Pineapple And Kumquat Powders Used To Combat The Oxidation Stress Induced By Potassium Bromate In Male Albino Rats.

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Abstract

Oxidative stress plays an important role in the etiology and pathogenesis of many chronic diseases. Dietary intake of antioxidants can inhibit or delay the oxidation of susceptible cellular substrates so prevent oxidative stress. The present study was designed to investigate potential protective and ameliorate effects of pineapple and kumquat powders against potassium bromate (KBrO3)-induced oxidative stress using experimental rats. Whole kumquat (seedless), kumquat seeds, pineapple flesh and pineapple leaves were analyzed for phenolic compounds. Measurement of potential of cytotoxicity against the liver carcinoma cell line (HEPG2) carried out by SRB assay of the ethanolic extract of whole kumquat seedless, kumquat seeds, whole kumquat, pineapple flesh, pineapple leaves, pineapple (flesh +leaves) and mixture of them.

Key words: Potassium bromate (KBrO3) – Oxidative stress – Pineapple – Kumquat- Liver enzymes –Antioxidant enzymes- Cancer cells - HEPG2

Introduction:

All living cells try to preserve a normal reduced environment. When this state is missing due to the production of reactive oxygen species (ROS) like free radicals, there is significant damage to the cell components like lipids, proteins and DNA. These cells are under oxidative stress **(Patten *et al.*, 2010)**.

Oxidative stress is a condition that reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to facilely detoxify (antioxidant defenses) the reactive intermediates or to repair the resulting damage **(Hampl *et al.,* 2012)**. Oxidative stress is a hallmark of numeral diseases including heart failure, Alzheimer's disease, sickle cell disease, parkinson's disease, myocardial infarction, diabetes, schizophrenia, fragile X syndrome, cardiovascular disease, cancer and chronic fatigue syndrome **(Krajcovicova-Kudlackova *et al.*, 2012)**.

Potassium bromate ($KBrO_3$) is an oxidizing agent that has been used as a food additive, mainly in the bread-making process. $KBrO_3$ increased production of reactive oxygen species and free radicals **(Ahmad and Mahmoud, 2012).** Many toxicological studies have suggested that $KBrO_3$ caused hepatotoxicity, nephrotoxicity, thyroid toxicity, neurotoxicity and carcinogenicity in experimental animals **(Omer *et al.*, 2012)**. Supporting the involvement of ROS in its action, several antioxidants (AO) have been shown to ameliorate the bromate-induced multiple organ toxicity **(Khan *et al.,* 2013).**

Dietary intake of antioxidants is vital to save cellular system from oxidative stress which present a risk factor for several chronic diseases **(Erukainure *et al.*, 2012)**.

Kumquat (*Citrus japonica*) is the smallest fruit of true citrus fruit. The plant family is *Rutaceae*. The flesh is sour; it is eaten together with the peel. **(Wang *et al.*, 2012)**. The oils from different parts of *C. japonica* have antioxidant activity **(Nouri and Shafaghatlonbar,2016).** Kumquat are an excellent source of natural antioxidants such as ascorbic acid, flavonoids, carotenoids and essential oils, which reduce the harmful effects of free radicals **(Aamer and El-Kholy, 2017).**

Pineapple (*Ananas comosus L.*) belongs to Family *Bromeliaceae*. It is a tropical to subtropical fruit. Pineapple contains several active phytochemicals such as chlorogenic acid, ananasate, beta-sitosterol, rutin, naringenin, campesterol, bromelain, vitamin A, B and C, glycosides and flavonoids. Pineapple is alexipharmic, antitussive, and antidiarrhea agents. It also known to possess anti-fertility and abortifacient activities, hepato-protective, and anti-depressant. **(Xie *et al*.,2007). Dougnon *et al*., (2009)** suggested that pineapple extract decreases hepatotoxicity of paracetamol in Wistar rats. **Dutta & Bhattacharyya (2013)** reported that pineapple leaves are used in traditional medicine for treatment numeral diseases and disorders. Pineapple leaves enriched with phenols, which have anti-hypolipidemic and antidiabetic effect.

The previous studies showed that different plant parts could be used successfully in treatment/prevention of different diseases. Thereupon, the experiment was conducted to study the effect of whole kumquat seedless, kumquat seeds, whole kumquat, pineapple flesh, pineapple leaves, pineapple (flesh +leaves) and mix from all former to combat the oxidation stress induced by potassium bromate in male albino rats. Measurement of potential of cytotoxicity against the liver carcinoma cell line (HEPG2) by SRB assay of the ethanolic extract of used plants was also in the scope of this work.

Material and Methods

Materials:

Plants: The tested plant in this investigation were kumquat *(Citrus japonica)* and pineapple (*Ananas comosus*) with its leaves, these plants were purchased from local market in Menoufia, Egypt.

Chemicals: Potassium bromate ($KBrO_3$) in the form of a white powder was purchased from El-Gomhoria Company for Drugs and Medical Equipments, Cairo, Egypt.

Diets: Diet consists of casein, sucrose, corn oil, choline chloride, vitamins mixture, mineral mixture, cellulose, and corn starch were purchased from El-Gomhoria Company for Drugs and Medical Equipments, Cairo, Egypt.

Experimental design:

Fifty-four (54) adult male albino rats were fed on basal diet for 7 days for acclimatization. Then, rats were randomly distributed into 9 equal groups, 6 rats each. Group 1(healthy rats) was fed on the basal diet and set as a negative control group (normal rats). The other 8 groups were injected by a single intraperitoneal dose of potassium bromate at dose of 125 mg/kg body weight for induction of oxidative stress according to the described method by **Khan and Sultana (2004).** All groups were fed for 4 weeks according to the following groups:

- **Group (1):** (Control " – ") rats (n=6) were fed on basal diet only.
- **Group (2):** (Control " + ") rats (n=6) were kept without any treatment and fed on basal diet after single intraperitoneal injection with $KBrO_3$ (125 mg/kg B.Wt).
- **Group (3):** (Whole kumquat seedless 7.5%) rats (n=6) were fed on basal diet containing 7.5% whole kumquat seedless after single intraperitoneal injection with $KBrO_3$ (125 mg/kg B.Wt).
- **Group (4):** (Kumquat seeds 7.5%) rats (n=6) were fed on basal diet containing 7.5% kumquat seeds after single intraperitoneal injection with $KBrO_3$ (125 mg/kg B.Wt).
- **Group (5):** (Whole kumquat 7.5%) rats (n=6) were fed on basal diet containing 7.5% whole kumquat after single intraperitoneal injection with $KBrO_3$ (125 mg/kg B.Wt).
- **Group (6):** (Pineapple flesh 7.5%) rats (n=6) were fed on basal diet containing 7.5% pineapple flesh after single intraperitoneal injection with $KBrO_3$ (125 mg/kg B.Wt).
- **Group (7):** (Pineapple leaves 7.5%) rats (n=6) were fed on basal diet containing 7.5% pineapple leaves after single intraperitoneal injection with $KBrO_3$ (125 mg/kg B.Wt).

