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Pumpkin Seeds
on Reducing the Spread of
Parasites among the Female
Preparatory Stage Students in
Sharkia**



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**The Effect of dried Pomegranate seeds and
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Abstract:

The current study aim is to study the effect of dried pomegranate seeds and pumpkin seeds anti parasites among the female preparatory students in Sharkia governorate, Egypt. This study was conducted on thirty female students age (13 years), classified into two groups (n=15) the study was assigned for eight weeks. Determination of some blood analysis such as CBC and liver functions as well as stool investigation were performed to detect parasites, Results showed the effect of dried pomegranate seeds and pumpkin seeds clearly to improve CBC analysis, especially dried pomegranate seeds which led to increase the Hb, Iron and Ferritin in the blood. In addition, parasites have been significantly affected, as they have reduced or eliminate completely in stool, causing the use of pomegranate and pumpkin seeds. Based on this result, it shows the vital role of dried pomegranate and pumpkin seeds to eliminate parasites as well as improve the overall health of girls.

Key words: Parasites, CBC, Liver function, Stool, pomegranate seeds, Pumpkin seeds.

Introduction:

Parasitic diseases have a significant influences great diversity of people in the world, according to reports from United States Centers for Disease Control and Prevention (CDC), which confirms the presence of more than a billion person infected at any one time (CDC 2017).

Parasites have a marked negative effect on children with depressed growth in a meta-analysis (Hall *et al.*, 2008). Intestinal parasitic infections are distributed virtually throughout the world, Amoebiasis, ascariasis, hookworm infection, and trichuriasis are among the ten most common infections in the world, in many countries blood loss, impaired work

capacity, and reduced growth rate due to intestinal parasitic infections constitute important health and social problems. Furthermore, other parasitic infections such as abdominal angiostrongyliasis, intestinal capillariasis, and strongyloidiasis are of local or regional public health concern (**WHO, 1987**).

Parasitic infections are a major public health problem worldwide; particularly in the developing countries and constituting the greatest cause of illness and disease (**Mehraj *et al.*, 2008**). These infections are usually associated with poor sanitary habits, lack of access to safe water and improper hygiene. The degree of each factor and the prevalence of infections vary from one region to the other (**Zagloul *et al.*, 2011**). The knowledge of intestinal parasitic infection extension in a given community is crucial for planning an efficient intervention programs.

A major problem medicinal chemists have synthesized a number of drugs which can be used against many is that many of these drugs were developed many years ago and some parasitic strains have become resistant to them. The development of new antiparasitic drugs has not been much of a priority for the pharmaceutical industry because many of the parasitic diseases occur in poor countries where the populations cannot afford to pay a high price for the drugs. Thus an investment in drug development against parasitic diseases is a risky affair.

A good starting point to find antiparasitic natural products would be traditional medicinal plants, such as those known from Asia, Africa or America (**Van Wyk and Wink 2004**). that have been employed to treat infections.

– Pomegranate (*Punicagranatum L.*) is one of the oldest known edible fruit that contains the highest concentration of total polyphenols in comparison with other fruits studied (**Fazaeli et al., 2013**). The seeds represent for 22% of total fruit (**Mohagheghi, et al., 2011**). Pomegranate has been known as a medical applications since ancient times, especially in Egypt, it was used for curing inflammation, diarrhea, intestinal worms, cough and infertility (**Lansky and Newman, 2007**).

Medjakovic and Jungbauer, (2012) showed that, pomegranate play important role as anti-parasitic properties and has been well applied as a blood tonic. Pumpkinseeds (*Cucurbitamoschata*) are easily available locally and rich sources of unsaturated oil, energy and vitamin E, (**Karanja et al., 2013**). The pumpkin seeds are used as a vermifuge, galactogogue, and anti-emetic, and to treat various other medical issues, in some countries (**Caili et al., 2006**). It is also traditional Chinese remedy for treatment of castodiasis, ascariasis and schistosomiasis (**Koik et al., 2005**).

Diaz et al. (2004) showed the anthelmintic anti-parasitic activity of pumpkin seeds.

Subject and Methods:

This study was carried out on thirty female students in the preparatory stage in sharkia governorate as a purposed sample. Blood and stool have taken before the start of experiment, female student's ≥ 13 years of age. The subject was randomly assigned into two groups (15 of each).

–Subject of Design:

-Material:

- Pomegranate (*Punicagranatum L*) was purchased from the Local market in Sharkia Governorate.
- Pumpkin (*Cucurbitamoschata*) dried samples of seed pumpkin was obtained from Haraz druggist (Cairo, Egypt).

