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Introduction

Nzo A [Pyrin] is an aromatic hydrocarbon that can be in a compound everywhere in Qatar. The substance with the formula C20H12 is one of the benzopyrene, formed from a benzene ring fused with pyrene **Kobayashi, (2017)**. Diol epoxide metabolites (known as BPDE) interact and are included in the first group of cancer by International Agency for Research on Cancer (IARC) in the eighteenth century **Jiao *et al*. (2012) and Kobayashi, (2017**). It is linked that benzo [a] Pirin produced tumors (cancer) in experimental animals in several different ways (orally, through the skin, inhalation, intra-trachea, intra-trachea, subcutaneous, intraperitoneal, for intravenous route). Benzo [a] Perrin had both a local and systemic carcinogenic effect (**Joseph and Jaiswal, 1975; Rajendran *et al*., 2018**).

The safflower plant (*Carthamus tinctorius L*), is a year old blooming herb, branched from the stellar family, and its native origin is the Middle East, but it is widely cultivated throughout Europe, China, India, and the United States, and is approximately one meter long and is It has shiny oval-shaped leaves, spruce edges that wrap around a smooth and flat stem, and produce flowers ranging in color from yellow to dark red (**Daniel *et al*., 2012**).

Much research indicates that safflower oil is used as a dietary supplement or as an alternative to other oils. Saffron contains nutrients including protein 16.18 g, fats 38.45 g, carbohydrates 34.29 g, monounsaturated fatty acids 4.84 g, multiple polyunsaturated fatty acids 28.22 g, calcium 78 mg, magnesium 353 mg, folate 160 micrograms, and many phenol compounds It is a derivative of serotonin. It also contributes to lowering the level of total cholesterol and low-density cholesterol. It was also found in the (**Journal of Nutritional Science and Vitaminology, 1991**) conducted in mice that consumed a high cholesterol regimen that phospholipids in saffron inhibited the high cholesterol in their blood and liver, and this effect depends on the level of these fats (**Chilliard *et al*., 2015**).

Saffron is containt in omega-6 reduces blood sugar levels. Reducing symptoms of Hepatitis C virus, lowering blood pressure in those with high levels, relieving constipation and relieving conditions of breathing problems, coughing, fever, and pain (**Ramsden *et al*., 2018**). Therefore,

the effect of fortification of pan bread with three levels of safflower powder on liver cancer induced by benzo [a] Pirinin albino rats were studied.

Material and Methods:

Materials:

- Benzo(a)pyrene ©: was acquired by Sigma Chemical Co. (St Louis, Mo, USA).
- Safflower (*Carthamus tinctorius*) flower, wheat flour (72 percent extract), wheat flour, sugar, and salt were purchased from the local market in Cairo as dried material. France-Egypt.
- **Animals:** Thirty six male albino rats, strain Sprague Dawley, weighing (150 ± 10 g) were bought from the Agricultural Research Centre's animal house in Giza, Egypt. The animals were kept in plastic cages, maintained on a natural light-dark cycle at room temperature of 26 ± 2°C, and fed on normal diet **(Reeves *et al.*, 1993).**
- **Methods:**

Pan bread making process: Pan bread was prepared according to **Lazaridou *et al.* (2007)**. Preparation of pan bread was carried out by using wheat flour (72% extraction), blender replaced separately with 20%, 30% and 40% Safflower powder according to the method of **AACC (2005)**.

Proximate analysis: The proximate nutritional qualities of the bread samples was carried out according to the method of **A.O.A.C (2000)** which covers for total protein, ash, fat, dietary fiber and carbohydrates, respectively.

Experimental design: Rats performed at the Food Technology Research Institute, Agriculture Research Centre, Giza, in the animal building. Five rats acted as a standard control group (-ve) after the acclimatization phase, while the other rats. Injected with one milliliter of a carcinogenic solution (10.08 mg/kg) containing benzo(a)pyrene s.c. In rats categorized in the previous study (Kallistratos and Fasske, 1976) into the positive control group (+ ve) and four treated rat groups, *as the following:-*

$B_{PCW:}$ Group fed on basal diet with 20% pan bread (100%wheat flour).