- **Group (8):** (Pineapple (flesh +leaves) 7.5%) rats (n=6) were fed on basal diet containing 7.5% pineapple (flesh +leaves) after single intraperitoneal injection with $KBrO_3$ (125 mg/kg B.Wt).
- **Group (9):** (Mix of all 7.5%) rats (n=6) were fed on basal diet containing 7.5% mix of all after single intraperitoneal injection with $KBrO_3$ (125 mg/kg B.Wt).

Biological Evaluation:

During the experimental period (28days), the consumed diet was daily recorded (feed intake), biological evaluation of the different diets was carried out by determination of body weight gain (BWG) and feed efficiency ratio (FER) according to **Chapman *et al.*, (1959).**

Blood Sampling:

At the end of the experiment, rats were fasted overnight and anesthetized with diethyl ether. Blood samples were collected in clean dry centrifuge tubes from hepatic portal vein; serum obtained by centrifugation was carefully aspirated, transferred into clean cuvette tubes and stored frozen at -20°C for analysis **(Malhotra, 2003).**

Serum samples were analyzed for determination the following Parameters:

Serum glutamate oxaloacetate transaminase S.GOT or (AST) was determined as Unit/L according to **Yound (1975),** S.GPT or (ALT) was determined as Unit/L according to **Yound (1975),** serum alkaline phosphatase (ALP) was determined U/L according to **(Tietz *et al.*, 1983),** total cholesterol was determined according to **Allain (1974),** enzymatic colorimetric determination of triglycerides was carried out according to **Fossati *et al*., (1980)**, determination of HDL was carried out according to the method of **Lopez (1977),** determination of LDL and VLDL was carried out according to the method of **Lee and Nieman (1996)**, atherognic index (AI) was calculated as the VLDL + LDL cholesterol / HDL ratio according to the formula of **Nakabayashi *et al*., (1995),** urea determination was according to the enzymatic method of **Malhotra (2003),** uric acid was determined according to the method described by **(Fossati *et al.,* 1980),** creatinine was determined according to the method described by **Bohmer (1971).**

Statistical Analysis:

The data were statistically analyzed using a computerized program by one way ANOVA .The results are presented as mean ± SD. Differences between treatments at $p \leq 0.05$were considered significant.

HPLC Identification of phenolic compounds:

Phenolic compounds fractions were extracted according to the method outlined by **(Ben-Hammouda *et al.,* 1995)**. Identification of individual phenolic compounds of the plant samples was performed on a Hewlett-Packard HPLC (Model 1100), using a hypersil C18 reversed-phase column (250 × 4.6 mm) with 5 mm particle size.

The sulphorhodamine (SRB) assay:

Measurement of potential cytotoxicity activity against the liver carcinoma cell line (HEPG2) by SRB assay of the ethanolic extracts of whole kumquat seedless, kumquat seeds, whole kumquat, pineapple flesh, pineapple leaves, pineapple (flesh +leaves) and mixture of them were tested using the method of **Skehane *et al.,* (1990)**.

Results and discussion:

A-Determination of phenolic compounds:

The obtained results in table (1) show the phenolic compounds of whole kumquat seedless, kumquat seeds, whole kumquat, pineapple flesh& pineapple leaves. The total number of 20 different phenolic compounds were assessed. Total phenolic compounds were highest (12258.84 ppm) in whole kumquat seedless, followed by kumquat seeds (4192.64 ppm), pineapple flesh (1927.3ppm) and then came the pineapple leaves (1807.96 ppm). The major phenolic compound for Whole kumquat seedless was myricetin (3322.27892 ppm), for kumquat seeds was rutin (2068.89853 ppm), for pineapple flesh was Myricetin (816.19165 ppm) &for pineapple leaves was myricetin (141.14020 ppm). **Sadek *et al.*, (2009)** showed that *F. margarita* peels may be regarded as a rich source of potentially bioactive polyphenols.These results agree with **Hossain & Rahman (2011)** who reported the pineapple fruit being rich in phenolics may provide a good source of antioxidant.

Table (1): The phenolic compounds (ppm) of whole kumquat seedless, kumquat seeds, pineapple flesh and pineapple leaves

N.	Test items	Whole kumquat seedless (ppm)	Kumquat seeds (ppm)	Pineapple flesh (ppm)	Pineapple leaves (ppm)
1	Gallic acid	371.27241	-	-	-
2	Catechol	-	-	1.44123	-
3	p-Hydroxy benzoic acid	79.1974	71.61801	1.72468	-
4	Caffeine	-	-	-	75.05245
5	Chlorgenic	-	-	-	38.39064
6	Vanillic acid	-	11.71693	-	42.56563
7	Caffeic acid	6.39881	8.84917	4.96949	21.05346
8	Syringic acid	8.32771	9.55383	7.98997	6.14724
9	Vanillin	-	2.06057	6.33506	13.50020
10	p-Coumaric acid	18.13794	5.61544	10.95876	64.12535
11	Ferulic acid	-	9.17528	1.46101	19.32588
12	Benzoic acid	792.31154	72.07983	-	-
13	Rutin	1.10476	2068.89853	363.81040	81.11621
14	Salicylic acid	2 841.66991	153.43038	4.92625	-
15	Cinnamic acid	-	17.87585	-	-
16	Myricetin	3322.27892	911.94184	816.19165	141.14020
17	Quercitin	-	-	39.87622	43.57509
18	Rosemarinic	845.88519	221.69447	-	3.69877
19	Neringein	2542.76253	548.93825	-	-
20	Kampherol	637.18118	-	-	-
Total		12258.84	4192.64	1927.3	1807.96

B- Biological changes:

Results of body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of experimental rats are presented in table (2). BWG, FI and FER of $KBrO_3$-intoxicated rats (positive control group) were decreased significantly, compared with those of the normal rats. On the other hand, all tested plants increased BWG except G7 leaves 7.5%& G8 (flesh +leaves) 7.5% decreased BWG because of high fiber content Best treatment for BWG recorded for G5 (whole kumquat 7.5%). All tested plants have significant increase FI compared to control (+). Best treatment for BWG, FI & FER recorded for G5 (whole kumquat 7.5%) which recorded 0.57 g/d compared to control (+) group. FER was highest in case of G5 (whole kumquat 7.5%)These results agree with **Tan *et al*., (2014)** who reported that kumquat (*Fortunella margarita Swingle*) fruit extract (FME) modified body weight gain to similarity with normal control group & **Rezq (2017)** who reported that FI, FBW and BWG of $KBrO_3$-intoxicated rats (positive control group) were decreased significantly, compared with those of the normal rats.

