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Potential Effect of Broccoli to Reduce the Side Effects of Cisplatin Chemotherapy in Adult Rats

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Abstract:

Multidrug resistance and various adverse side effects have long been major problems in cancer chemotherapy. The discovery of bioactive components in foods is exciting, suggesting the possibility of improved public health through diet. Broccoli floret are an excellent source of health-promoting phytochemicals such as vitamins, glucosinolates, and phenolics. The present study aimed to examine the therapeutic effects broccoli powder to reduce the side effects of cisplatin chemotherapy in adult rats. Twenty four adult male albino rats were randomly divided into two main groups; the first main group served as a negative control and the second main group (18 rats) were injected with a single intraperitoneal dose of cisplatin (CP) (10 mg/kg of B.W.). This group were divided into three subgroups, subgroup 1 served as positive control group, subgroup 2 and 3 were fed on the diet containing 2.5% and 5% broccoli powder. After completing the treatment period, animals were sacrificed under diethyl ether anesthesia. Blood samples were collected for the biochemical analysis. Results showed that group rats fed on 5% broccoli floret recorded the highest effective to reduce SGPT, SGOT, ALP, T.P, TC and TG level compared with group rats fed on 2.5% broccoli floret. Also, 5% broccoli improvement all tested oxidative enzyme (SOD, NAD and CAT) was observed. In conclusion, group rats fed on 5% broccoli floret markedly the side effects of cancer patients treated with cisplatin.

Key words: Chemotherapy, Cancer, broccoli, antioxidant status, Biochemical analysis.

INTRODUCTION

Chemotherapy has improved the quality life of cancer patients and provides them hope for healing (**Weijl *et al.*, 2004**). Although, the chemotherapy treatment has some side effects and toxicity (**WHO, 1990**). Cisplatin (CP) is one of the major chemotherapy drug that is widely using for the treatment for number of different types of cancer as testicular, ovarian, bladder, head and neck, and non-small cell lung cancer as treatment, it contains platinum, it is used alone or in combination with other medications to slow or stop cancer cell growth (**Pasari and Tchounwou, 2014**). Cisplatin has caused a decreasing of plasma antioxidant levels in patients which may cause failure of antioxidant defense system against oxidative damage (**Srivastava *et al.*, 2010**). In recent years, increasing attention has been given to fruits and vegetables in the context of healthy diets because they are good sources of nutrients, dietary fiber, minerals, carotenoids, and phenolic compounds (**Severini *et al.*, 2016** and **Soares *et al.*, 2019**). Epidemiological studies have demonstrated that a diet rich in cruciferous vegetables, including broccoli, can reduce the risk of cancer and cardiovascular diseases (**Higdon *et al.*, 2007, Zhang *et al.*, 2011**).

Broccoli is a good source of polyphenolic compounds (**Domínguez *et al.*, 2010**) with high antioxidant activity, and it could play a significant role in the prevention of diseases associated with oxidative stress, such as cardiovascular and neurodegenerative diseases as well as cancer (**Scalbert and Jiménez 2005**). Polyphenols demonstrate multidirectional antioxidant activity. Polyphenols are one of the most numerous and widespread compounds in the plant world (**D'Archivio *et al.*, 2007**). This group includes more than 8000 different compounds, more than half of which are flavonoids. They make a significant contribution to the daily human diet. It is postulated that the ability of polyphenolic compounds to prevent disease is primarily due to their ability to reduce the levels of Reactive Oxygen Species (ROS) in cells (**Akhlaghi and Bandy, 2009**). The antioxidant activity of polyphenols may be direct, resulting from reactions with free radicals and their subsequent removal (polyphenols act as “scavengers” of free radicals) or from chelating of metal ions. It may also be indirect, resulting from the ability of these compounds to modulate the activity of oxidative stress enzymes, increase expression of genes encoding antioxidant enzymes, or exert protective action against other

antioxidants, such as vitamin C and α -tocopherols (**Pandey and Rizvi, 2009**). So the purpose of this study was to investigate the possible therapeutic role of broccoli on biochemical changes as a side effect of using cisplatin in adult rats.