-Methods:

I-Preparation of pomegranate seeds:

Pomegranate was cleaned with clean water, and leaved it for seeds, then dry it in a drying oven at 60C for 3 days, finally grumbled to become a powder .

11- Experimental design;

All female students infected with parasites were enrolled in the study and randomly classified into two groups as following:

Group (I): Treated with 5g/daily of dry Pomegranate seeds before breakfast.

Group (II): Treated with 10g/daily of Pumpkinseeds before breakfast.

III- Laboratory investigation:

These laboratory investigations were evaluated before, middle and after the experiment as following:

1-Stool analysis:

Stool samples were obtained from subjects at three time (zero time, middle and final stage) then, the analysis and detection of parasites

was carried out according to the method described by **Giovanna Raso et al., (2004)**.

2- Chemical Composition of Pumpkin seeds: Moisture, protein, fat, fiber and ash contents were determined in pumpkin seeds according to **ISOIEC 17025 - (2005)**. Carbohydrate was calculated by difference according to the equation of **Chatfield and Adamas (1940)**.

3-Blood analysis (CBC): Red blood cells count (RBC), White blood cells count (WBC), hemoglobin (Hb) and platelets count (PLT) were determined according to the method described by **Dacie and Lewis, (1998)**. WBCs (total and differential) were determined according to the method described by **Koda-kimble et al., (2001)**.

4- Determination of Liver Enzymes activity:

AST and ALT were determined according to **Chawla, (2003)** and **Srivastava et al., (2002)**.

IV- Statistical analysis Enzymes:

The obtained data was statistically analyzed by SPSS program, version 14. Results were presented as mean \pm SD by T. test analysis used to determine if there is a significant difference between the means of the beginning and end of the experiment at $p < 0.05$ and $p < 0.01$ (**Spss, 2000**).

Results and discussion:

Table (1): Proximate composition % dry basis of dried pomegranate and pumpkin seeds

Constituents	dried pomegranate seeds	pumpkin seeds
Protein(g/100g)	6.39g	39.6 g
Crude fiber(g/100g)	9.10g	26.7 g
Fat(g/100g)	2.75g	44.4 g
Ash(g/100g)	2.99g	5.1 g
Moisture(g/100g)	7.39	16.5 g
Total carbohydrates	80.48g	5.6 g

Table (1) summarized the results of chemical composition of dried pomegranate seeds. The values of dried pomegranate seeds chemical composition were 6.39 g , 9.10 g , 2.75g , 2.99 g, 7.39 g and 80.48g for Protein, crude fiber, fat, Ash, Moisture, and Total carbohydrates respectively. Total carbohydrates =100- (protein + Fat + Ash +Moisture fiber) **Merrill et al. (1973)**. These results agree with **AOAC (2012)** recorded of pomegranate seeds were rich in carbohydrates, crude fiber, Moisture, carbohydrates and Protein.

Also, Table (1) Summarized the results of chemical composition of pumpkin seeds (dry basis). The values of pumpkin seeds chemical composition were 39.6 g, 26.7g, 44.4 g, 5.1 g, 16.5 g and 8.67 g Protein, crude fiber, fat, Ash, Moisture, and Total carbohydrates respectively. Total carbohydrates =100- (protein + Fat + Ash +Moisture + crude fiber) **Merrill et al. (1973)**. These results agree with **ISOIEC**

17025–(2005) that reported of pumpkin seeds were rich in fat, Protein and crude fiber.

Table (2): Detection of parasites in stool for three stages (zero time, middle and final stage) of group1 (dried pomegranate seeds)

Zero time			The middle stage			Final stage		
Cysts1	Ova1	Larva1	Cysts2	Ova2	Larva2	Cysts3	Ova3	Larva3
Entamoeba (+)								
Entamoeba (++)				Enterobius (+)				
	Ascaris (+)			Ascaris (+)			Ascaris (+)	
Entamoeba (+)								
Entamoeba (+)	Ascaris (+)							
Entamoeba (++)	Ascaris (+)							
		Ascaris (+)	Entamoeba (+)	Enterobius (++)			Enterobius (++)	
Entamoeba (Few)								
Entamoeba (+)				Ascaris (+)				
Entamoeba (++)				Ascaris (+)			Ascaris (+)	
Entamoeba (+)								
Entamoeba (++)				Ascaris (+)				

	Enterobius (few)							
Entamoeba (++)				Ascaris (+)				
	Ascaris (+)			Ascaris (+)			Ascaris (+)	

Table (2) presents the result of analysis to detect the presence of parasites in the stool for three stages (zero time, middle and final stage) of group 1 who ate dried pomegranate seeds. A clear improvement can be noticed elimination or reduction of parasites in the stool as a result of eating dried pomegranate seeds. Entamoeba (few) in cysts 1 was 6.7% of subject in zero time while in cysts 2 and cysts 3 were not present 0%. Whereas Entamoeba (+) in cysts 1 was 33.3 %, then in cysts 2 became 6.7% of subject and in the last stage, it has clearly decreased (0) %.