***B**PFS1:* Group fed on basal diet with 20% pan bread fortified with 20% safflower powder.*

***B**PFS2:* Group fed on basal diet with 20% pan bread fortified with 30% safflower powder.*

***B**PFS3:* Group fed on basal diet with 20% pan bread fortified with 40% safflower powder.*

The second biological assessment of the diets evaluated at the end of the 6-week experiment was conducted by assessing total feed consumption, body weight gain (BWG) and the Feed efficiency ratio (FER).

Biochemical analysis:

Determination of liver enzymes: The activity of serum alanine and aspartate aminotransferases (ALT & AST) and alkaline phosphatase (ALP) enzymes was Determined according to (**Reitman and Frankel, 1957**).

Determination of serum lipids: The method of assaying the total lipids **(Kaplan, 1984).** Serum total cholesterol (TC) was according to **(Henry, *et al.*, 1974).** The Serum triglycerides (TG) were determined according to **Fossati and Prencie (1982).** Method Serum high density lipoprotein cholesterol (HDL-cholesterol) was according to **Burstein, (1970).** The low density lipoprotein cholesterol (LDL-cholesterol) concentration in serum was estimated by the equation as follows: LDL-cholesterol (mg/dl) = Total cholesterol — HDL cholesterol — (TG/5)

Determination of kidney functions:

Measurement of serum urea and creatinine were done according to the method of **Patton and Crouch, (1977).**

Determination of serum antioxidant parameters:

Operation of superoxide dismutase(SOD), complete potential of antioxidants (TAC), malondialdehyde (MDA), and tumor necrosis factor (TNF-α) were determined according to **Nishikimi *et al.* (1972), Cao *et al.* (1996), Ohkawa *et al.* (1979) and Thorell and Lanner, (1973),** respectively.

Statistical analysis:

The collected data were analyzed using computerized statistics (SPSS). Effects of various treatments were analyzed using the one-way ANOVA (Variance Analysis) method using the multi-range method of Duncan, and $p<0.05$ was used to suggest significance between different groups **(SAS 1988).**

Results and Discussion:

Proximate analysis constituent of safflower flowers**:**

Table (1) showed that, moisture content increased gradually by increasing level of safflower powder in bread. Results showed a significant increase ($p<0.05$) Ash and dietary fiber content in pan bread which Fortified with safflower increased, than that of un-fortified pan bread (control) (B_{PCW}). Both ash and dietary fiber tendency to increase according to the increments of safflower powder in pan bread fortified due to the higher content of safflower powder in ash and dietary fiber compared to wheat flour.

Table 1: Chemical composition of control and fortified pan bread with safflower powder

Parameters	B_{PCW}	B_{PFS1}	B_{PFS2}	B_{PFS3}
Moisture	**32.70**	**35.16**	**36.90**	**39.10**
Protein	**10.95**	**10.20**	**10.97**	**12.50**
Fat	**4.67**	**4.14**	**3.91**	**2.13**
Ash	**1.34**	**2.41**	**2.47**	**3.61**
Dietary fiber	**1.54**	**3.83**	**5.01**	**6.82**
Carbohydrate	48.84	**44.26**	**40.75**	**36.85**

Nutritional characters of control and fortified pan bread with safflower powder

Table (2) indicates a steady decrease in body weight gain, feed intake and FER In the positive control group (+ve) by about, 67.10%, 23.70% and + 2.722 relative to normal control group (-ve). Statistical data showed that control (+ ve) rat group showed a substantial decrease in body weight gain , feed intake and feed efficiency ratio, whereas All treatments with pan bread Which fortified with safflower powder showed significant increases in these parameters compared to control (+ ve) group at three levels, and no significant difference compared to control (-ve) group.

Table (2): Effect of safflower powder on feed intake, body weight and Feed efficiency ratio in rats received benzo(a)pyrene

Groups	Weight gain (g)	%	Feed intake (g/d)	%	FER	%
Contr. (-ve)	96.76± 8.11 a	-	17.54± 2.20 a	-	0. 187± 0.03a	-
Contr. (+ve)	29.64± 8.11d	- 67.10	13.34± 2.20d	- 23.70	0.67± 0.06e	+ 2.722
B_{PCW}	86.13± 9.13 c	- 13.80	16.46± 2.32 a	- 7.13	0.185± 0.04ab	- 1.07
B_{PFS1}	87.27± 9.17 c	- 10.11	16.35± 2.21 a	- 7.19	0.183± 0.03b	- 2.14
B_{PFS2}	88.04± 9.17 b	- 9.44	16.08± 2.92 a	- 7.02	0.185± 0.04 ab	- 1.07
B_{PFS3}	93.44± 9.17 b	- 6.40	16.66± 2.32 a	- 7.13	0.180± 0.04 c	- 3.74

