## Anticestodal activity of the crude aqueous extract of pumpkin seed (Cucurbita pepo) against the dwarf tapeworm (Hymenolepis nana) in mice

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#### Abstract

The anticestodal activity of the crude aqueous extract of *Cucurbita pepo* seeds was carried out on mice experimentally infected with *Hymenolepis nana*. Significant effects were obtained by reducing the number of eggs and worms when compared with the control group. Five concentrations of *C. pepo* aqueous extracts were used (125, 250, 375, 500 and 625 mg/kg). These concentrations produced deparasitization rates of eggs of 52.7/, 61.6/, 84.2/, 89.4/ and 100/, respectively at the fourth day of the treatment. Also, more potent

effects were obtained with highest concentrations especially 500 mg/kg and 625 mg/kg which gave a total eradication of H. nana eggs and worms at the fifth and fourth days of the experiment, respectively.

### Introduction

*Hymenolepis nana*, the dwarf tapeworm, commonly infects both human beings and rodents (1). It is more common in children than in adults, and of high frequency in certain families and institutions and family groups (2). The mode of transmission of this tapeworm is by ingestion of contaminated grain and flour with beetles and by autoinfection which is very common in children in day-care centers (3). *H. nana* is one of the important pathological intestinal parasites and children are especially at risk of infection (2).

The major control strategy adopted against helminth parasites is the use of anthelmintics (4). However, the high cost of modern anthelmintics has limited the effective control of these parasites. In some cases, widespread intensive use of sometimes low quality anthelmintics (5), has led to the development of resistance and hence a reduction in the usefulness of available anthelmintics (6). Although the use of alternate drugs was also advocated as a measure to avoid the development of resistant strains of helminth parasites, and as a mean of reducing the cost of controlling helminthic diseases (7), the emergence of resistant strains of pathogenic helminths have stimulated the desire to search for additional chemotherapeutic agents that might allow more efficient control of helminth parasites (6,8). A practical solution to this is to develop effective drugs from reasonably less expensive and available raw materials. Therefore, there has been an increased interest in natural plant derived broad spectrum toxicants (9,10).

Seeds of gourd (*Cucurbita pepo*) have principal medical properties. They are used for diuretic, tonic, fattening cure, sore chests, bronchitis, fever, allays and they are good for

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kidney and brain (9). The aim of this study was to test the anticestodal activity of crude aqueous extract of C. pepo seeds against experimental *H. nana* infection in mice.

#### **Materials and Methods**

Animals: Male BALB/c mice, weighing between 18-26 gm, were obtained from the Animal House of College of Medicine at Al-Nahrain University. The mice were used at 8-10 weeks of age. These animals were housed under convenient conditions with food and water provided *ad libitum*. The total number of mice used in experimental treatment was 60 mice.

Plant collection and preparation: The seeds of *C. pepo* were obtained from a local market in Baghdad. They were dried and powdered with a grinder. An amount of 50 gm of seed powder was dissolved in 500 ml of distilled water and the mixture was boiled for 45 minutes. Then, it was kept a side off the hot plate for 30 minutes to allow it to infuse. After that, it was filtered through a cheese cloth. The filtrate was centrifuged at 750g for ten minutes. Then, it was filtered through a filter paper (Whatman No. 1). The liquid extract was evaporated to dryness in a vacuum by using a rotary evaporator coupled to a thermoregulator. The solid extract obtained was refrigerated at 4 C° until required (4).

Evaluation of tolerated doses of the extraction: The dose that did not produce any sign of toxicity (referred to as maximum tolerated doses; MTD) was determined orally by administering serial doses (100-700 mg/kg) of the extract to mice.

Experimental design: Adult worms of *H. nana* were collected in 0.9? warm PBS (pH =7.4) from intestine of previously infected mice which were maintained in laboratory. Eggs and gravid segments were taken from them. Each of the 60 mice was infected orally by stomach tube. Dose of infection was determined as 2000 eggs in 0.1 ml of 0.85? NaCl.

Ten days post infection, fresh faecal samples from each infected mice were collected by squeezing them out of the rectum and were examined by simple flotation. Mice not shedding ova of *H. nana* were discarded from the experiments.

Anticestodal activity was determined by administering various doses of the crude aqueous extract of *C. pepo* orally to the mice group. Group 1, 2, 3, 4 and 5 (treatment groups) received the extract at 125, 250, 375, 500 and 625 mg/kg, respectively, while group 6 served as control and received normal saline via the same route. All treatment doses were given after three days of infection and for six days. Eggs were counted daily from feces while worms were also counted after three days from the beginning of the treatment and the end of the experiments (after six days) by killing and dissection of five mice from each group. Percentage of deparasitization was calculated by using the formula (11):

Deparasitization (/) = [  $N-n/N \times 100$  ]

Where: "N" and "n" represented number of eggs or adult worm found in untreated control mice and treated mice, respectively.

