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ANTI-PROSTATE AND COLON CANCER AND DIURETIC EFFECTS OF QURANI PLANTS' MIXTURE (A NEW PHARMACEUTICAL PRODUCT)

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ABSTRACT

Objective: QURANI plants' mixture is a new pharmaceutical product composed of some edible and medicinal plants (15 plants) mentioned in the Holy Quran (in a certain percentage, according to that is mentioned in Patent no. 1429/2013, presented to the Academy of Scientific Research and Technology, Egypt in 11/9/2013). The main aim of this work is to determine the cytotoxic effect of this extract against both human prostate (PC3) and colon (HCT116) cancer cell lines and also to evaluate the diuretic effect of this extract.

Methods: *In vitro* evaluation of the ethanolic extract of the QURANI plants' mixture regarding its cytotoxic effect against both human prostate (PC3) and colon (HCT116) cancer cell lines was carried out. *In vivo* studies of the diuretic effect of daily feeding rats with 2, 4 and 8 g/kg of the QURANI plants' mixture was performed also.

Results: *In vitro* studies of the ethanolic extract of the QURANI plants' mixture revealed the cytotoxic effect of this extract against both human prostate (PC3) and colon (HCT116) cancer cell lines. Inhibition caused by using 100 µl/ml (this volume is related to 25 µg d. w. of QURANI plants' mixture) of this extract reached to 36.300±0.085 and 76.300±0.045 against HCT116 and PC3 cell lines, respectively. IC₅₀ of this extract against PC3 cell line equals 44.60 µl/ml (this volume is related to 11.15 µg d. w. of QURANI plants' mixture), but it can not be detected in case of HCT116 cell line using these investigated concentrations of this extract. *In vivo* studies of the diuretic effect of daily feeding rats with 2, 4 and 8 g/kg of the QURANI plants' mixture showed that, the amount of urine increased by increasing the amount of this mixture when compared to the control group. Results of kidney functions and urine analyses revealed that, this mixture has not any bad side effects in this regard, moreover, rats feeding with the QURANI plants' mixture have better kidney functions (urea and creatinine are lower in case of rats feeding with the QURANI plants' mixture than those of control group). Rats fed with this mixture are also having normal urine physical and chemical characters compared to the control group.

Conclusion: Results of anticancer and diuretic effects of the QURANI plants' mixture will lead us to more biological and chemical investigations of this new, cheap and safe pharmaceutical natural product.

Keywords: Cytotoxicity, Colon cancer, Prostate cancer, Edible and medicinal plants, QURANI plants' mixture, Diuretic activity, Urine analyses, Kidney functions.

INTRODUCTION

The presented work is a part of the submitted patent no. 1429/2013 (A new Pharmaceutical product from Plants mentioned in the Holy Quran), presented to the Academy of Scientific Research and Technology, Egypt in 11/9/2013. This patent is aimed at the production of a new, cheap and safe pharmaceutical product, this product is composed of QURANI plants' mixture (15 plants) in different percentages, this mixture is valuable against many dangerous diseases (with no toxicity and too little side effects), results will be published in a series of successive papers [1-2].

These 15 plants, those used to prepare this new mixture were cited in the holy Quran as follows: Sûrat Al-Baqarah (The Cow): (61, 266); Sûrat Al-An'âm (The Cattle): (99, 141); Sûrat Ar-Ra'd (The Thunder): (4); Sûrat An-Nahl: (11); Sûrat Al-Kahf (The Cave): (32); Sûrat Maryam (Mary): (23-26); Sûrat Al-Anbiyâ (The Prophets): (47); Sûrat Al-Mu'minûn (The Believers): (18-20); Sûrat An-Nûr (The Light): (35); Sûrat Ash-Shu'arâ (The Poets): (146-148); Sûrat Luqmân: (16); Sûrat Saba' (Sheba): (16); Sûrat Yâ-Sîn: (33-35, 57); Sûrat As-Sâffât (Those Ranged in Ranks): (146); Sûrat Sâd: (51); Sûrat Az-Zukhruf (The Gold Adornments): (73); Sûrat Qâf: (10); Sûrat At-Tûr (The Mount): (22); Sûrat Ar-Rahmân (The Most Gracious): (10-13, 37, 52, 68); Sûrat Al-Wâqi'ah (The Event): (20, 28-29, 32, 89); Sûrat Al-Insân or Ad-Dhr (Man or Time): (17); Sûrat Al-Mursalât (Those Sent Forth): (42); Sûrat An-Naba' (The Great News): (32); Sûrat 'Abasa (He Frowned): (27-31); Sûrat At-Tîn (The Fig): (1-2) (Reference number. 1).