As for entamoeba (++) in cysts 1 was 33.3% but in cysts 2 and cysts 3 was 0%. This result agree with **Amrutesh, (2011)** who reported that pomegranate bark and flower infusions treat diarrhea, intestinal worms, bleeding noses and ulcers . From the same table, it can noticed that, the ratio of Ascaris (+) in Ova 1 was 26.7% then, increased by 40% in ova 2 but the percent of presence of ascaris (+) in ova 3 reached to 20%, this finding demonstrates the role of pomegranate seeds to reduce parasites . with respect to ascaris (+) in larva 1 was 6.7 %, but it was affected by eating dried pomegranate seeds in the two stages (larva 2 and 3) where they completely disappeared from the stool.

Pomegranate has risen to fame for its medical applications since ancient times. Pomegranate has been regarded as the most significant medication even in the writings of Hippocrates. Health beneficial properties of pomegranate were known to ancient civilizations. In Egypt, it was used for curing inflammation, diarrhea, intestinal worms, cough and infertility Lansky and Newman, (2007).

Table (3): Detection of parasites in stool for three stages (zero time, middle and final stage) of group2 (pumpkin seeds)

Ziro time			The middle stage			Final stage		
Cysts1	Ova1	Larva1	Cysts2	Ova2	Larva2	Cysts3	Ova3	Larva3
Entamoeba (+)								
Entamoeba (++)			Entamoeba(+)					
		Ascaris		Ascaris (+)			Ascaris (+)	
Entamoeba (few)	Ascaris (few)	(few)						
Entamoeba (++)				Ascaris (+)				
Entamoeba (+)	Ascaris (few)							
Entamoeba (+)			Entamoeba(+)			Entamoeba(+)		
Entamoeba (+)	Enterobius (+)	Ascaris		Ascaris (+)				
Entamoeba (+)	Ascaris (+)	(few)						
Entamoeba (+)								
	Ascaris (+)							
Entamoeba (+)	Enterobius (+)			Enterobius (+)			Enterobius (+)	
Entamoeba (++)				Ascaris (+)			Ascaris (+)	
	Enterobius (++)						Ova3	
Entamoeba (++)	Enterobius (+)		Cysts2	Ova2		Cysts3		

Table (3) presents the result of analysis to detect the presence of parasites in the stool for three stages (zero time , middle and final stage) of group 2 who ate pumpkin seeds, it can noticed that elimination or reduction of parasites in the stool as a result of eating pumpkin seeds, pumpkin seeds are a potential vermifuge. Eaten fresh or roasted, they help relieve abdominal cramps and distension due to intestinal worms (**Cailli et al., 2006**).

As for entamoeba (few) in cysts 1 was 6.7 % in zero time while in cysts 2 and cysts 3 were not present (0%) .

Whereas entamoeba (+) in cysts 1 was 46.7%, then decreased to 13.3% and 6.7% in cysts 2 and the last stage respectively.

As for Entamoeba (++) in Cysts 1 was 26.7 %, but in Cysts 2 and Cysts 3, it has clearly decreased. This result agrees with **Diaz et al., (2004)** who showed the anthelmintic anti-parasitic activity of pumpkin seeds.

From the same table, it can noticed that, the ratio of ascaris (Few) in ova1 was 13.3 % while in ova 2 and ova 3 were not present (0%).

With respect to ascaris (+) in ova 1 was 13.3% pumpkin seeds did not have the effect of decreasing ascaris(+) but on the contrary, the percentage of presence of ascaris (+) in ova 2 reached 26.7% and then decreased to 13.3 % in last stage (ova 3). Also ascaris (Few) in larva1 was 13.3% while in larva2 and larva3 were not present (0%). This result shows a strong pumpkin seeds effect to eliminate ascaris. Pumpkin seeds are also an important traditional Chinese medicine in the treatment of cestodiasis, ascariasis, and schistosomiasis (**Koike et al., 2005**). In

Middle America, a vermifugal preparation containing shelled and powdered pumpkin seeds is used to expel intestinal worms, including tapeworms **Morton, (1981)**.

With respect to enterobius (+) in ova 1 was 6.7% but it was affected by eating pumpkin seeds in the two stages (ova 2 and 3), where they completely disappeared from the stool. While enterobius (++) in ova 1 was 6.7%, but in ova 2 and ova 3 were not present (0%).