Table (2): Body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of normal rats and intoxicated rats with $KBrO_3$ (n=6 rats/groups)

Groups \ Parameter		BWG(g/d) Mean ± SD	FI(g) Mean ± SD	FER(g/day) Mean ± SD
Control	**G1 (– ve)**	$1^{a} \pm 0.02$	$34.02^{a} \pm 0.13$	$.029^{a} \pm 6.5\times10^{-4}$
	G2 (+ ve)	$.22^{e} \pm 0.01$	$23.66^{h} \pm 0.05$	$.009^{c} \pm 4\times10^{-4}$
Kumquat	**G3** Whole seedless 7.5%	$.36^{c} \pm 0.02$	$33.33^{c} \pm 0.04$	$.011^{c} \pm 0.001$
	G4 Seeds 7.5%	$.32^{cd} \pm 0.01$	$33^{ef} \pm 0.17$	$.01^{c} \pm 3\times10^{-4}$
	G5 Whole kumquat 7.5%	$.57^{b} \pm 0.02$	$33.59^{b} \pm 0.01$	$.017^{b} \pm 1\times10^{-3}$
Pineapple	**G6** Flesh 7.5%	$.33^{cd} \pm 0.04$	$32.87^{f} \pm 0.02$	$.006^{d} \pm 0.0015$
	G7 Leaves 7.5%	$.07^{f} \pm 0.03$	$33.13^{de} \pm 0.02$	$.001^{f} \pm 2\times10^{-4}$
	G8 (Flesh +leaves) 7.5%	$.18^{e} \pm 0.05$	$29.42^{g} \pm 0.07$	$.002^{e} \pm 1\times10^{-4}$
Mix of all	**G9** (Whole kumquat+ pineapple flesh + pineapple leaves) 7.5%	$.29^{d} \pm 0.01$	$33.24^{cd} \pm 0.01$	$.009^{c} \pm 3\times10^{-4}$
	LSD	0.0502	0.1351	0.00158

Values with different letters indicate significant differences Between the groups (P<0.05), and vice versa. LSD: least significant Differences (P<0.05).

C- Relative organs weights:

Data presented in table (3), show the relative organ weight (liver, heart, kidneys, spleen and lungs). These results denoted that there was a significant increase in relative liver, kidneys, spleen, heart and lungs weights of

$KBrO_3$-intoxicated rats control (+) group compared to normal rats control (-) group. All plants diets G3, G4, G5, G6, G7, G8 & G9 had significant decrease in liver, heart, kidney, spleen and lungs weight (g). The highest limit decrease obtained for G5 (whole kumquat 7.5%) in liver, kidneys and lungs weights(g) & G3 (whole kumquat seedless 7.5%) in heart and spleen weights (g).

Table (3): Relative organs weights of normal rats and intoxicated rats with $KBrO_3$(n=6 rats/groups)

Groups	Parameter	Liver(g) Mean ± SD	Heart(g) Mean ± SD	Kidneys(g) Mean ± SD	Spleen(g) Mean ± SD	Lungs(g) Mean ± SD
Control	G1 (– ve)	$2.35^{f}\pm 0.021$	$.553^{a}\pm 0.0095$	$.97^{e}\pm 0.01$	$.324^{d}\pm 0.016$	$1.2^{a}\pm 0.03$
	G2 (+ ve)	$3.62^{a}\pm 0.04$	$.853^{b}\pm 0.009$	$1.96^{a}\pm 0.03$	$.56^{e}\pm 0.014$	$2.34^{f}\pm 0.04$
Kumquat	G3 Whole seedless 7.5%	$2.96^{d}\pm 0.05$	$.571^{a}\pm 0.012$	$1.11^{d}\pm 0.02$	$.404^{c}\pm 0.024$	$1.29^{g}\pm 0.06$
	G4 Seeds 7.5%	$3.58^{a}\pm 0.04$	$.735^{cd}\pm 0.016$	$1.84^{b}\pm 0.04$	$.552^{a}\pm 0.017$	$2.00^{b}\pm 0.05$
	G5 Whole kumquat 7.5%	$2.42^{a}\pm 0.02$	$.570^{a}\pm 0.014$	$1.00^{e}\pm 0.015$	$.383^{c}\pm 0.01$	$1.3^{g}\pm 0.03$
Pineapple	G6 Flesh 7.5%	$3.18^{b}\pm 0.037$	$.841^{b}\pm 0.015$	$1.8^{b}\pm 0.03$	$.531^{a}\pm 0.011$	$1.78^{c}\pm 0.025$
	G7 Leaves 7.5%	$3.00^{d}\pm 0.03$	$.749^{cd}\pm 0.127$	$1.85^{b}\pm 0.1$	$.553^{a}\pm 0.034$	$1.73^{c}\pm 0.04$
	G8 (Flesh+leaves) 7.5%	$3.06^{c}\pm 0.036$	$.583^{a}\pm 0.016$	$1.247^{c}\pm 0.105$	$.482^{b}\pm 0.013$	$1.38^{e}\pm 0.01$
Mix of all	G9 (Whole kumquat+ pineapple flesh + pineapple leaves) 7.5%	$3.2^{b}\pm 0.021$	$.802^{bc}\pm 0.015$	$1.8^{b}\pm 0.03$	$.511^{ab}\pm 0.03$	$1.5^{d}\pm 0.04$
	LSD	0.0578	0.0758	0.092	0.035	0.066

Values with different letters indicate significant differences Between the groups ($P<0.05$), and vice versa. LSD: least significant Differences ($P<0.05$).