The following is a simple introduction of some important plants contained in this mixture regarding their medicinal importance and their chemical composition.

Ginger has been used extensively in folk medicine to treat common ailments. Ginger has a number of chemical constituents like [6]-Gingerol, [6]-Shagol, Methyl [6] - isogingerol, Paradol which are responsible to provide different pharmacological actions. Now scientific evidences in support of some of these beneficial properties are budding which would shore up their conservation. The ginger bears an enormous number of pharmacological activities such as: cardio protective, anti-inflammatory, anti-microbial, antioxidant, anti-proliferative, neuro-protective and hepatoprotective properties which have been proved. Among those, neuro-protective activity as well as the effect of ginger against colon cancer has facilitated the extent of the further research with a positive outcome. Since there is no good medicine till now for the treatment of these diseases, so researchers may do a lot of research on ginger for finding out less toxic and more potent drugs for the better treatment of these dangerous diseases [3].

Garlic (*Allium sativum*) qualifies as a great vegetable because not only is it an indispensable cooking ingredient, it can also be delightfully eaten. The *Allium* genus belongs to the Liliaceae family comprising onions, leeks, shallots, asparagus etc. Garlic is primarily used as an herb to enhance many food dishes in various cultures. It contains many substances which studies have shown to act together to prevent various diseases such as hypertension, cancer and it has been shown to reduce plasma concentration of cholesterol and low-density lipoprotein in the blood and age-related conditions. Initial reports of the antimicrobial activity of garlic showed that, Allicin (allyl-2-propene thiosulfinate); a notable flavonoid in garlic, is formed when garlic cloves are crushed. Garlic also contains some sulphur-containing compounds such as alliin, ajoene, diallylsulphide, dithin, Sallylcysteine, enzymes as well as some non sulphur

containing compounds including vitamin B, proteins, minerals, saponins and flavonoids [4-5]. Many researches have reported a phytoalexin called allixin in garlic [6-7].

Vitis vinifera L. Contains many chemical constituents such as: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, sugars, sterols, amino acids, and minerals. *Vitis vinifera* L. is considered as a natural antioxidant source. Grapes are also used as demulcent, laxative, refrigerant, stomachic, diuretic and cooling. Moreover, it is useful in bilious dyspepsia, haemorrhage, dysuria, in chronic bronchitis, heart diseases and gout. Grape juice is given to children to prevent constipation. Dried grapes or raisins are useful in thirst attendant on fevers, cough, catarrh, jaundice, and in sub-acute cases of enlarged liver and spleen [8].

A date palm (*Phoenix dactylifera* L.) fruit is an important component of the diet in most of the hot arid and semi arid regions of the world. Date palm fruits were found to contain carbohydrates (44-88%), fats (0.2-0.4%), proteins (2.3-5.6%), fibers (6.4-11.5%), minerals and vitamins [9]. Carbohydrates in dates are mostly in the form of fructose and glucose, which are easily absorbed by the human body [10]. Interestingly, dates contain higher concentrations of proteins when compared to other cultivars of fruits such as apples, oranges, bananas and grapes (containing 0.3, 0.7 and 1.0% proteins, respectively) [11]. Twenty-three different amino acids were found in date's proteins. Many of which are not found in the most popular fruits [9]. Several studies in the literature concluded that, the aqueous extracts of dates have potent antioxidant and antimutagenic activity [12-13]. *Phoenix dactylifera* L. was reported to have the second highest antioxidant activity among 28 fruits commonly consumed in China [14]. Antioxidants have received increased attention by nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative diseases such as cancer, cardiovascular diseases and aging [15-16]. The most effective antioxidants in this respect appear to be the flavonoids and phenolics. Because of their metal-chelating and radical-scavenging properties, phenolics were considered effective inhibitors of lipid peroxidation [12]. Dates were found to be a high source of antioxidants, anthocyanins, carotenoids and phenolics [17].