Table (4): Mean \pm SD of CBC analysis for group 1 (dried pomegranate seeds) before and after the experiment

Parameters	Before	After	P.Value	Sig
Hb	10.427 \pm 2.755	11.540 \pm .546	.127	N.S
RBCs	4.167 \pm .302	4.340 \pm .331	.158	N.S
Ferretin	22.13 \pm 10.134	24.93 \pm 11.386	.003	*
Iron	54.40 \pm 8.253	58.87 \pm 8.887	.005	*
Platelet	279.87 \pm 84.908	254.27 \pm 68.993	.011	*
WBCs	6.587 \pm 1.935	6.673 \pm 2.587	.780	N.S
Basiophil	.133 \pm .163	.000 \pm .0000	.007	*
EOsinophil	3.07 \pm .884	2.33 \pm .488	.016	*
Monocytes	5.98 \pm 1.75	2.67 \pm 1.404	.000	**
Lymphocytes	40.593 \pm 7.149	37.62 \pm 5.671	.302	N.S
Neutrophil	50.31 \pm 8.201	59.23 \pm 5.113	.000	**

N.S = non-significant S.D = Standard Deviation, * = Significant at P < 0.05 ** = Significant at P < 0.01

The results in table (4) showed a significant difference in some analyses between before and after eating dried pomegranate seeds.

As for Hband RBCs level, increased insignificantly after eating pomegranate seeds. It can notice that the ferretin level increased significantly by 12.7 % compared to the beginning of the experiment.

In the same context, the rate of iron increased significantly at the end of the experiment by 8.22 %, while Platelet level decreased significantly at $p < 0.05$ compared to the beginning of the experiment. This result means that pomegranate contributes to the increase of iron stocks in the body and agree with some studies indicated to pomegranate aril juice provides about 16% of an adult's daily vitamin C requirement per 100 ml serving, and is a good source of vitamin B5 (Pantothenic acid), potassium and polyphenols, such as tannins and flavinoids (**Hotez et al., 2008**), (**WHO 2005**), (**WHO 2008 and Belizario et al., 2009**).

On the other side, Neutrophil increased significantly at end of the experiment from $(50.31 \pm 8.20$ to $59.23 \pm 5.11)$ respectively. Eosinophil (acidophils) are a variety of white blood cells and one of the immune system components responsible for combating multicellular parasites and certain infection in vertebrates. Monocytes influence the process of adaptive immunity. The efficacy of pomegranate extract was tested in *Vivo* and it was found to exhibit a potent protective activity in acute oxidative tissue **Kaur et al., (2006)**.

Also helps to reduce symptoms of anemia **Easton A. (1999)**. Regarding Basophil as a type of white blood cells decreased significantly by 100 % after eating pomegranate seeds throughout the experiment. Also, Eosinophil and Monocytes recorded a significant decrease in their level at the end of the experiment by 24.10% and 55.35% respectively .

Table (5): Mean \pm SD of CBC analysis for group 2 (pumpkinSeeds) before and after the experiment

Parameters	Before	After	P.value	Sig
Hb	10.740 ± .821	11.187 ± .789	.032	*
RBCs	4.220 ± .472	4.207 ± .417	.881	N.S
Ferretin	30.93 ± 15.540	33.60 ± 15.747	.000	**
Iron	55.73 ± 12.068	59.13 ± 10.569	.009	*
Platelet	263.00 ± 65.603	280.93 ± 80.423	.294	N.S
WBCs	6.467 ± .874	6.056 ± .832	.143	NS
Basiophil	.520 ± .365	.000 ± .000	.000	**
EOsinophil	3.99 ± .487	2.47 ± .516	.000	**
Monocytes	7.69 ± 2.235	2.77 ± 1.075	.000	**
Lymphocytes	43.120 ± 2.792	44.26 ± 6.695	.531	N.S
Neutrophil	44.25 ± 3.825	51.79 ± 6.260	.000	**

S.D=Standard Deviation, * = Significant at P < 0.05 ** = Significant at P < 0.01 N.S = non-significant

The result in table showed a significant difference in some analyses between before and after eating pumpkin seeds.

It can notice that the hemoglobin level increased by 4.16 % compared to the beginning of the experiment. In the same context, the rate of Ferretin and Iron increased significantly at the end of the experiment (8.63 % and 6.10%, respectively). This results agreed with *Lazos, (1986)* who showed that, Pumpkin seeds are excellent sources of both oil (37.8–45.4%) and protein (25.2–37%). Regarding Basiophil, decreased significantly by 100 % after eating pumpkin seeds throughout the experiment

Also, Eosinophil and Monocytes recorded a significant decrease in their level at the end of the experiment by (38.09% and 63.98 %, respectively).