Values are expressed as mean ± SD, n=6, mean values are different at $p<0.05$ in each column with different superscripts (a, b , c, ..) and vice versa.. B_{PCW}: pan bread of 100% wheat flour, B_{PFS1}: pan bread fortified with 20% safflower powder, B_{PFS2}: pan bread fortified with 30% safflower powder. B_{PFS3}: pan bread fortified with 40% safflower powder.

Effect of pan bread fortified with safflower powder on lipid profile in rats received benzo(a)pyrene

The effects of **fortified pan bread with safflower powder** at three levels (20, 30 & 40%) on serum lipid profile for **benzo(a)pyrene** injection induced cancer liver are shown in Table (3). Cancer liver induced by **benzo(a)pyrene** injection caused significant rise in total lipids, triglycerides, total cholesterol and LDL-C while, there was significant decrease in HDL-C.

Treatment with **fortified pan bread with safflower powder** at three different dose levels induced significant decreasing in serum total lipids, triglycerides and total cholesterol at three levels administration while The best result of lipid profile was at the high level 40%. . In this respect, **benzo(a)pyrene**, an antibiotic with broad spectrum of effect against many clinically important bacteria, possess inherent cancer liver effects and induces micro vesicular steatosis by increasing the of accumulation triglyceride, inhibiting β-oxidation of free fatty acids, secretion of lipoprotein from the liver and mitochondrial lipid metabolism **(Helal, *et al.*, 2012)**. **Safflower** is to be effective in ameliorating the undue effects of the useful antibiotic. As an antioxidant **safflower**, contributes to liver and pancreatic health by helping the body to more quickly remove toxins.

It has also being linked to lowered or normalized cholesterol levels **(Daniel, *et al*., 2019).**

Effect of pan bread fortified with safflower powder on liver function in rats received benzo(a)pyrene With regard to liver function, LT4 led to a remarkable increase in serum AST, ALP and ALP levels in positive control (+ve) group as comparing with (-ve) group. Feeding rats on fortified pan bread with safflower powder with different premixes decreased activities of AST, ALP and ALK levels of cancer liver rats near to the level of the control (-ve). Though, the significant elevation witnessed in AST, ALP and ALK levels of cancer liver rats (Table 4). The best result was 40 per cent high, followed by 30%. According to our findings, **(Helal, *et al*., 2012)** documented cancer liver oxidative damage and increased levels of AST , ALT, ALP, total bilirubin and reduced total protein. Also, caused histopathological alterations. Therefore, the reduction in serum levels of AST, ALT, and ALP by safflower powder treatment is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by **benzo(a)pyrene** injection. This result shows that, with cancer liver recovery, the serum transaminase levels return to normal (**Chilliard *et al*., 2015**).

Effect of pan bread fortified with safflower powder on kidney function in rats received benzo(a)pyrene

Table (5) Results showed kidneys function tests were elevated by benzo(a)pyrene injection in (+ve) control group. Whereas uric acid, urea and creatinine levels were found to be significantly lowered by fortified pan bread with safflower powder treatment at three different dose levels. The best result was at high levels 40% followed by 30%. Also, Astragalin effect may prevent the progression of chronic renal disease and had anti-inflammatory activity which reduced infiltration of inflammatory cells **(Ansari *et al*., 2012 and Ni *et al*., 1919)**.

Effect of pan bread fortified with safflower powder on SOD, Total antioxidants, MDA and TNF-α in rats received benzo(a)pyrene

The effects of safflower powder at two levels on serum antioxidant parameters in benzo(a)pyrene injection induced cancer liver are shown in Table (6). Benzo(a)pyrene injected cancer liver induced a substantial decrease in serum superoxide dismutase SOD, total antioxidant levels

MDA, and TNFα. Significant increasing was noticed by l fortified pan bread with safflower powder treatment at high level 40% followed by 3% level.