Material and Methods

Materials

Cisplatin (Unistin 50 ml /50 mg Vial) was purchased from Eimc United Pharmaceutical Company, Badr City, Cairo, Egypt. Broccoli (Free from blemishes or obvious defects) were obtained from a super market in Shibein El-Kom, Menoufia Governorate, Egypt. Casein, cellulose, vitamin mixture, mineral mixture, corn oil and corn starch were obtained from Morgahn Co., Cairo, Egypt. Aloxan was purchased from El-Gomhoria Co., Cairo, Egypt. Gentamycin (aminoglycosides antibiotics) obtained from Memphis Co. from Pharm. Chem. Ind., Cairo., Egypt. Twenty four female Sprague- Dawley albino rats $200\pm 5g$ were purchased from Conjunctivitis Eye Institute Giza, Egypt.

Methods

Preparations of broccoli

Broccoli floret was washed and cut to regular slides. After that, broccoli was dried in oven under vacuum $50^{\circ}C$. The broccoli was grinded in an electric mill and passed through 80 screen sieves. The fine powder was kept in plastic package and stored at $-20^{\circ}C$ until used.

Animals

Rats were housed individually in wire cages under the normal laboratory conditions. Rats were fed on standard diet for one week as an adaptation period. Diet was introduced to rats in special food cups to avoid scattering of food. Also, water was provided to rats by glass tubes projecting through the wire cages from an inverted bottle supported to one side of the cage. Food and fresh water were provided checked daily. Standard diet was prepared from fine ingredients according to **Reeves et al., (1993)**. All animals were received care in compliance with the Egyptian rules for animal protection.

Chemical composition

The samples were analyzed for chemical composition (moisture, crude protein, fat, crude fiber, and ash) using the **A.O.A.C., (2012)**. The determination of total phenols was carried out by using Folin-Ciocalteu's described by **Chun et al., (2003)**. Total phenolic compounds were expressed as mg gallic acid equivalents / 100 g dry weight sample.

Experimental groups

Rats fed on basic meal for 7 consecutive days to make adjustment and rats were randomly divided into two main groups, the first group, normal control group (n=6) fed standard diet. All rats in the other group (CP groups, n=18) were injected with a single intraperitoneal dose of (CP) (10 mg/kg of B.W.) which is enough to induce testicular toxicity in rats (**Silva et al., 2001 and Tohamy et al., 2003**). Injured rats were divided into three subgroups (6 rats each) as follow: Subgroup 1 served as positive control group, subgroup 2 served as 2.5% broccoli powder and subgroup 3 served as 5% broccoli powder from the weight of the diet.

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 30 minutes, then centrifuged for 10 minutes at 4000 rpm to separate the serum, which were carefully as pirated and transferred into clean cuvette tube and stored frozen in deep freezer till analysis according to method described by **Schermer (1967)**.

Biochemical analysis

Serum total cholesterol was determined according to the colorimetric method described by **Thomas (1992)**. Serum triglyceride was determined by enzymatic method using kits according to the **Young (1975) and Fossati (1982)**. HDL-c was determined according to the method described by **Friedewaid (1972) and Grodon and Amer (1977)**. VLDL-c was calculated in mg/dl according to **Lee and Nieman (1996)** using the following formula: $VLDL-c \text{ (mg/dl)} = \text{Triglycerides}/5$, LDL-c was calculated in mg/dl according to **Lee and Nieman (1996)** as follows: $LDL-c \text{ (mg/dl)} = \text{Total cholesterol} - \text{HDL-c} - \text{VLDL-c}$, Determination of serum alanine amino transferase (ALT) and serum asparatate amino transferase (AST), were carried out according to the method of **Hafkenschaid (1979) and Clinica Chimica Acta (1980)** respectively. Serum urea and serum creatinine were determined by enzymatic method according to (**Henry (1974) and Patton & Crouch 1977**). SOD activity in the serum was measured by the method described by **Sun et al., (1988)**. Serum samples were used for determination of

malondialdehyde (MDA) as a measure of lipid peroxidation according to **Yoshioka *et al.*, (1979)** and Catalase (CAT) according to **Hu, (1994)**.