D- Biochemical data changes:

1-Liver enzymes activities:

Data presented in table (4) show the effect of feeding by tested plants (whole kumquat seedless, kumquat seeds, whole kumquat, pineapple flesh, pineapple leaves, pineapple "flesh +leaves" and mix from all) for $KBrO_3$-intoxicated rats on liver enzymes (AST, ALT& ALP) and AST/ALT ratio. Results in table (4) showed significant elevations in serum activity of AST, ALT and ALP enzymes and AST/ALT ratio compared with those of the normal rats. All plants diets G3, G4, G5, G6, G7, G8 & G9 had significant ameliorations in serum activity of AST, ALT and ALP enzymes and AST/ALT ratio compared with those of the positive control rats. The highest decreased limit of ALP obtained for G5 (whole kumquat 7.5%) with non-significant difference with control (-) group. These results are in agreement with **Okafor *et al., (*2011)** who reported that treatment with pineapple extract for 28 days was observed to significantly decrease the enzyme levels, signifying the modulatory effect of the extract on the hepatic biomarkers and its hepato-protective potentials. The best formula showing maximum numerical reductions of AST activity was observed for G5 (whole kumquat 7.5%). The highest decreased limit in ALT (U/L) obtained for G5 (whole kumquat 7.5%) with non-significant difference with control (-) group. **Saxena & Panjwani (2014)** found that oral treatment with HEAC (hydro-alcoholic fruit extract of *Ananas comosus*) decreased serum ALP, AST&ALT of all the $KBrO_3$-induced rats. All intoxicated rats with $KBrO_3$ and fed on all tested plants showed significant decreases in ratio of (AST/ALT) ranging from -13.99 % to -29.51% of control (+) group.

Table (4): Serum activity of AST, ALT& ALP and AST/ALT ratio in normal rats and intoxicated rats with $KBrO_3$(n=6 rats/groups)

Groups	Parameter	AST (U/L) Mean ± SD	ALT (U/L) Mean ± SD	ALP(U/L) Mean ± SD	AST/AIT Mean ± SD
Control	**G1** (– ve)	$40^{g} \pm 2.00$	$27^{e} \pm 1.00$	$.97^{e} \pm 0.01$	$1.48^{a} \pm 0.01$
	G2 (+ ve)	$202^{a} \pm 3.00$	$83^{a} \pm 1.00$	$1.96^{a} \pm 0.03$	$2.43^{g} \pm 0.095$
kumquat	**G3** Whole seedless 7.5%	$61^{ef} \pm 1.00$	$31^{de} \pm 2.5$	$1.11^{d} \pm 0.02$	$1.977^{bc} \pm 0.13$
	G4 Seeds 7.5%	$82^{k} \pm 2.00$	$40^{b} \pm 2.4$	$1.84^{b} \pm 0.04$	$2.05^{b} \pm 0.155$
	G5 Whole kumquat 7.5%	$58^{f} \pm 1.00$	$28^{e} \pm 1.3$	$1.00^{e} \pm 0.015$	$2.07^{b} \pm 0.06$
pineapple	**G6** Flesh 7.5%	$65^{d} \pm 1.00$	$36^{c} \pm 4.1$	$1.8^{b} \pm 0.03$	$1.823^{bc} \pm 0.18$
	G7 Leaves 7.5%	$72^{b} \pm 3.00$	$42^{b} \pm 0.1$	$1.85^{b} \pm 0.1$	$1.713^{c} \pm 0.075$
	G8 (Flesh+leaves) 7.5%	$60^{ef} \pm 2.00$	$33^{cd} \pm 0.9$	$1.247^{c} \pm 0.105$	$1.82^{bc} \pm 0.11$
Mix of all	**G9** (Whole kumquat+ pineapple flesh + pineapple leaves) 7.5%	$90^{c} \pm 3.00$	$43^{b} \pm 1.00$	$1.8^{b} \pm 0.03$	$2.09^{b} \pm 0.105$
	LSD	3.706	3.35	0.092	0.194

Values with different letters indicate significant differences Between the groups (P<0.05), and vice versa. LSD: least significant Differences (P<0.05).

2-Lipids fraction of serum:

Data presented in table (5), show the effect of feeding by plant diets on serum lipids fractions. It could be observed that $KBrO_3$ intoxication accompanied by the rise of TC, TG, VLDL, LDL, AI ratio. All intoxicated rats with KBrO3 and fed on all tested plants diets (G3, G4, G5, G6, G7, G8 & G9) had significant decreases in serum total cholesterol (TC) (mg/dl) ranging from -4.04% to -26.26% compared to control (+) group. The highest decreased limit obtained for G5 (whole kumquat 7.5%)& G8 (pineapple (flesh +leaves) 7.5%), with non-significant difference with control (-) group, **Ji-lie *et a*l., (2008)** stands by whole kumquat reduce TC values

All Experimental diets (G3, G4, G5, G6, G7, G8 & G9) presented a significant decrease in serum triglycerides

(TG) (mg/dl) ranging from -2.78 % to -23.61% of control (+) group. Moreover, G3 (whole kumquat seedless 7.5%), G5 (whole kumquat 7.5%) & G8 (pineapple (flesh +leaves) 7.5%) decreased TG more than control (-) group record. G3 (whole kumquat seedless 7.5%) showed the highest decreased limit in serum triglycerides (TG) (mg/dl), with significant difference with the other groups.

VLDL in serum was appreciably increased by $KBrO_3$ intoxication while decreased by nutritional intervention using experimental diets (G3, G4, G5, G6, G7, G8 & G9) which ranging from -2.78% to -23.16% of control (+) group. The highest decreased limit obtained for G3 (whole kumquat seedless 7.5%) with significant difference compared to the other groups.

It is obvious $KBrO_3$ intoxication lowered considerably the level of good cholesterol (from 49.5 to 35.4 mg/dl). On the contrary feeding experimental diets (G3, G4, G5, G6, G7, G8 & G9) reversed such change, taking into consideration that the highest increased limit obtained for G5 (whole kumquat 7.5%) with significant difference with them.

Lien *et al*., (2009) demonstrated that blood HDL-C increased in obese mice when treated with different extract fractions from kumquat (*Fortunella japonica*) peels.The intake of plants diets lowered appreciably the LDL level. Best treatment recorded for G5 (whole kumquat 7.5%) &G8 (pineapple (flesh +leaves) 7.5%) which revealed non-significant different LDL content in comparison with control (-) group.

Studies of **Ji-lie *et al*., 2008 ; Lien *et al*., (2009)** agree with results of present work in whole kumquat group decreased plasma LDL cholesterol levels.

Nutritional intervention with experimental diets (G3, G4, G5, G6, G7, G8 & G9) lowered greatly the AI, in particular for the G5 (whole kumquat 7.5%) & G8 (pineapple (flesh +leaves) 7.5%) which recorded -66.83% & -67.94% less AI compared to control (+) group. Moreover, G5 (whole kumquat 7.5%) & G8 (pineapple (flesh +leaves) 7.5%) reversed no significantly different AI value compared to control (-) rats.

Allam *et al*., (2015) showed that treatment with whole kumquat reduced AI, VLDL, LDL, TG &TC compared to control (+) group in hypercholesterolemic rats.