Banana is the common name for the fruit of the herbaceous plant of the family *Musaceae* and genus *Musa*. They are native to the tropical region of Southeast Asia. Bananas are mostly cultivated for the fruit and also for fiber and ornamental use. About 170 countries produce bananas in the world. In the developing world banana are considered as staple food [18]. According to the Food and Agriculture Organization of the United Nations (FAO), banana is the main fruit in international trade and one of the most popular fruits in the world. This fruit industry is an important source of income, employment and export earnings for developing countries, in Latin America, Asia and Africa, and is responsible for creating many jobs, both in agricultural and urban areas [19]. It provides instant energy, rich source of fiber, minerals and vitamins [18]. Banana fruit is rich in nutrients with good flavor, is widely consumed throughout the world [20]. It has about 200 volatile components which include phyto-nutrients, sterols and fatty acids. Traditionally it is considered good for any gastric irritations, ulcers, constipation. Its starch provides a protective layer in the stomach while its non-digestible fiber is good for cholesterol lowering and constipation. Native Africans use inner part of banana peel for insect bites and skin problems. In Ayurveda they suggest eating of banana peel for diabetics as it is a good source of potassium, but it does not contain sugars which are more in the edible portion. Ancient uses and recent studies have shown that bananas have good antioxidant properties and correcting electrolyte imbalance [18].

MATERIAL AND METHODS

Plant material

Fifteen edible and medicinal plants mentioned in the Holy Quran were purchased from the Egyptian market, these plants were washed carefully with distilled water and surface sterilized by 70 % ethanol for 20-30 seconds, then they cut to small pieces, dried at room temperature (25°C) till complete dryness, then these plants

were grinding to give fine powder, then mixed in a certain percentage [1-2].

Cytotoxic effect of ethanolic extract of the Qurani plants' mixture (a new pharmaceutical product) against PC3 and HCT116 human cancer cell lines (Measurement of Potential cytotoxicity by SRB assay)

Potential cytotoxicity of the ethanolic extract of the Qurani plants' mixture (1:10 w/v, this extract was dried using rotary evaporator, then dissolved in sterile dist. water, the final examined volume was belonging to 250 mg d. w. mixture /ml dist. Water) was tested using the modified method of SKEhan *et al.*, 1990 (21) as follows:

- Cells (PC3, HCT116) were plated in 96-multiwell plate (10⁴cells/well) for 24 hours before the treatment with the extract to allow the attachment of cells to the wall of the plate.
- Different concentrations of the tested extract (0.00, 12.50, 25.00, 50.00 and 100.00 µl/ml) were added to the cells monolayer, 6 replicates wells were prepared for each individual dose.
- Monolayer cells were incubated with the extract for 48 hours at 37°C and in atmosphere of 5% CO₂.
- After 48 hours, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain.
- Excess stain was washed with acetic acid and the attached stain was recovered with Tris EDTA buffer.
- Color intensity was measured in an ELISA reader.
- The relation between surviving fraction and drug conc. is plotted to get the survival curve of each tumor cell line after the specified compound.
- IC₅₀ of this extract against both PC3, HCT116 cell lines were calculated using these survival curves.

Animals and diet

Thirty adult female albino rats weighing 125-140 g were obtained from the Animal House of the National Research Centre, Dokki, Giza, Egypt. Animals were divided into five groups, each group consisted of six animals, rats were held (during 26-30 August 2014) in the metabolic cages (at the normal environment in the Animal House of the National Research Centre) and fasted for 18 hours. Then all groups were allowed for water and fed with their normal basal diet (containing 23 % protein). Diet was purchased from Milado Company, Egypt.

Diuretic effect of the Qurani plants' mixture

The previously mentioned five groups of rats are divided as follows: 1- The first group was left as negative control receiving 1 ml saline and fed with their normal basal diet. 2- Three other groups were daily feeding with 2, 4 and 8 g/kg of the Qurani plants' mixture, respectively, in addition to their normal basal diet. 3- The last group was injected intramuscularly with the standard diuretic agent (Lafurex, Furosemide Ampules; 40 mg/kg/day). Volume of urine was estimated for all these five groups after 1, 2, 3 and 4 days of administration in addition to urine analyses and kidney functions' estimation were carried out for all these five groups at the end of the 4th day of the study (8). All animal treatments were conducted according to the Ethics Committee of the National Research Center and in accordance with the recommendations for the proper care and use of laboratory animals (NIH Publication No. 85-23, revised 1985) in accordance with international ethical considerations.