Statistical analysis

Results were expressed as the mean \pm SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (**Artimage and Berry, 1987**).

RESULTS AND DISCUSSION

Chemical composition of broccoli florets

Data presented in Table (1) show the chemical composition of fresh broccoli florets as weight wet. It is clear to notice that the percentage of moisture, protein, fat, ash, fiber and carbohydrate which were 90.8%, 2.28%, 0.54%, 0.34%, 1.10% and 4.93%, respectively. While on dry weight broccoli floret had higher significantly ($p \leq 0.05$) in carbohydrate than the fresh broccoli floret which was (53.62) followed by protein, fiber, moisture, fat and ash which were 24.8, 12, 8.65, 5.93 and 3.68, respectively. These results are in agreement with **U.S.D.A (2001)** which concluded that the content of broccoli from moisture was (90), protein (2.57), fat (0.34), ash (0.83) and carbohydrate (3.81). In another study, **Sigmond *et al.*, (2010)** showed carbohydrate, fiber, ash, moisture, protein and fat of broccoli were 53.62, 11.00, 2.68, 2.40, 24.50 and 5.5%, respectively.

Identification of phenolics compounds in broccoli floret

Data tabulated in Table (2) show the identification of phenolic compounds of broccoli. It is clear to mention that the highest phenolic compounds in broccoli floret were gallic acid, pyrogallic acid and chlorogenic acid. The values were 192.43, 186.72 and 117.38 mg/100g DW, respectively. While, the lowest broccoli values were ferulic acid, caffeic acid and vanillin. The values were 9.96, 6.26 and 5.08 mg/100g DW, respectively. These results are in agreement with **Gaafar *et al.*, (2013)** who reported that the values of phenolic compounds were gallic acid (192), pyrogallic acid (186), chlorogenic acid (117), caffeic acid (6.26), ferulic acid (9.96) and vallon (5.08) mg/100g DW, respectively.

Effect of broccoli powder on liver functions of cisplatin tested rats

Data in Table (3) explained the effect of broccoli powder on liver function of cisplatin treated rats. Rats were received cisplatin alone (positive control group) had significantly ($p \leq 0.05$) increased in serum

SGPT, SGOT, ALP and T.P level when compared with negative group (normal rats). These results are in agreement with **John et al., (2017)** who reported that transient elevations of liver enzymes have been reported to be associated with cisplatin administration at the recommended doses. Also, **Mika and Guruvayoorappan, (2003)** observed that administration of cisplatin led to increasing in the (SGOT) level. Group fed on 5% broccoli floret more effective to reduced SGPT, SGOT, ALP and T.P levels compared with group fed on 2.5% broccoli floret. The mean values were 66.8,120,139 and1.9U/L followed by 81,149.9,147and2.9U/L, respectively. These findings are supported by **Robbins et al. (2011) and Yoshida et al. (2015)**, how reported that cruciferous vegetables like broccoli may increase livers natural detoxification enzymes which protect it from damage and improve blood levels of liver enzymes. Also, **Abdalraheem and Salam (2018)**, who observed that broccoli which helps the liver to produce detoxifying enzymes, they also contain sulfur compounds that aid with liver health.