Table (5): Lipids fractions in serum of normal rats and intoxicated rats with $KBrO_3$(n=6 rats/groups)

Groups	Parameter	TC (mg/dl) Mean ± SD	T G (mg/dl) Mean ± SD	VLDLc (mg/dl) Mean ± SD	HDLc (mg/dl) Mean ± SD	LDLc (mg/dl) Mean ± SD	AI Mean ± SD
Control	G1 (–ve)	$76^{fg} \pm 1.00$	$60.67^{d} \pm 1.04$	$12.13^{d} \pm 0.208$	$49.5^{a} \pm 0.6$	$14.37^{g} \pm 0.603$	$.537^{g} \pm 0.003$
	G2 (+ ve)	$99^{a} \pm 0.3$	$72^{a} \pm 1.5$	$14.4^{a} \pm 0.3$	$35.4^{h} \pm 0.3$	$49.2^{a} \pm 0.3$	$1.8^{a} \pm 0.03$
Kumquat	G3 Whole seedless 7.5%	$87^{d} \pm 2.00$	$55^{f} \pm 1.5$	$11^{f} \pm 0.3$	$45.1^{cd} \pm 0.5$	$30.9^{d} \pm 2.2$	$0.93^{e} \pm 0.07$
	G4 Seeds 7.5%	$95^{b} \pm 1.00$	$64^{c} \pm 0.5$	$12.8^{c} \pm 0.1$	$39.6^{f} \pm 0.5$	$42.6^{b} \pm 0.6$	$1.397^{b} \pm 0.006$
	G5 Whole kumquat 7.5%	$76^{fg} \pm 0.5$	$58^{e} \pm 1.3$	$11.6^{e} \pm 0.26$	$47.6^{b} \pm 1.3$	$16.8^{g} \pm 2.06$	$0.597^{g} \pm 0.055$
Pineapple	G6 Flesh 7.5%	$80^{e} \pm 2.00$	$66^{c} \pm 1.00$	$13.2^{c} \pm 0.2$	$44.3^{d} \pm 0.4$	$22.5^{f} \pm 1.8$	$0.807^{f} \pm 0.025$
	G7 Leaves 7.5%	$92^{c} \pm 2.00$	$69^{b} \pm 0.7$	$13.8^{b} \pm 0.14$	$41.2^{e} \pm 1.2$	$37^{c} \pm 0.66$	$1.233^{c} \pm 0.015$
	G8 (Flesh+leaves) 7.5%	$73^{g} \pm 2.00$	$59^{de} \pm 2.00$	$11.8^{de} \pm 0.4$	$46.2^{c} \pm 0.8$	$14.9^{g} \pm 2.406$	$0.577^{g} \pm 0.07$
Mix of all	G9 (Whole kumquat+ pineapple flesh + pineapple leaves) 7.5%	$78^{ef} \pm 1.00$	$70^{ab} \pm 1.00$	$14^{ab} \pm 0.2$	$38^{g} \pm 0.9$	$26^{e} \pm 0.1$	$1.053^{d} \pm 0.025$
	LSD	2.515	2.14	0.4281	1.364	2.517	0.071

Values with different letters indicate significant differences Between the groups (P<0.05), and vice versa. LSD: least significant Differences (P<0.05).

d-Kidney function:

The results illustrated in table (6) indicate the serum creatinine (mg/dl), urea (mg/dl) &uric acid (mg/dl) of experimental rats. It could be noticed that $KBrO_3$ intoxication raised serum creatinine (mg/dl), urea (mg/dl) &uric acid (mg/dl). All rats of experimental diets (G3, G4, G5, G6, G7, G8 & G9) showed significant decreases in serum creatinine (mg/dl), urea (mg/dl) &uric acid (mg/dl). Taking into consideration that the highest decreased limit of serum creatinine (mg/dl) & urea (mg/dl) obtained for G3 (whole kumquat seedless 7.5%).

G6 (pineapple flesh 7.5%) record -68 % decrease serum uric acid (mg/dl) compared to control (+) group with non-significant difference with G4 (kumquat seeds 7.5%) & G8 (pineapple (flesh +leaves) 7.5%). **Rezq (2017)** reported that $KBrO_3$-intoxicated positive rats have significant increase in serum levels of urea nitrogen (UN), creatinine (Cr)& uric acid (UA) compared with those of normal control rats.

Table (6): Kidney function (creatinine (mg/dl), Urea (mg/dl) &uric acid (mg/dl)) in serum of normal rats and intoxicated rats with $KBrO_3$(n=6 rats/groups)

Groups	Parameter	Creatinine (mg/dl) Mean ± SD	Urea (mg/dl) Mean ± SD	Uric acid (mg/dl) Mean ± SD
Control	**G1** (– ve)	$.71^{b} \pm 0.07$	$20^{g} \pm 2.00$	$3^{d} \pm 0.1$
	G2 (+ ve)	$3.5^{a} \pm 0.33$	$44^{a} \pm 3.00$	$7.5^{a} \pm 0.2$
Kumquat	**G3** Whole seedless 7.5%	$.673^{b} \pm 0.312$	$32^{de} \pm 2.00$	$3.2^{cd} \pm 0.1$
	G4 Seeds 7.5%	$1.053^{b} \pm 0.07$	$36^{bcd} \pm 1.00$	$3.3^{bcd} \pm 0.3$
	G5 Whole kumquat 7.5%	$.943^{b} \pm 0.129$	$27^{f} \pm 2.00$	$2.9^{d} \pm 0.3$
Pineapple	**G6** Flesh 7.5%	$.957^{b} \pm 0.127$	$35^{cd} \pm 3.00$	$2.4^{bcd} \pm 0.4$
	G7 Leaves 7.5%	$1.03^{b} \pm 0.026$	$38^{bc} \pm 1.00$	$3.6^{bc} \pm 0.1$
	G8 (Flesh +leaves) 7.5%	$.967^{b} \pm 0.067$	$29^{ef} \pm 2.00$	$3.3^{bcd} \pm 0.1$
Mix of all	**G9** (Whole kumquat+ pineapple flesh + pineapple leaves) 7.5%	$.983^{b} \pm 0.133$	$40^{b} \pm 1.00$	$3.8^{b} \pm 0.2$
	LSD	0.298	3.478	0.388

Values with different letters indicate significant differences Between the groups ($P<0.05$), and vice versa. LSD: least significant Differences ($P<0.05$).

E- Serum protein fractions:

The results of table (7) show serum protein fractions (total protein (g/dl), albumin (g/dl), globulin (g/dl) & Alb/Glb ratio of experimental rats. It is evident that T. protein & albumin (g/dl) degenerated due to $KBrO_3$ intoxication, while were raised by feeding tested plants, in particular G5 (whole kumquat 7.5%)which recorded the highest increase of T. protein, albumin& Alb/Glb ratio. $KBrO_3$ intoxication elevated the globulin (from 3.6 to 3.9 g / dl). moreover, G3 (whole kumquat seedless 7.5%), G5 (whole kumquat 7.5%), G6 (pineapple flesh 7.5%) & G8 (pineapple (flesh +leaves) 7.5%) decreased the globulin to (3.2, 3, 3.4& 3.2 g / dl) respectively than 3.6 (g / dl) for control (-) group.