On the other hand, significant increasing was noticed in malondialdehyde MDA and tumor necrosis factor TNF-α of (+ve) control group. Treatment with fortified pan bread with safflower powder at three levels significantly attenuated the increased levels of MDA and TNF-α. The higher level 40% had the better result than 30% level. Also, several reports have shown the hepatoprotective effect of vitamin C against the cancer liver caused by some drugs (**Kobayashi, 2017**). Hence, vitamin C can function as an antioxidant by scavenging ROS, reducing oxidative stress and associated complications. In addition, vitamin C as a safflower powder improves vitamin E's antioxidant capacities, indicating that the main role of vitamin C is to recycle tocopheroxyl radicals and glutathione, and to prevent lipid peroxidation and hepatocellular harm (**Ramsden *et al.*, 2018**).

Data are expressed as mean, n = 3. $\mathbf{B_{PCW}}$: pan bread of 100% wheat flour, $\mathbf{B_{PFS1}}$: pan bread fortified with 20% safflower powder, $\mathbf{B_{PFS2}}$: pan bread fortified with 30% safflower powder. $\mathbf{B_{PFS3}}$: pan bread fortified with 40% safflower powder.

Table (3): Effect of pan bread fortified with safflower powder on lipid profile in rats received benzo(a) pyrene

Groups	TC mg/dl	%	TG mg/dl	%	LDL-c mg/dl	%	HDL-c mg/dl	%
Control (-ve)	75.49± 3.86 ^{e}	-	45.16± 3.28 ^{c}	-	38.30± 5. 25 ^{d}	-	28.35± 4.71 ^{a}	-
Control (+ve)	121.46± 3.84 ^{a}	+ 60.89	75.43± 8.15 ^{a}	+ 67.03	86.31± 8.83 ^{a}	+ 125.35	19.47± 1.18 ^{e}	- 31.32
$\mathbf{B_{PCW}}$	81.27± 5. 52 ^{b}	+ 7.65	51.19± 6.46 ^{b}	+ 13.35	46.98± 5.75 ^{b}	+ 22.66	24.03± 0. 37 ^{c}	- 15.24
$\mathbf{B_{PFS1}}$	80.14± 3.65 ^{b}	+ 6.16	49.18± 5.795 ^{b}	+ 8.90	42.65± 1.74 ^{c}	+ 11.36	27. 71 ± 2.36 ^{b}	- 2.26
$\mathbf{B_{PFS2}}$	77.46± 4.65 ^{c}	+ 2.61	47.76± 4.75 ^{b}	+ 5.76	40.97± 4.74 ^{c}	+ 6.97	26.92 ± 3.36 ^{b}	- 5.04
$\mathbf{B_{PFS3}}$	77.11± 3.66 ^{c}	+ 2.14	47.19± 4.75 ^{b}	+ 4.49	40.72± 4.74 ^{c}	+ 6.32	26. 96 ± 2.36 ^{b}	- 4. 90

Values are expressed as mean ± SD, n=6, mean values are different at $p<0.05$ in each column with different superscripts (a, b , c, ..) and vice versa.. $\mathbf{B_{PCW}}$: pan bread of 100% wheat flour, $\mathbf{B_{PFS1}}$: pan bread fortified with 20% safflower powder, $\mathbf{B_{PFS2}}$: pan bread fortified with 30% safflower powder, $\mathbf{B_{PFS3}}$: pan bread fortified with 40% safflower powder.

Table (4): Effect of pan bread fortified with safflower powder on liver function in rats received benzo(a)pyrene

Groups	AST (U/L)	%	ALT (U/L)	%	Alk (µ /ml)	%
Control (-ve)	43.82± 2.11 d	-	22. 51± 1. 78 d	-	40.71± 5.02 b	-
Control (+ve)	84.07± 5.58 a	+ 91.85	46.36± 3. 27 a	+ 105.95	60.78± 7.01 a	+94.30
B_{PCW}	42.86± 2.75 d	- 2.19	22.46± 2. 13 d	- 0.22	48.75± 4.81 b	-0.99
B_{PFS1}	50.08± 4.28 b	+ 14.28	27.99± 3.43 b	+ 24.34	45.91± 4.31 b	0.43
B_{PFS2}	46.82± 3.02 c	+ 6.84	26.73± 2.14 b	+ 18.75	43.41± 4.31 b	0.44