Effect of broccoli powder on kidney functions of tested rats

The obtained results of Table (4) reflected that the effect of broccoli powder on kidney function of cisplatin treated rats. Kidney removes metabolic wastes such as urea, uric acid, and creatinine. The concentrations of the metabolites increase in blood during renal diseases or renal damage may due to high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels (**Anwar and Meki, 2003**). The serum urea and creatinine levels of positive control group had higher than negative control group with significant difference ($p \leq 0.05$). These results are agreement with **Lubomir et al. (2008)** who reported that cisplatin can induce apoptosis and necrosis of kidney cells in a dose-dependent manner. Renal damage is associated with several patterns of histological changes; this damage is clinically manifested as increase in urea, serum creatinine, disturbances in serum electrolytes and acute renal failure. Rats which received 2.5% broccoli had recorded more effective to reduce creatinine, urea and uric acid levels, followed by 5% broccoli group.

Effect of broccoli powder on total cholesterol and triglycerides level of tested rats

Data in Table (5) revealed the effect of broccoli powder on total cholesterol and triglycerides level of cisplatin treated group. Group rats administrated 5% broccoli represented the highest ($p \leq 0.05$)

improvement percentage in serum total cholesterol and triglycerides (75 and 74 mg/dl) compared to positive control group followed by 2.5% broccoli which were improve by (93 and 82 mg/dl), respectively compared to control positive group. These results were agreement with **Bernhard (2016)** who reported that broccoli had cholesterol lowering properties; the fiber in broccoli lowers cholesterol by binding with bile acids in the digestive tract and excreting it out of the body. Likewise **Alexopoulos et al., (1992)** showed that patients who responded favorably to chemotherapy demonstrated a significant increase in serum total cholesterol and LDL cholesterol values, who exhibited a no significant decrease in both of these parameters. Serum triglycerides tended to increase after effective chemotherapy.

Also, **Raghavan et al., (1992)** and **Boes et al., (2015)**, reported that elevated levels of cholesterol, LDL-C and TG have been reported in patients on cisplatin therapy. Elevated levels of total cholesterol reported by **Salem et al. (2013)** who reported that ameliorative effect of cauliflower and broccoli against development of changes in lipid profile and vascular pathology were attributed in partly to lowering the serum level of total lipid, total cholesterol, HDL-C, LDL-C and VLDL-C and decrease the expression of VEGF in smooth muscle cells of blood vessel wall.

Effect of broccoli powder on oxidative stress level of cisplatin tested rats

Data in Table (6) showed the effect of broccoli powder on oxidative stress level of cisplatin treated rats, the data revealed that the mean value of positive control group was significantly lower ($p \leq 0.05$) than the mean value of negative control group, which were 0.019 and 0.023U/L, respectively. The mean values of group 5% broccoli and 2.5% broccoli showed significantly difference ($p \leq 0.05$) when compared with positive control group. The values were 0.002 and 0.003 U/L, respectively. These results were agreeing with result obtained by **Sadzuka et al., (1992)** who reported that increases in lipid peroxide in kidneys of rats treated with cisplatin were examined in relation to decreases in the activities of superoxide dismutase (SOD) in the kidney and the liver were significantly decreased after cisplatin administration. This was the largest decrease among these enzymes in the tissues examined. SOD activity significantly decreased only in the kidney. Likewise **Chiesa, (2017)** mentioned that broccoli florets are naturally

rich in SOD. They are also excellent sources of vitamin C and contain small amounts of essential trace minerals that boost SOD production, including copper, manganese and zinc.

In MDA, negative control group had lower significantly ($p \leq 0.05$) which was (0.11) than cisplatin groups. On the contrary, positive control group had higher significantly ($p \leq 0.05$) which was (0.28) than negative and other cisplatin groups followed by broccoli 5% and broccoli 2.5% which were (0.14 and 0.23 nmol/l) respectively. These results are in agreement with **khoshbin et al. (2015)**, who reported that levels of MDA in patients with breast cancer that had undergone chemotherapy and radiotherapy. The comparison of the mean of MDA levels showed an increasing trend from beginning to end of treatment. Likewise **Dixon et al., (2011)** who showed that blood tests showed that health markers significantly improved in those who took the broccoli sprout powder, including decreases in the oxidative stress index, decreased blood levels of oxidized LDL (bad) cholesterol, and decreased levels of MDA higher MDA indicates more oxidation. Broccoli sprout powder improved total antioxidant capacity as well.