Saxena & Panjwani, (2014) showed that administration of HEAC (hydro-alcoholic fruit extract of *Ananas comosus*) to ISO injected rats showed a significant increase in total protein level as compared to ISO injected rats due to presence of antioxidant.

Table (7): Serum protein fractions (total protein (g/dl), albumin (g/dl), globulin (g/dl) & Alb/Glb ratio in serum of normal rats and intoxicated rats with $KBrO_3$(n=6 rats/groups)

Groups	Parameter	Total protein (g/dl) Mean ± SD	Albumin (g/dl) Mean ± SD	Globulin (g/dl) Mean ± SD	Alb/Glb Mean ± SD
Control	G1 (– ve)	$7.4^{a} \pm 0.05$	$3.8^{ab} \pm 0.1$	$3.6^{bc} \pm 0.05$	$1.058^{b} \pm 0.043$
	G2 (+ ve)	$4.9^{f} \pm 0.1$	$1^{g} \pm 0.2$	$3.9^{ab} \pm 0.1$	$.259^{e} \pm 0.058$
Kumquat	G3 Whole seedless 7.5%	$6.5^{c} \pm 0.2$	$3.3^{c} \pm 0.02$	$3.2^{cd} \pm 0.18$	$1.033^{b} \pm 0.055$
	G4 Seeds 7.5%	$5.4^{e} \pm 0.1$	$1.8^{e} \pm 0.09$	$3.6^{bc} \pm 0.01$	$.5^{d} \pm 0.02$
	G5 Whole kumquat 7.5%	$7^{b} \pm 0.3$	$4^{a} \pm 0.2$	$3^{d} \pm 0.1$	$1.33^{a} \pm 0.02$
Pineapple	G6 Flesh 7.5%	$6.1^{d} \pm 0.1$	$2.7^{d} \pm 0.3$	$3.4^{cd} \pm 0.4$	$.807^{c} \pm 0.186$
	G7 Leaves 7.5%	$5^{f} \pm 0.2$	$1.5^{f} \pm 0.1$	$3.5^{bc} \pm 0.1$	$.43^{d} \pm 0.01$
	G8 (Flesh+leaves) 7.5%	$6.8^{b} \pm 0.1$	$3.6^{b} \pm 0.08$	$3.2^{cd} \pm 0.02$	$1.127^{b} \pm 0.015$
Mix of all	G9 (Whole kumquat + pineapple flesh + pineapple leaves) 7.5%	$5.3^{e} \pm 0.1$	$1.2^{g} \pm 0.13$	$3.9^{ab} \pm 0.23$	$.307^{e} \pm 0.045$
	LSD	0.2697	0.269	0.3016	0.1224

Values with different letters indicate significant differences Between the groups ($P<0.05$), and vice versa.LSD:least significant Differences ($P<0.05$).

G-Antioxidants enzymes:

Data of table (8) show the effect of feeding by experimental diets on serum levels of antioxidants enzymes (CAT(mmol/L), SOD (mmol/L) & GPX(ng/ml)) in $KBrO_3$-intoxicated rats.

It is obvious that due to $KBrO_3$ intoxication CAT(mmol/L), SOD (mmol/L) & GPX(ng/ml) activity reduced. All rats of tested plants showed significant increase in serum levels of CAT(mmol/L) ranging from +13.74 % to + 52.67 % of control (+) group. G8 (pineapple (flesh +leaves) 7.5%)showed the highest increased limit in serum levels of CAT (mmol/L) compared to all diets formulae, with non-significant difference with control (-) group.

Feeding on experimental diets (G3, G4, G5, G6, G7, G8 & G9) raised greatly the SOD activity, in particular that of G8 (pineapple (flesh +leaves) 7.5%) +75.75 % increase in comparison with control (+) group. G5 (whole kumquat 7.5%) & G8 (pineapple (flesh +leaves) 7.5%) reversed higher increase SOD activity compared with control (+) group.

Experimental diets (G3, G4, G5, G6, G7, G8 & G9) showed significant increasing in serum levels of GPX (ng/ml) ranging from +25.5 % to +75.75 % of control (+) group, taking into consideration that the highest increased limit obtained for G8 (pineapple (flesh +leaves) 7.5%), with no significant differences with G5 (whole kumquat 7.5%)& control (-) group. **Cho *et al*., (2005)** showed that pineapple is an excellent source of the trace mineral manganese, which is an essential cofactor in a number of enzymes important in energy production and antioxidant defenses. For example, the key oxidative enzyme superoxide dismutase, which disarms free radicals produced within the mitochondria (the energy production factories within our cells), requires manganese. **Okafor *et al.*, (2011)** found that methanolic extract of pineapple peels under alcohol–induced oxidative stress can positive modulation of catalase activities. **Rezq (2017)** reported that KBrO3-intoxicated positive rats have significant decrease GPX activity in serum compared with those of normal control rats.

Table (8): Antioxidants enzymes (CAT(mmol/L), SOD (mmol/L) & GPX(ng/ml)) activity of normal rats and intoxicated rats with $KBrO_3$(n=6 rats/groups)

Groups	Parameter	CAT (mmol/L) Mean ± SD	SOD (mmol/L) Mean ± SD	GPX (ng/ml) Mean ± SD
Control	G1 (– ve)	.202^{a} ± 0.002	60.42^{a} ± 1.06	.75^{a} ± .02
	G2 (+ ve)	.131^{c} ± 0.016	46.2^{d} ± 1.23	.4^{e} ± 0.004
Kumquat	G3 Whole seedless 7.5%	.162^{abc} ±0.026	56.2^{b} ±2.04	.66^{b} ±0.041
	G4 Seeds 7.5%	.141^{c} ± 0.009	51.34^{c} ± 0.95	.572^{c} ± 0.027
	G5 Whole kumquat 7.5%	.192^{ab} ± 0.011	58.34^{ab} ± 1.32	.681^{b} ± 0.052
Pineapple	G6 Flesh 7.5%	.161^{abc} ± 0.009	55.94^{b} ± 0.94	.564^{c} ± 0.023
	G7 Leaves 7.5%	.153^{bc} ± 0.022	51.00^{c} ± 0.86	.502^{d} ± 0.032
	G8 (Flesh +leaves) 7.5%	.2^{a} ± 0.015	59.01^{ab} ± 1.52	.703^{ab} ± 0.024
Mix of all	G9 (Whole kumquat+ pineapple flesh + pineapple leaves) 7.5%	.149^{ab} ± 0.024	58.14^{ab} ± 1.02	.671^{b} ± 0.012
	LSD	0.0286	2.171	0.0506

Values with different letters indicate significant differences Between the groups (P<0.05), and vice versa. LSD: least significant Differences (P<0.05).