On the other side, Neutrophil increased significantly at end of the experiment from (44.25 ± 3.825 to 51.79 ± 6.260). This results agreed

with Iwo, *et al.*, (2014) and Matthews *et al.*, (2016) who showed that Pumpkin seeds have received considerable attention because of their health protective values, such as anti cholesteremic, antioxidant, anticancer, and anti-inflammatory effects.

There were no significant differences in the analysis of RBCs, Platelet, WBCs, and lymphocytes.

Table (6): Mean \pm SD of liver function analysis for group 1 (Dried Pomegranate Seeds) before and after the experiment

LIVER.F	Before	After	P.value	Sig
Albumin	5.140 \pm .213	5.007 \pm .175	.003	*
ALT	17.200 \pm 1.207	17.067 \pm 1.099	.670	N.S
AST	19.333 \pm .976	18.467 \pm .990	.007	*
Direct Bilirubin	.120 \pm .041	.153 \pm .052	.055	N.S
Total Bilirubin	.627 \pm .158	.693 \pm .103	.019	*

SD= Standard Deviation, N.S= non-significant, *=significant at $p < 0.05$

From the result recorded in Table (6) a significant changes were found in liver function analyzes before and after intervention for girls who ate (dried pomegranate seeds), it can noticed that, albumin and AST level decreased significantly ($P < 0.05$) by 2.59% and 4.48% compared to the beginning of the experiment. While bilirubin level increased significantly at the end of experiment by 10.5%. on the other side, ALT and Direct

bilirubin did not record significant differences between the beginning and end of the experiment. **Kaur *et al.*, (2006)** reported that pomegranate flowers to possess potent antioxidant and hepatoprotective property .

This result agree with that the pomegranate seeds as anti tumoral (**Hamad and Al-Momene, 2009**) or anti hepatotoxic (**Celik *et al.*, 2009**) and improve cardiovascular health (**Davidson *et al.*, 2009**).

Table (7): Mean \pm SD of liver function analysis for group 2 (pumpkin seeds) before and after the experiment

Liver .F	Before	After	P.value	Sig
Albumin	4.927 \pm .215	4.980 \pm .126	.418	N.S
ALT	17.467 \pm 1.246	17.667 \pm .724	.550	N.S
AST	18.067 \pm 2.120	17.867 \pm 1.125	.619	N.S
DirectBilirubin	.160 \pm .051	.147 \pm .052	.546	N.S
Total Bilirubin	.653 \pm .125	.660 \pm .074	.792	N.S

SD= Standard Deviation, NS= non-significant

Data in Table (7) revealed that no significant changes were found in liver function analyzes before and after intervention for girls ate pumpkin Seeds.

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تأثير بذور الرمان المجفف وبذور القرع علي الحد من انتشار الطفيليات لطالبات المرحلة
الإعدادية في محافظة الشرقية

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ملخص البحث

تهدف الدراسة لمعرفة تأثير بذور كلا من الرمان المجفف والقرع المجفف المضادة للطفيليات بين طالبات المرحلة الإعدادية في محافظة الشرقية بمصر. وأجريت الدراسة على ثلاثين طالبة قسمت إلى مجموعتين بكل مجموعة (15) طالبة (لمدة ثمانية أسابيع. وتم إجراء بعض التحاليل المعملية للدم مثل (صورة الدم كاملة، ووظائف الكبد) وفحص لعينات من البراز للتعرف على الطفيليات الموجودة، وقد أظهرت النتائج تحسن في تحليل الدم للطالبات الأتى تناولنا بذور الرمان المجفف وبذور القرع على وظهر تحسن ملحوظ للمجموعة المعالجه ببذور الرمان المجفف. أدى إلى ارتفاع ملحوظ في نسبة الهيموجلوبين والحديد في الدم، وأكد ذلك أيضاً معدل انتشار الطفيليات بالبراز وانخفاض ملحوظ بالمجاميع المعالجه ببذور الرمان المجفف وبذور القرع. وأوضحت الدراسة الدور الحيوى لبذور الرمان المجفف وبذور القرع في الحد او الإقلال من نشاط الطفيليات مما يؤكد دورهم الفعال المضاد للطفيليات بالإضافة إلى تحسن كلى في صحة البنات.

الكلمات الدالة: الطفيليات المعوية، بذور الرمان المجفف، بذور القرع - CBC Stool - Liver function -