{ab}± 0.1	+279	3.499^{bc}± 0.05	-8.00
G2 (+ ve)	5.00^{f}± 0.1		0.99^{g}± 0.2		3.9^{ab}± 0.1	———
G3Neem leaves 5%	5.9^{d}± 0.1	+24.49	2.7^{d}± 0.3	+170	3.4^{cd}± 0.4	-12.82
G4Neem leaves 10%	5^{f}± 0.2	+2.04	1.5^{f}± 0.1	+50	3.5^{bc} ± 0.1	-10.26
G5Neem leaves 15%	6.3^{b}± 0.1	+38.78	3.6^{b}± 0.08	+260	3.2^{cd}± 0.02	-17.95

Table 6. illustrates the influence of various levels of neem leaves powder (5%,10% and 15%) for KBrO3-intoxicated rats on Lipids fraction of serum (TC, TG, and VLDLc)

Groups Parameters	TC (mg/dl) Mean ± SD	%Change of C(+)ve	TG (mg/dl) Mean ± SD	%Change of C(+)ve	VLDLc (mg/dl) Mean ± SD	%Change of C(+)ve
G1 (-ve)	75.2 ^{f}± 1.00	-22.9	50.9^{d}± 1.04	-16.1	11.99^{d}±0.208	-16.76
G2 (+ ve)	1^{a}±0.3		72^{a}± 1.5	———	15.1± 0.3	———
G3Neem leaves 5%	80^{e}± 2.00	-19.19	66^{c}± 1.00	-8.33	13.2^{c}± 0.2	-8.33
G4Neem leaves 10%	92^{c}± 2.00	-7.07	69^{b}± 0.7	-4.17	13.8^{b}± 0.14	-4.18
G5Neem leaves 15%	73^{g}± 2.00	-26.26	59^{de}± 2.00	-18.06	11.8^{de}± 0.4	-17.99

Table 7. illustrates the effect of various concentration of neem leaves powder (5%,10% and 15%) for KBrO3-intoxicated rats on kidney function of serum (Creatinine, Urea, & Uric a‘)

Groups Parameters	Creatinine (mg/dl) Mean ± SD	%Change of C(+)ve	Urea (mg/dl) Mean ± SD	%Change of C(+)ve	Uric acid (mg/dl) Mean ± SD	%Change of C(+)ve p
G1 (-ve)	0.69^{b}± 0.07	-80. 1	19.99^{g}±2.00	-53.98	2.98^{d}±0.1	-59
G2 (+ ve)	3.49^{a}±0.33		43.9^{a}±3.00		7.48^{a}±0.2	———
G3Neem leaves 5%	0.957^{b}± 0.127	-72.65	35^{cd}± 3.00	-20.45	2.4^{bcd}± 0.4	-68
G4Neem leaves 10%	1.03^{b}± 0.026	-70.57	38^{bc}± 1.00	-13.64	3.6^{bc}±0.1	-52
G5Neem leaves 15%	0.967^{b}± 0.067	-72.37	29^{ef}± 2.00	-34.09	3.3^{bcd}± 0.1	-56

Table 8. illustrates the influence of various concentrations of neem leaves powder (5%,10% and 15%) for KBrO3-intoxicated rats on antioxidants enzymes of serum (Serum catalase activity, Serum superoxide dismutase activity) mmol/L, & Glutathione peroxidase activity) ng/ml

Groups Parameters	CAT (mmol/L) Mean ± SD	%Change of C(+)ve	SOD (mmol/L) Mean ± SD	%Change of C(+)ve	GPX (ng/mt) Mean ± SD	%Change of C(+)ve
G1 (-ve)	0.199^{a}±0.002	+53.9	61.1^{a}± 1.06	+85.4	0.749^{a}±.02	+88.1
G2 (+ ve)	0.129^{e}± 0.016	———	45.9^{d}± 1.23	———	0.39^{e}±0.004	
G3Neem leaves 5%	0.16^{cbc}± 0.009	+22.19	55.94^{b}± 0.94	+41	0.564^{c}±0.023	+41
G4Neem leaves 10%	0.15^{bc}± 0.022	+16.79	51.00^{c}± 0.86	+25.5	0.502^{d}± 0.032	+24.5
G5Neem leaves 15%	0.2^{a}±0.015	+52.67	59.01^{ab}± 1.52	+75.75	0.703^{ab}± 0.024	+76.75

-49.71% less ALP than that detected for control (+) group, with significant variance among them. All experimental groups showed significant reduction in ALP activity (U/L) ranging from -43.16% to -46.43% of control (+) group, considering that the greatest diminished limit attained for G4 (10% neem leaves) showed nonsignificant variance with control (-) group.