SRB:

Effect of kumquat and pineapple fruits on human liver cancer cells:

The effect of whole kumquat seedless, kumquat seeds, whole kumquat, pineapple flesh, pineapple leaves, pineapple (flesh +leaves) and mix of all are shown in table (9) and photos (1-7).

Table (9) : Median lethal concentration (LC50) of whole kumquat seedless, kumquat seeds, whole kumquat, pineapple flesh, pineapple leaves, pineapple (flesh +leaves) and mix of all that kills 50% of human liver cancer cells (surviving cells)

Concentration (ug/ml)	Survival fraction of cancer cell HEPG2						
	Whole kumquat seedless	Kumquat seeds	Whole kumquat	Pineapple flesh	Pineapple leaves	Pineapple (flesh +leaves)	Mix of all
0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
12.500	0.786	0.859	0.682	0.850	0.832	0.741	0.682
25.000	0.736	0.605	0.568	0.609	0.691	0.455	0.591
50.000	0.373	0.264	0.264	0.236	0.259	0.214	0.218
100.000	0.295	0.218	0.273	0.227	0.245	0.214	0.245
IC50(ug/ml)	40.7	32	30	31.7	36	23	31

HEPG2 (liver carcinoma cell line). IC50 (dose of the tested compound which reduces survival cells to 50%).

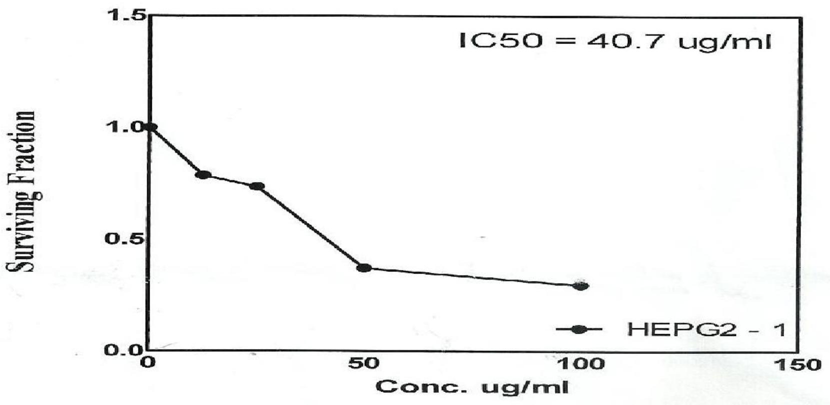

Figure (1): Median lethal concentration (LC50) of kumquat seedlessthat kills 50 of human liver cancer cells (surviving cells)

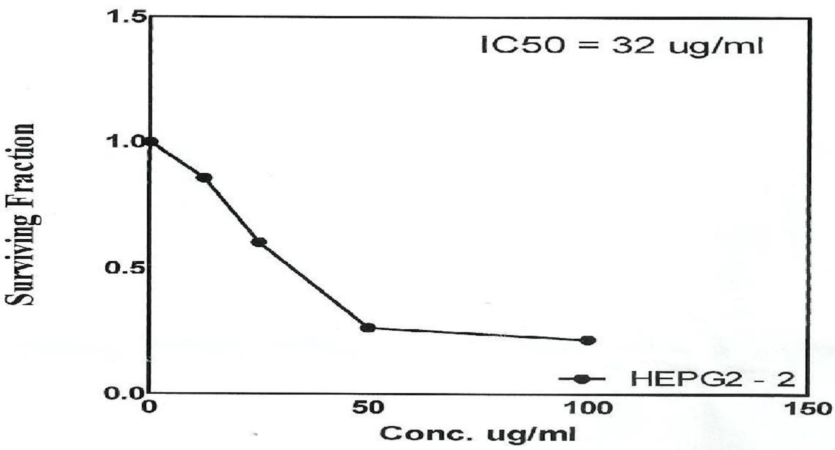

Figure (2): Median lethal concentration (LC50) of kumquat seeds that kills 50% of human liver cancer cells (surviving cells)

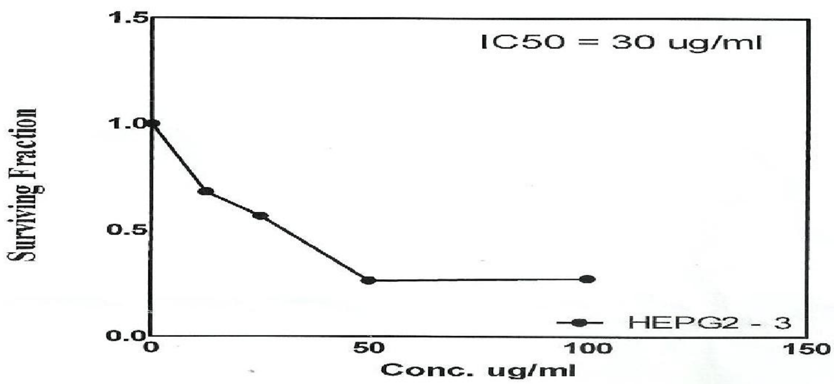

Figure (3): Median lethal concentration (LC50) of whole kumquat that kills 50% of human liver cancer cells (surviving cells)

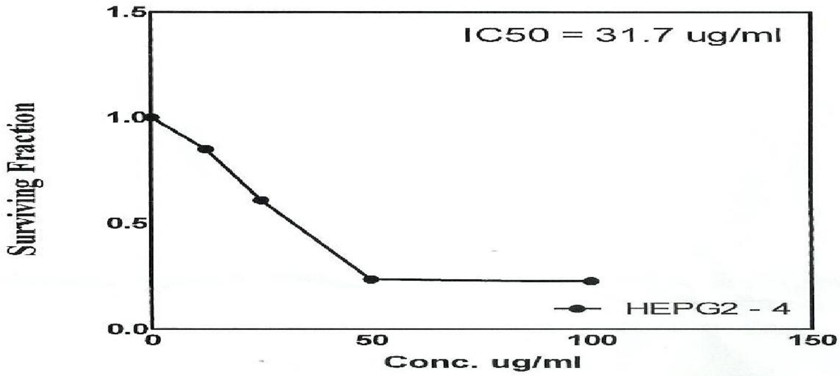

Figure (4): Median lethal concentration (LC50) of pineapple flesh that kills 50% of human liver cancer cells (surviving cells)