Values are expressed as mean ± SD, n=6, mean values are different at p<0.05 in each column with different superscripts (a, b , c, ..) and vice versa.. B_{PCW}: pan bread of 100% wheat flour, B_{PFS1}: pan bread fortified with 20% safflower powder, B_{PFS2}: pan bread fortified with 30% safflower powder. B_{PFS3}: : pan bread fortified with 40% safflower powder. Alk: Alkaline phosphatase

Table (5): Effect of pan bread fortified with safflower powder on kidney function in rats received benzo(a)pyrene

Groups	Uric acid mg/dl	%	Urea mg/dl	%	Creatinine mg/dl	%
Control (-ve)	2.73± 0.78 ^{b}	-	42.67± 3.06 ^{c}	-	1.02± 0.07 ^{b}	-
Control (+ve)	5.20± 0.52 ^{a}	+ 125.83	70.03± 2.65 ^{a}	+ 70.40	1.97± 0.31 ^{a}	- 43.54
B_{PCW}	4.92± 0.57 ^{a}	+ 9.93	65.0± 7.64 ^{ab}	- 1.47	1.37± 0.15 ^{b}	- 0.99
B_{PFS1}	2.92± 0.72 ^{b}	+ 97.35	61.67± 6.35 ^{ab}	+ 9.63	1.41± 0.22 ^{b}	- 27.43
B_{PFS2}	2.80± 0.93 ^{b}	+ 44.88	56.01± 3.21 ^{bc}	+ 7.08	1.17± 0.06 ^{b}	- 11.13

Values are expressed as mean ± SD, n=6, Mean values in each column having different superscript (a, b,c,..) are significant at p<0.05 by different and vice versa. **B_{PCW}**: pan bread of 100% wheat flour, **B_{PFS1}**: pan

bread fortified with 20% safflower powder, **B_{PFS2}**: pan bread fortified with 30% safflower powder. **B_{PFS3}** : pan bread fortified with 40% safflower powder.

Table (5): Effect of pan bread fortified with safflower powder on SOD, Total antioxidants, MDA and TNF-α in rats received benzo(a)pyrene

Groups	SOD u/ml	%	Total antioxidants mmol/L	%	MDA mmol/L	%	TNF-α (pg/ml)	%
Control (-ve)	2.73± 0.78 ^{b}	-	42.67± 3.06 ^{d}	-	1.02± 0.07 ^{c}	-	3.08± 0.29 ^{d}	-
Control (+ve)	5.20± 0.52 ^{a}	+ 125.83	70.03± 2.65 ^{a}	+ 70.40	7.97± 0.31 ^{a}	- 90.74	9.99± 1.01 ^{a}	- 22.48
B_{PCW}	4.92± 0.57 ^{a}	+ 9.93	65.0± 7.64 ^{ab}	- 1.47	4.37± 0.15 ^{b}	- 8.17	7.63± 0.52 ^{b}	- 7.54
B_{PFS1}	2.92± 0.72 ^{b}	+ 97.35	61.67± 6.35 ^{ab}	+ 9.63	3.41± 0.22 ^{b}	- 9.43	5.11± 0.08 ^{b}	- 4.90
B_{PFS2}	2.80± 0.93 ^{b}	+ 44.88	56.01± 3.21 ^{c}	+ 7.08	2.17± 0.06 ^{b}	- 10.38	4.56± 0.08 ^{c}	- 5.45

Values are expressed as mean ± SD, n=6, mean values are different at p<0.05 in each column with different superscripts (a, b , c, ..) and vice versa.B_{PCW}: pan bread of 100% wheat flour, **B_{PFS1}**: pan bread fortified with 20% safflower powder, **B_{PFS2}**: pan bread fortified with 30% safflower powder. **B_{PFS3}**: : pan bread fortified with 40% safflower powder.