Statistical analysis

Results were expressed as mean ± SD, they were analyzed by one way ANOVA. The differences between means were tested at P < 0.05 by least significant test (LSD). In all statistical tests, the probability level (P < 0.05) was considered significant. Spearman correlation coefficient was used to determine the relationship between different variables. All analysis was made by SPSS version 16.0 for windows

(Statistical package for Social Science, Chicago, USA). Replicate numbers in these experiments are 6 replicates.

RESULTS AND DISCUSSION

Cytotoxic effect of the ethanolic extract of the Qurani plants' mixture (a new pharmaceutical product) against PC3 and HCT116 cell lines:

In vitro studies of ethanolic extract of the QURANI plants' mixture revealed the cytotoxic effect of this extract against both

human prostate (PC3) and colon (HCT116) cancer cell lines. Inhibition caused by using 100 µl/ml (this volume is related to 25 µg d. w. of the QURANI plants' mixture) of this extract reached to 36.300±0.085 and 76.300±0.045 against HCT116 and PC3 cell lines, respectively.

IC₅₀ of this extract against PC3 cell line equals 44.60 µl/ml (this volume is related to 11.15 µg d. w. of QURANI plants' mixture), but it can not be detected in case of HCT116 cell line using these investigated concentrations of this extract (Table. 1 and Figures: 1-2).

Table 1: Cytotoxicity of the ethanolic extract of the Qurani plants' mixture (a new pharmaceutical product) against PC3 and HCT116 cell lines (n=6)

Concentrations µL/ml	Surviving rate (Mean±SD)		% of inhibition (Mean±SD)	
	PC3	HCT116	PC3	HCT116
0.000	1.000±0.043	1.000±0.072	0.000± 0.043	0.000±0.072
12.500	0.919±0.078	0.974±0.079	8.100±0.078	3.000±0.079
25.000	0.711±0.069	0.877±0.036	28.900±0.069	12.300±0.036
50.000	0.443±0.065	0.834±0.091	55.700±0.065	16.600±0.091
100.000	0.237±0.045	0.637±0.085	76.300±0.045	36.300±0.085

DRUG CYTOTOXICITY

CONC: uL/ml	PC3		
	Mean	SD	N
0.000	1.000	0.043	6
12.500	0.919	0.078	6
25.000	0.711	0.069	6
50.000	0.443	0.065	6
100.000	0.237	0.045	6

PC3

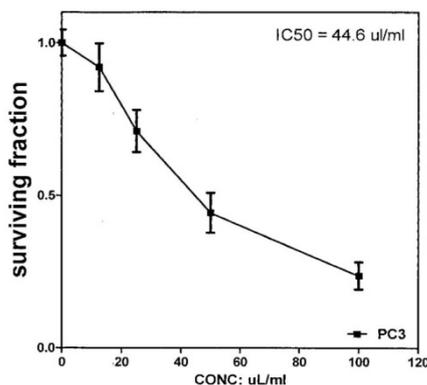


Fig. 1: IC₅₀ of PC₃ by the ethanolic extract of the Qurani plants' mixture (a new pharmaceutical product)

DRUG CYTOTOXICITY

CONC: uL/ml	HCT-		
	Mean	SD	N
0.000	1.000	0.072	6
12.500	0.974	0.079	6
25.000	0.877	0.036	6
50.000	0.834	0.091	6
100.000	0.637	0.085	6

HCT116

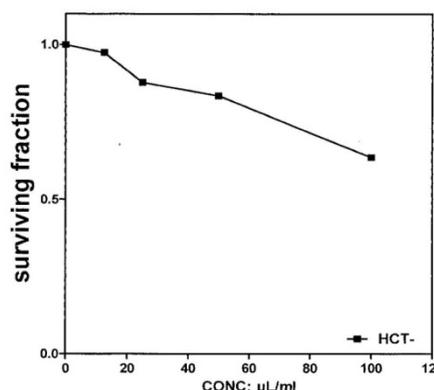


Fig. 2: IC₅₀ of HCT116 by the ethanolic extract of the Qurani plants' mixture (a new pharmaceutical product)