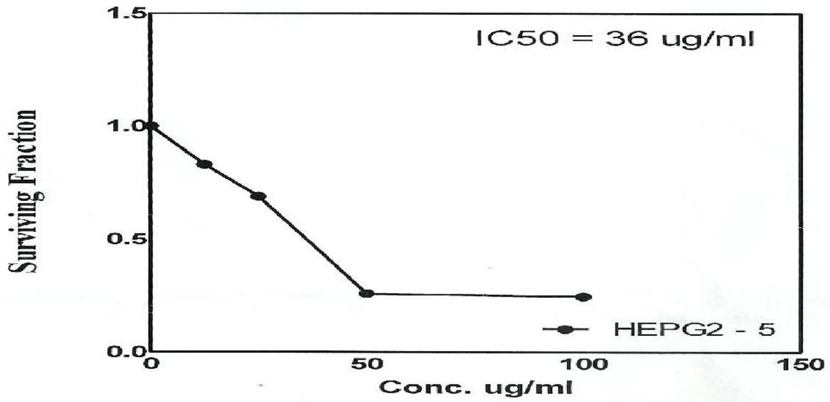

Figure (5): Median lethal concentration (LC50) of pineapple leaves that kills 50% of human liver cancer cells (surviving cells)

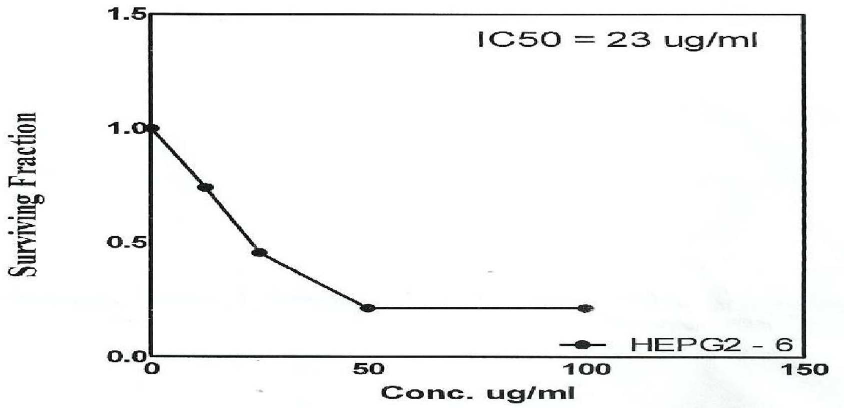

Figure (6): Median lethal concentration (LC50) of pineapple flesh and Leaves that kills 50% of human liver cancer cells (surviving cells)

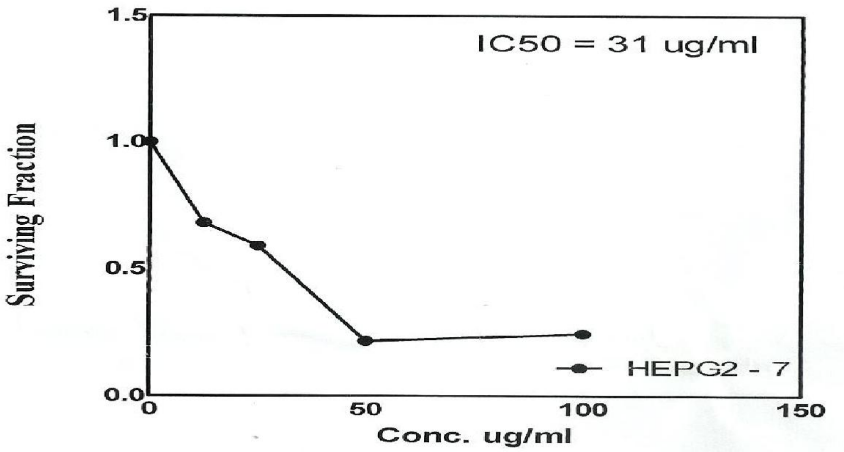

Figure (7): Median lethal concentration (LC50) the mixture of all above studied treatments that kills 50% of human liver cancer cells (surviving cells)

The previous mentioned table results indicated firstly that all treatments had apoptotic action on human cancer liver cells. Apoptosis occurs normally during development and aging and a homeostatic mechanism to maintain cell population in tissues. Irradiation or drugs used for cancer chemotherapy results in DNA damage in some cells, which can lead to apoptotic death through so called p53 dependent pathway **(Elmore, 2007)**. Meanwhile no thing is found in the previous literature saying that kumquat or pineapple plants have any toxicity. On the other side data of table (9) as illustrated in Fibagures (1-7) indicate the value of kumquat seedlees and kumquat seeds as well as pineapple flesh and pineapple leaves to combat human liver cancer.

It is evident that surviving action was lowest (best treatment) for pineapple flesh plus pineapple leaves (Table 9) showing a level of 23 HEPG2-1 while for plant groups the range was 31.7 to 40.7 HEPG. Best group for kumquat was that of either kumquat seeds or kumquat flesh with seeds (32&30 HEPG2-1 respectively). Best treatments based on biological and biochemical parameters recorded for kumquat flesh with seeds, followed by pineapple flesh plus leaves. Concerning HEPG2-1 best results recorded for pineapple flesh with leaves followed by kumquat with seeds then kumquat seeds. This may be explained on basis of findings reportedby **Elmore (2007)**. The numerous parameters encouraging apoptosis and death of cancer cells may not be exactly that encouraging metabolism and avoid deterioration of the organs functions. This do not exclude that many parameters are the same.

Anyhow pineapple flesh plus leaves followed by kumquat flesh with seeds proved to show appreciable cancer cells apoptosis.

Baez *et al.*, (2007) reported that the *in vivo* antitumoral/antileukemic activity was evaluated using the following panel of tumor lines: P-388 leukemia, sarcoma (S-37), Ehrlich ascitic tumor (EAT), Lewis lung carcinoma (LLC), MBF10 melanoma and ADC-755 mammary adenocarcinoma. Bromelain shown efficient anti cancerous effects on above all cell line. Bromelain potentially interferes with tumor metastasis progression at a variety of pivotal points. Bromelain inhibits cell surface adhesion proteins that are essential in cell adhesion, migration and inflammation. This inhibition is predominantly due to suppression of NF-κB activation. Furthermore, bromelain inhibits the invasiveness of human cancer cells by suppressing matrix metalloproteinase (MMP)-9 expression through inhibiting activator protein 1 (AP-1) and NF-κB signaling pathways (**Rathnavelu *et al.*,2016**).

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