Statistical analysis: Eggs and worms were expressed as mean  $\pm$  SE. The significance of difference between the means was determined by student t-test using a computer software package (SPSS for Windows Release 6.0) and regarded as significant when p > 0.05.

### Results

The results of *in vivo* treatment of *H. nana* (eggs and adult worms) to different concentrations of plant extracts are given in tables (1 and 2). The extracts exhibited a significant anticestodal effect (p > 0.05) when they reduced sharply the number of eggs and worms, especially the highest concentrations (500 and 625 mg/kg) which gave total eradication from infection on the day 5 and 4, respectively of the experiments.

There was a significant difference (p > 0.05) in the number of eggs and worms between treated mice and the control which still showing an increase in growth of the worms (Tables 1 and 2). Lower concentrations also gave good response in reducing the number of eggs and worms when compared with untreated group.

Deparasitization rates are demonstrated in Tables (3 and 4). The extracts produced a consistently high anticestodal effect (p > 0.05). At concentrations of 500 and 625 mg/kg, deparasitization rates were 100% at day 5 and 4, respectively. The rate continued in decrease with the increase concentration in and duration of the experiments (Table 3).

Table (4) showed deparasitization rates on number of worms at the third and sixth day of experiments. All concentrations gave a good suppressive effect on worm burden and they were all significant (p > 0.05) when compared with the control.

#### Discussion

The phytochemical analysis (seeds of *Cucurbita pepo* demonstrated the occurrence of triterpenes (cucurbitacins), carotenoids, fatty acids, minerals, tocopherol and lignins (12). Studies have shown that pumpkin seeds are potents of the drug indomethacin relieving chronic rheumatoid arthritis (13). It is likely that this effect is due to the essential fatty acid profiles, rich antioxidant content and the synergistic effects of other minor components that pumpkin seeds have such as high levels of vitamin E, including all forms of the tocopherol family (13,14). It was reported by (15) that pumpkin seeds were used as anti-inflammatory and cardioprotective activity. The extracts of pumpkin seeds suppressed mitogen- induced neopterin production and tryptophan degradation in a dose-dependent way (13,15). Data demonstrated capacity of pumpkin extracts to modulate immunobiochemical pathways induced by interferon- $\gamma$ . Findings imply an immunoregulatory potential of compounds contained in pumpkin seed (15).

There were signals in public medicine and researches that *Cucurbita* was used as antimicrobial, insecticidal and antiparasites. In a related study on crude ethanolic extracts (CEE), two species of Cucurbitaceae (*C. maxima and Momoridica charantia*) were assayed for antimalarial activity. The CEE of dry *C. maxima* seeds showed strong antimalarial activity in infected mice (16). Extracts of some commonly used plant materials of ethanoveterinary importance in Pakistan, screened for their *in vitro* anthelmintic activity, revealed that the aqueous and ethanol extracts of *C. maxima* seeds have exhibited good anthelmintic activity against *Moniezia expansa, Fasciolopsis buski*, Ascaris *lumbricoides* and *Hymenolepis diminuta* (17). In a study on *in vitro* effects of *C. pepo* on many microorganisms, (18) reported that *C. pepo* aqueous extract showed significant antimicrobial activity on *Bacillus subtilis, Escherichia coli, Aspergillus niger* and *A. flaves*. Human studies conducted in China have shown that pumpkin seeds are helpful for people with acute schitosomiasis (15). Significant in vitro effect of extract of *C. pepo* on the motility of mature *Haemonchus contortus* of sheep was reported (19). Also, pumpkin seeds showed high effect on pig nodular worm *(Oesophagostomum spp.)* (20).

The seeds of *C. pepo* are noted as an affective remedy against Taenia, a parasitic worm that is paralyzed by the action of cucurbitine (3- amino- 3- carboxypyrrolidine) contained in the skin of the seeds (9). Cucurbitine is an amino acid that has shown antiparasitic activity in vitro (20,21). Cucurbitine possesses an unusual amino acid which is the main and most active chemical principle essential for antihelmintic capability of eliminating worm activity displayed by the pumpkin seeds remedy. This acid is concentrated only in the seeds of *Cucurbita* species (22,23).

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Table (1): Mean daily eggs counts ( $\pm$  SE) in mice infected with H. nana and treated with various doses of crude aqueous extract of C .pepo seeds.