Serum protein fractions

Total protein (T. protein)

The outcomes of Table (5) indicate the serum total protein of experimental vermin. ~~It is obvious that~~ degeneration of the T. protein occurred as a result of KBrO3 intoxication (from 7.4 to 4.9 gram per deciliters), whereas it was elevated by feeding the examined plants, particularly G5 (fifteen percent neem leaves), which exhibited ~~the greatest~~ elevation.

Albumin (A)

Data in Table (5) shows the albumin content (A) in serum of experimental rats. It might be observed that control (-) group showed +279% over that observed for control (+) group, with significant variance among them. All experimental groups showed pronounced elevation in serum concentration of albumin (g/dl) varying from +50% to +260 % of control (+) group, considering that the greatest elevated limit attained in G5 (15% neem leaves) with an insignificant variance of control (-) group.

Globulin (G)

Outcomes of Table (4) show the globulin fraction in serum of experimental rats. KBrO3-intoxication increased the globulin (from 3.49to 3.9 gram per In manuscript please change gram/ dL Furthermore, G3 (5% neem leaves) & G5 (15% neem leaves) decreased the globulin to (3.4 & 3.2 gram per deciliters), correspondingly than 3.49 (gram per deciliters) for control (-) group. The lowest levels revealed for G5 were (15% neem leaves).

Lipids fraction of serum

Total cholesterol (TC)

The data in Table 6 demonstrate the total serum cholesterol concentration in experimental rats. KB1O3 intoxication was seen to be associated with an increase in TC. The control (-) group exhibited a reduction of 22.9 percent compared to the control (+) group, with a statistically significant variance between them. All intoxicated rats that

were administered with KBrO3 and given the studied plant diets (G3, G4, and G5) exhibited significant reductions in serum total cholesterol (mg/dl), varying from -7.07 percent to -26.26 percent in comparison with the control (+) group. The maximum reduced limit achieved for G5 was fifteen percent neem leaves.

Triglycerides (TG)

The TG of experimental rats is illustrated in the same table. The control (-) group exhibited a significant distinction in comparison to control (+) group, as evidenced by a -16.1 percent decrease in their results. The serum triglycerides (mg/dl), of the control (+) group were significantly reduced by all experimental diets (G3, G4, and G5), with a range of -4.17 percent to -18.06 percent. Additionally, G5 (fifteen percent neem leaves) recorded a greaterGreater TG reduction than the control group G4 (10% neem leaves) demonstrated the greatest reduced limit in serum TG (mg/dl), with a significant distinction with the other groups.

VLDL-c

Data in Table (6) indicates the VLDLc in serum of experimental mice. It could be observed that VLDLc in serum was considerably elevated by KBrO3 intoxication and reduced by nutritional intervention utilizing experimental diets (G3, G4 and G5) which ranging from -8.33% to -17.99% of control positive group. The greatest reduced limit attained for G5 is (15% neem leaves) with significantly greater variance than the other groups.

Kidney function

Serum creatinine

The serum creatinine of experimental rats is indicated by the outcomes presented in Table (7). The serum creatinine was observed to increase as a result of KBrO3 intoxication. The control (-) group exhibited a significant distinction in comparison to control (+) group, as shown by a -80.1 percent decrease in their data. The serum creatinine (mg/dl), of all rats in the experimental diets (G3, G4, and G5) demonstrated a significant reduction, varying from -70.57 percent to -72.65 percent, compared to control (+) group. The control (-) group didn't exhibit a significant distinction. The lowest serum creatinine level (milligrams per deciliters) was reported for G4 (ten percent neem leaves).

Urea

The influence of feeding the examined plant on serum urea (milligrams per deciliters) is illustrated in Table (7). It has been detected that the control (-) group exhibited a significant variance in concentrations of urea, with a -53.98% reduction in comparison to the control (+) group. The serum urea (milligrams per deciliters) of the control (+) group was significantly reduced by experimental diets (G3, G4, and G5), with the greatest decrease observed in G4 (ten percent neem leaves). The range of the reduction was from -13.64 percent to -34.09 percent.

Uric acid

The results shown in Table (7) illustrate the uric a' level in serum of experimental rats. KBrO3 intoxication raised considerably the serum uric acid (2.4 to 3.6 mg/dl). Due to plants diets intakes, the level decreased appreciably, especially in case of G3 (5% neem leaves) which recorded -68 % decrease in comparison with control (+) group.

Antioxidants enzymes

Serum catalase (CAT) activity (mmol/L)

The data in Table (8) illustrates the influence of experimental diets on the serum concentration of CAT (mmol per liter) in rats that have been intoxicated with KBrO3. It was observed that the control (-) group demonstrated a significant distinction in comparison to control (+) group, with a +53.9% increase. All rats from the tested plants exhibited a substantial rise in serum CAT (mmol per Liter) levels, with a range of +16.79% to +52.67% compared to control (+) group. In comparison with all other diet diets, G5 (15% neem leaves) exhibited the most significant rise in serum CAT concentration (mmol per Liter), with an insignificant variance from the control (-) group.