Positive control group had significantly lower ($p \leq 0.05$) in CAT level compared with normal control group and treated group were showed a significantly ($p \leq 0.05$) difference between them, broccoli 5% recorded more effective to increase CAT followed by broccoli 2.5% which were (0.16 and 0.15 ng/ml) respectively. Similar trend was observed for **Noori and Mahboob (2010)** who showed that cisplatin-induced oxidative stress was indicated by increased level of tissue MDA and decreased level of tissue SOD and Catalase, Therefore, including broccoli is rich in polyphenols. Eating plenty of these green leafy vegetables help to produce of catalase which have anticancer effects when fed a diet of these vegetables

Table (1): Proximate chemical composition of broccoli

Constitutes (%)	W/W	D/W
Moisture	90.80	8.65
Protein	2.28	24.80
Fat	0.54	5.90
Ash	0.34	3.68
Fiber	1.10	12.00
Carbohydrates	4.93	53.62

W/W= Wet weight

D/W= Dry weight

Table (2): Identification of phenolic compounds in broccoli

Phenolic compounds	Concentrations (Mg/100g DW)
Pyrogalllic acid	186.72
Gallic acid	192.43
Chlorogenic acid	117.38
Caffeic acid	6.26
Ferulic acid	9.96
Rutin	12.27
P-Coumaric acid	19.19
Vanillin	5.08
Para-hydroxy benzoic	20.09

Table (3): Effect of broccoli powder on liver functions on cisplatin treated rats

Groups Treatments	Negative group (normal rats)	Cisplatin groups		
		Positive control group (Broccoli 0%)	Rats fed on broccoli (2.5%)	Rats fed on broccoli (5%)
SGPT (U/L)	54.60 ^d ±1.7	113.0 ^a ±4	81.0 ^b ±3.7	66.80 ^c ±1.5
SGOT (U/L)	101.0 ^d ±3.9	168.70 ^a ±5.9	149.90 ^b ±3.7	120.0 ^c ±1.8
ALP (U/L)	84.40 ^d ±3.5	284.0 ^a ±4.3	147.40 ^b ±3.2	139.0 ^c ±6.96
T.PROT(U/L)	1.64 ^d ±0.27	3.90 ^a ±0.15	2.90 ^b ±0.16	1.90 ^c ±0.20

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test ($a > b > c > d > e$).

Table (4): Effect of broccoli powder on kidney functions of tested rats

Groups Treatments	Negative group (normal rats)	Cisplatin groups		
		Broccoli 0% Positive group	Rats fed on broccoli (2.5%)	Rats fed on broccoli (5%)
Urea (mg/dl)	70.4 ^d ±2.3	165.2 ^a ±3.9	139 ^b ±4	75.6 ^c ±3.9
Creatinine (mg/dl)	0.59 ^d ±0.055	1.89 ^a ±0.02	1.7 ^b ±0.068	1.12 ^c ±0.016

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test ($a > b > c > d > e$).

Table (5): Effect of broccoli powder on total cholesterol and triglycerides level of tested rats

Groups Treatments	Negative group (normal rats)	Cisplatin groups		
		Broccoli 0% Positive group	Rats fed on broccoli (2.5%)	Rats fed on broccoli (5%)
Cholesterol (mg/dl)	113.30 ^d ±1.9	165.0 ^a ±2.5	155.0 ^b ±2.8	124.0 ^c ±3.7
Triglycerides (mg/dl)	97.0 ^d ±1.6	143.60 ^a ±4.2	118.0 ^b ±3.5	107.70 ^c ±2.7
HDL-c	48.0 ^a ±1.7	16.60 ^d ±2	28.0 ^c ±2.4	38.0 ^b ±3.1
LDL-c	47.10 ^d ±2.8	124.0 ^a ±4.2	99.50 ^b ±3.3	62.0 ^c ±2.8
VLDL-c	19.0 ^d ±0.4	28.80 ^a ±0.9	25.50 ^b ±0.6	21.0 ^c ±0.8

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test ($a > b > c > d > e$).