Diuretic effect of the Qurani plants' mixture

In vivo studies of the diuretic effect of daily feeding rats with 2, 4 and 8 g/kg of the QURANI plants' mixture showed that, the amount of urine increased by increasing the amount of this mixture when compared to the control group. It was observed that, the average daily increase in the volume of urine of the rat (compared to the control group) reached to 1.85 ml/day after feeding with 8 g/kg of the Qurani plants' mixture.

Results of kidney functions and urine analyses revealed that, this mixture has not any bad side effects in this regard, moreover rats feeding with the QURANI plants' mixture have better kidney functions (urea and creatinine are lower in case of rats those daily feeding with 2, 4 and 8 g/kg of the QURANI plants' mixture than those of control group). Rats fed with this mixture are also having normal urine physical and chemical characters compared to the control group (Tables: 2-4).

Table 2: Amount of urine during 4 days (1, 2, 3 = 2, 4 and 8 g/kg of the Qurani plants' mixture, respectively, 4= Control and 5 = Positive control (Lafurex: Furosemide Ampules, 40 mg, 40 mg/kg/day), n= 6 rats

Treatments	Days				Average volume/day	Average daily volume/rat
	1	2	3	4		
1	7.50	70.00	20.0	25.00	30.63	6.13
2	35.00	45.00	70.00	45.00	49.00	9.80
3	60.00	70.00	57.00	40.00	56.75	11.35
4	35.00	45.00	50.00	60.00	47.50	9.500
5	10.00	17.00	15.00	25.00	16.75	3.35

Table 3 (a, b, c and d): Urine analyses during 4 days (1, 2, 3 = 2, 4 and 8 g/kg of the Qurani plants' mixture, respectively, 4= Control and 5 = Positive control (Lafurex: Furosemide Ampules, 40 mg, 40 mg/kg/day)**a. The 1st day**

N°	Color	SP. GR.	pH	Sugar	Acetone	Albumin	Bile Pig.	Blood	Pus	Crystals
1	Yellow	1000	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	Yellow	1000	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3	Yellow	1025	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4	Yellow	1000	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5	Yellow	1000	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil

b. The 2nd day

N°	Color	SP. GR.	pH	Sugar	Acetone	Albumin	Bile Pig.	Blood	Pus	Crystals
1	Yellow	1000	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	Yellow	1000	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3	Yellow	1025	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4	Yellow	1000	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5	Yellow	1000	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil

c. The 3rd day

N°	Color	SP. GR.	pH	Sugar	Acetone	Albumin	Bile Pig.	Blood	Pus	Crystals
1	Yellow	1005	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	Yellow	1015	7	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3	Yellow	1015	7	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4	Yellow	1010	8	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5	Yellow	1005	9	Nil	Nil	Nil	Nil	Nil	Nil	Nil

d. The 4th day:

N°	Color	SP. GR.	pH	Sugar	Acetone	Albumin	Bile Pig.	Blood	Pus	Crystals
1	Yellow	1015	7	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	Yellow	1015	7	Nil	Nil	Nil	Nil	Nil	Nil	Nil
3	Yellow	1025	7	Nil	Nil	Nil	Nil	Nil	Nil	Nil
4	Yellow	1005	9	Nil	Nil	Nil	Nil	Nil	Nil	Nil
5	Yellow	1005	9	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 4: Kidney functions analyses after 4 days (1, 2, 3 = 2, 4 and 8 g/kg of the Qurani plants mixture, respectively, 4= Control and 5 = Positive control (Lafurex: Furosemide Ampules, 40 mg, 40 mg/kg/day), n= 6 rats.

Test	1	2	3	4	5
Urea	37.50±3.30	43.00±2.00	44.20±2.15	44.20±2.50	59.00±4.10
Creatinine	0.96±0.07	1.05±0.10	1.08±0.08	1.22±0.05	1.13±0.09

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CONFLICT OF INTERESTS

Declared None

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