Serum superoxide dismutase (SOD) activity (mmol/L)

The SOD activity in the serum of experimental rats is demonstrated in Table (8). It is evident that the SOD activity decreased from +75.75 to +25.5 (mmol per Liter) as a result of KBrO3 intoxication. However, the SOD activity was significantly elevated by the administration of experimental diets (G3, G4, and G5), with the G5 (fifteen percent neem leaves) exhibiting a +75.75 % rise in compared to the control (+) group.

Glutathione peroxidase (GPX) activity (ng/ml)

The results in Table (8) illustrate the GPX activity in serum of experimental mice. It is clear that due to KBrO3 intoxication the GPX

activity reduced remarkably from 0.50 to 0.70(ng/ml) with significant difference between them. The experimental diets (G3, G4, and G5) exhibited a significant rise in serum GPX (nanograms per milliliter) concentration, with a range of +24.5% to +76.75% compared to control (+) group. The greatest rise has been observed in the G5 diet, which contained 15% neem leaves.

DISCUSSION

Scientists could develop neem treatments for human use to combat oxidative stress-related conditions, such as liver and kidney diseases. The presence of bioactive compounds like nimbidiol and quercetin may provide antioxidant and anti-inflammatory benefits, potentially offering a natural alternative to synthetic drugs. Further research is needed to explore the efficacy and safety of neem extracts in human trials, paving the way for new therapeutic applications. These results support the findings of [35] they observed that diabetic mice nourished with neem leaf extract showed a sharp increase in FI, FBW and BWG(g) levels than the (+) control group nourished with basal diet. Also[14] stated that the serum activity of ALP AST, & ALT enzymes was significantly elevated in KBrO3-intoxicated rats (positive control group) in compared to that of normal mice. [37]observed that the smallest serum concentration ALT AST, & ALP was documented for diabetic mice nourished on (5% neem leaves) with significant variance (p-value less than 0.001) than (+) control nourished on basal diet. [14] It was stated that the serum activity of ALP AST, & ALT enzymes was significantly elevated in KBrO3-intoxicated rats (positive control group) in comparison to that of normal mice. [38] Illustrated that administration of a hydroalcoholic extract of neem leaves to rats injected with ISO led to a significant elevation in the total protein concentration han rats injected with ISO, which was attributed to the presence of antioxidants. The outcomes attained are in agreement with those of [13] they demonstrated that prior treatment with an alcoholic neem leaf extract significantly reduced serum cholesterol, triglyceride, LDLc and VLDLc concentration and elevated HDLc levels in rats with ISO-induced myocardial infarction. In addition,[12] found that pineapple lowered total cholesterol, triacylglycerol and LDLc in rats and mice. This influence was due to the presence of bromelain, which has a lipolytic and proteolytic effect, and the high fiber content in the raw leaves, which induces a cholesterol-lowering effect. The outcomes of the current work are in line with those of [12] they concluded that diabetic mice fed with different neem leaves had diminished uric a‘ and creatinine concentration (mg/dl) compared to basal diet-fed control rats. [39]Revealed that a methanolic extract of neem leaves may positively regulate catalase activities in response to alcohol-induced oxidative stress. [40]Found that liver antioxidant concentration, including hepatic glutathione (P-value equal 0.003), SOD (P-value less than 0.001), and lipid peroxidation (P-value equal 0.002), were restored following therapy, which supports these findings. [41]Found that the serum activity of glutathione peroxidase catalase enzymes, superoxide dismutase and total antioxidant declined significantly in KBrO3-poisoned mice in ompared with normal rats.

CONCLUSION

Based on the biochemical results and the results of oxidative enzymes, it is clear that the leaves of the neem leaves have a high ability to get rid of free radicals and harmful components present in the body of living animals (rats). This is due to them containing biologically active substances that have the ability to reduce oxidative stress. In the end of present work, we can submit the following recommendation may be submitted people should planteded neem tree in the streets to could be used to combat various metal intoxications like cadmium, arsenic. Neem has been reported to possess some medicinal properties as such, it can be used to improve health and nutrition. More researches should be carried out to show the other benefit of neem tree. Neem leaves It not only has a positive effect in lowering the lipid levels but also alters the levels of the liver enzymes and hence can also improve the liver functions.

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The Science Research Ethics Committee of the Faculty of Home Economics has approved the research protocol #04-SREC-10-2021.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

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Not Applicable.

Author Contributions

Lobna Saad : designed the research; Lobna Saad and Hajir Altoom performed the research; Sultan Mashnafi and Elgaili Abdelrahman Omer: contributed to data & sample collection; Yousif jumma and Billgis Siddig : contributed analytic tools and analyzed the data; Lobna Saad, Sultan Mashnafi , Yousif jumma , Elgaili Abdelrahman Omer and Hajir Altoom : wrote the paper.

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