Dose Day	125 mg/k g	250mg/kg	375 mg/k g	500 mg/k g	625mg/kg	control
1	411 ± 7.54	372.2±29.3	323.2±5.8	301±15.16	201 ± 7.41	496 ± 15.16
2	380.4±7.63	333 ± 4.69	265.2±7.92	209 ± 2.64	127.8±7.69	508 ± 5.43
3	327.8±7.72	300.6±4.72	179 ± 5.87	113 ± 2.91	60.6±4.92	524.2 ± 3.76
4	277.2±9.83	224.8±6.49	92.4 ± 5.77	62.2 ± 4.96	0	586 ± 9.61
5	259.8±4.32	102.8±2.58	$41.2 \pm 6.68$	0	0	570.4 ± 7.95
6	193.2±2.38	95.4±3.84	10.2 ± 1.92	0	0	563.4±4.77

Table (2): Mean daily worms counts ( $\pm$  SE) in mice infected with H. nana and treated with various doses of crude aqueous extract of C .pepo seeds.

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125mg/kg	250mg/kg	375 mg/kg	500 mg/kg	625mg/kg	control
$17 \pm 4.14$	15.2±1.92	10.66±1.63	7±2.52	3.16±1.6	20±1.78
$11 \pm 2.34$	$5.2 \pm 1.3$	$1.16 \pm 0.75$	0	0	25±3.74
	125mg/kg 17±4.14 11±2.34	125mg/kg 250mg/kg   17±4.14 15.2±1.92   11±2.34 5.2±1.3	125mg/kg 250mg/kg 375mg/kg   17±4.14 15.2±1.92 10.66±1.63   11±2.34 5.2±1.3 1.16±0.75	125mg/kg   250mg/kg   375mg/kg   500mg/kg     17±4.14   15.2±1.92   10.66±1.63   7±2.52     11±2.34   5.2±1.3   1.16±0.75   0	125mg/kg   250mg/kg   375mg/kg   500mg/kg   625mg/kg     17±4.14   15.2±1.92   10.66±1.63   7±2.52   3.16±1.6     11±2.34   5.2±1.3   1.16±0.75   0   0

Table(3): Effect of various doses of crude aqueous extract of C .pepo seeds on deparasitization rate of eggs of H. nana

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Dose	125mg/kg	250mg/kg	375 mg/kg		625mg/kg
Day				500mg/kg	
1	20.7%	33.3%	34.9%	39.3%	59.5%
2	25.2%	34.4%	47.8%	58.9%	74.8%
3	37.5%	42.7%	65.9%	78.4%	88.4%
4	52.7%	61.6%	84.2%	89.4%	100%
5	54.5%	82.0%	92.8%	100%	100%
6	65.7%	83.1%	98.2%	100%	100%

Dose Group	125 mg/kg	250mg/kg	37 5m g/kg	500mg/kg	625mg/k g
After 3 days	15%	24%	46%	65%	84.2%
After 6 days	56%	79.2%	95.4%	100%	100%

Table (4): Effect of various doses of crude aqueous extract of C .pepo seeds on deparasitization rate of worms of H. nana

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# الفعالية الطاردة للديدان الشريطية للمستخلص المائي الخام لبذور القرع Hymenolepis ضد الدودة الشريطية القزمة Cucurbita pepo في الفئران

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#### الذلاصة

درست النعالية الطاردة للديدان الشريطية للمستخلص المائي الخام لبذور نبات الترع *Cucurbita pepo في* النئران المصابة مختبريا بالدودة الشريطية *Hymenolepis nana و*قد تم الحصول على تأثيرات معنوية من خلال تقليل أعداد البيوض والديدان عند مقارنته مع مجموعة السيطرة.استعملت خمسة تراكيز من المستخلص المائي الخام لنبات C.pepo وهي ( 125 ، 250 ، 375، 500 و25 6ملغم /كغم)، وسجلت هذه التراكيز نسبا لطرد الطنيليات وهي(7,52 % ، 6, 16% ، 2,84 % ، 4,88% و 100%) وكانت ذا قيمة معنوية عند مستوى احتمالية 50,0 حو لدى مقارنتها بمجموعة السيطرة ( غير المعالجة) عند اليوم الرابع من العلاج. كما أظهرت التراكيز العالية ولاسيما 525 و 100ملغم /كغم فعالية اكبر في طرد جميع الطنيليات سواء البيوض أو الديدان البالغة عند اليوم الرابع والخامس للتجربة على التوالي. قسم علوم الحياة، كلية التربية (الرازي)،جامعة ديالى،ديالى،العراق \* قسم علوم الحياة، كلية التربية ( ابن الهيثم)، جامعة بغداد، بغداد، العراق