Abstract:

The current investigation was carried out to examine the possible potential protective effects of safflower against benzo [a] pirin -induced cancer liver. This study was conducted on thirty six male albino the rats divided into two main groups; the first main groups group (n= 6 rats) was fed on the basal diet And used as anegative control (–ve) normal rats. The rats of the second main group (n= 30 rats) fed basal diet and injected benzo[a] Pirin (10.08 mg/kg) in the previous cancer liver induction study and then divided into four subgroups (n=6 rats) as follows: group (2) fed basal diet without any treatment and used as a positive control (C+ve)Groups (3,4,5&6) fed on basal diet containing 20% pan bread fortified B_{PCW}, $B_{PFS1,}$ B_{PFS2}, B_{PFS3} & B_{PFS4} for 28 days. Results indicated that, injected benzo [a] pirin cancer liver groups showed high significant increase in serum AST, ALT, ALP, total lipids, total cholesterol, triglycerides, LDL cholesterol, urea, creatinine, MDA and tumor

necrosis factor TNF,α. While body weight gain, feed intake, feed efficiency ratio, total protein, HDL, SOD and total antioxidants were significantly decreased compared to control positive group. Treatment with pan bread fortified with safflower powder attenuated these adverse effects and ameliorated the evaluated biochemical parameters. The best result was pan bread for B_{PFS} at high level 40% , 30 % followed by 20% fortified with safflower powder diets compered to pan bread with 100% B_{PCW}. In conclusion, the results demonstrate that safflower has a potent protective effect against benzo [a] pirin -induced cancer liver injury in rats. The results also revealed that the effect of pan bread with safflower powder on the liver cancer may be attributed to its antioxidant and free radical scavenger.

Key words: bread with- Cancer- safflower- wheat flour

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تأثير الخبز المدعم بعشبة العصفر علي سرطان الكبد الناتج عن البنزوبيرين فى الفئران

الملخص العربي

أجريت الدراسه الحاليه للتعرف علي التائثير الوقائي لعشبه العصفرضد سرطان الكبد الناجم عن الإصابة بمادة البينزوبيرين٠اجريت هذه الدراسةعلى سته و ثلاثون من ذكور الفئران البيضاء وقسمت الى مجموعتين رئيسيتين٠المجموعة الرئيسية الأولى(٦فأر)تغذت على الوجبة القياسية وظلت كمجموعة ضابطة سالبة، اما المجموعة الرئيسية الثانية(٣٠فأر)تغذت على الوجبة القياسية مع الحقن بمادة البينزوبيرين (١٠٠٨ملجم/كجم)لاحداث سرطان الكبد ثم قسمت الى اربع مجموعات فرعية (٦فأر)على النحوالتالى:المجموعة(١)تغذت على الوجبة القياسية وظلت كمجموعة ضابطة موجبة. و(٣,٤,٥,٦) والتي تغذت على الوجبة القياسية بالإضافة الى الغذاء المحتوى على٢٠%من الخبز القالب المدعم بعشبة العصفر بنسب مختلفة ٤٠.٣٠.٢٠ % لمدة ٢٨ يوم. أظهرت النتائج ان المجموعات المصابة بسرطان الكبد الناتج عن الإصابة بمادة البينزوبيرين زيادة معنوية عالية في سيرم ALP،ALT،AST، الدهون الكليه، الكوليسترول الكلي، الدهون الثلاثيه،LDL، اليوريا ،الكرياتنين،MDA،وعامل نخر الورم TNF،α.في حين زياده وزن الجسم وتناول المأخوز من الوجبه ، ونسبه كفائه الغذاء، البروتين الكلي ،HDL،SOD.وانخفضت مضادات الاكسده الكليه بشكل ملحوظ مقارنه بالمجموعه الكنترول الموجبه .بينما المجموعات التي عوملت بالخبز المدعم بالعصفر خفضت من الاثار السلبيه وادت الي تحسن في المعايير الكيموحيويه ، وكانت افضل النتائج التي تناولت الخبز القالب المدعم بعشبه العصفر عند مستوي عالي من التركيز ٤٠%ثم يليه٣٠%ويليه ٢٠%ثم يليه الخبز الكنترول الغير مدعم ١٠٠%بدقايق القمح. وفي النهايه أظهرت النتائج ان عبه العصفر لها تأثير وقائي قوي ضد الاصابه بسرطان الكبد الناجم عن ماده البينزوبيرين ،كما أوضحت النتائج ان تاثير الخبز القالب المدعم بسحوق العصفر علي سرطان الكبد يمكن ان يرجع الي ارتفاعه في مضادات الاكسده وكاسح أيضا للشقوق الحره في الجسم.