Table (6): Effect of broccoli powder on oxidative stress level of tested rats

Groups Treatments	Negative group (normal rats)	Cisplatin groups		
		Broccoli 0% Positive group	Rats fed on broccoli (2.5%)	Rats fed on broccoli (5%)
SOD (U/L)	0.95 ^a ±0.023	0.14 ^d ±0.019	0.19 ^c ±0.003	0.23 ^b ±0.002
MDA(nmol/l)	0.11 ^d ±0.003	0.28 ^a ±0.002	0.23 ^b ±0.003	0.14 ^c ±0.002
CAT(ng/ml)	0.26 ^a ±0.005	0.13 ^b ±0.004	0.15 ^b ±0.008	0.16 ^b ±0.003

Data are expressed as mean ± SD. Values within a row having different superscripts are significantly different ($p \leq 0.05$); where the small letters indicate significant among dietary treatment groups as indicated by one-way ANOVA followed by Duncan's multiple range test ($a > b > c > d > e$).

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التأثير المحتمل للبروكلي لخفض التأثيرات الجانبية للسيسبلاتين كعلاج كيماوى فى الفئران البالغة

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الملخص العربى:

دائما كانت مقاومة الادوية المختلفة والاثار الجانبية المتعددة من المشاكل الرئيسية فى العلاج الكيماوى للسرطان. ويعد اكتشاف المركبات الفعالة بيولوجيا فى الاطعمة امرا مفيد للغاية ، مما يشير الى امكانية تحسين الصحة العامة من خلال النظام الغذائى. البروكلى هو مصدر مهم للمواد الكيميائية الفعالة فى النباتات المعززة للصحة مثل الفيتامينات والجلوكوزينات والفينولات. هدفت هذه الدراسة الى فحص الاثار العلاجية لمسحوق زهور البروكلى لتقليل الاثار الجانبية للعلاج الكيماوى للسيسبلاتين فى الفئران البالغة. تم تقسيم أربعة وعشرون من ذكور الفئران البيضاء البالغة عشوائيا الى مجموعتين رئيسيتين. المجموعة الاولى وهى المجموعة السالبة والمجموعة الثانية (18 فأر) تم حقنها بجرعة واحدة داخل الصفاق من سيسبلاتين (cp) بجرعة (10مجم/كجم من وزن الجسم) وتم تقسيم هذه المجموعة الى ثلاث مجموعات فرعية . المجموعة الاولى الايجابية او المجموعة الضابطة، المجموعة الفرعية 2،3 تم تغذيتها على نظام غذائى 2،5% ، 5% مسحوق زهور بروكلى وبعد انتهاء فترة العلاج تم ذبح هذه الفئران تحت تخدير ثنائى ايثيل اثير وتم جمع عينات الدم لاستخدامها فى التحاليل البيوكيميائية وأظهرت نتائج تلك المجموعات ان الفئران التى تم تغذيتها على 5% بروكلى أعلى فاعلية فى خفض مستوى أنزيم TC, TG, SGPT, SGOT, ALP, TP , مقارنة بالمجموعة التى تتغذى على 2،5% من البروكلى ، ونتيجة لذلك ان المجموعات التى تغذت على 5% بروكلى كان لها تأثير على الفئران الذين عولجوا بالسيسبلاتين. ايضا استخدام تركيز 5% من زهور البروكلى أدى إلى تحسين وظائف الأنزيمات المؤكسدة (SOD, NAD, CAT).

الكلمات المفتاحية: العلاج الكيماوى ، السرطان ، مضادات الاكسدة ، التحاليل الكيميائية الحيوية

