Feed efficiency, some blood parameters and In-vitro chemoprevention of prickly pear (Opuntia ficus indica L.) seeds oil growing in Egypt

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Although chemoprevention has shown promise in some epithelial cancers, currently available preventive agents are limited and the agents are costly, generally with side effects. Cancer chemoprevention is a new approach in cancer prevention, in which chemical agents are used to prevent cancer in normal and/or high-risk populations. To find out the best natural product that can be used in chemoprevention of cancer, we tested the hexane extract of prickly pear seeds, for its feed efficiency, some blood profiles and anticancer effects in cultured cells and in an animal model. The objective of this research was to evaluate the effect of prickly pear seeds oil supplemented diet on rats on feed intake and body weight, feed efficiency ratio and protein efficiency ratio of rats were determined during 60 days of treatment and as well as to find a natural product that can be used in natural agent of cancer control. Hexane extract of prickly pear seeds were used to treat immortalized liver and colon cancer cells. Hexane extract of prickly pear seeds were used at six concentrations from (0.0, 0.01, 0.1, 1, 10 and 100 μm) to treat cells. Growth inhibition, apoptosis induction, and cell cycle changes were analyzed in the cultured cells; the suppression of cells growth in rats was evaluated. Immunohistochemistry staining of tissue samples from animal cells were performed to examine the gene expression. The obtained results indicated a significant decrease in serum glucose concentration (29.9%) over the control group. However, an increase in the concentration of glycogen was noted in liver. Blood cholesterol and low density lipoprotein (LDL)-cholesterol decreased in the treated group. High density lipoprotein (HDL)-cholesterol concentration remained unaltered during the treatment. These findings support the nutritional value of prickly pear seeds as a natural source of edible oil containing essential fatty acids and reinforce the possibility of prickly pear as a new crop for Egypt especially in semi-arid regions and newly reclaimed land where conventional crops are difficult to establish and Cancer cells exposed to prickly pear extracts had a significant increase in apoptosis and growth inhibition in both immortalized epithelial cells and cancer cells in a dose- and time-dependent manner.

Keywords: Prickly pear seed oil, feed efficiency ratio, glucose and glycogen, cholesterol, HDL, LDL, Rats, In-vitro natural prevention

INTRODUCTION

Opuntia ficus-indica (L.)”OVI”, a member of the Cactaceae family, is a tropical or subtropical plant originally grown in South America and cultivated in dry regions as an important nutrient and food source (Trombetta et al., 2006). In Egypt,
the fruits are consumed exclusively as fresh fruit, while the seeds are usually discarded (Saenz, 2000). The extracted pigments from prickly pear fruits are used as additives in food, cosmetic and pharmaceutical preparations (Piga, 2004).

The literature contains much information concerning the juice and cladode of prickly pear, but little on the nutritional value of the seed oil (Oliveira et al., 2001). The prickly pear seed oil composition and its chemical characteristics were investigated by (Salvo et al., 2002). In 2003, Coskuner and Tekin studied the seed composition of prickly pear fruits during the maturation period, whereas Ramadan and Morsel (2003) compared the seed and pulp oil composition. All the authors have agreed that \textit{Opuntia ficus-indica} seed oil was rich in polyunsaturated fatty acids and vitamins and may potentially be included in animal and human diets. However, data on the nutritional value of prickly pear oil are at present unknown.

The goal of cancer prevention is to delay or block the processes of initiation and progression from pre-cancerous cells into cancer. Cancer chemoprevention, which targets normal and high risk populations, involves the use of drugs or other chemical agents to inhibit, delay, or reverse cancer development (Keloff et al., 1999a and Keloff et al., 1999b). There has been significant success in the study of cancer prevention and chemoprevention in the last 20 years and, as a result, the incidences of certain types of cancer have decreased due to prevention techniques and improved screening technology (Keloff et al., 1999a and Keloff et al., 1999b). Prickly (\textit{Opuntia}) has been used for many years as a common vegetable and as medicine by the Native Americans and Mexicans (Cornett, 2000) and (Tesoriere et al., 2004). Prickly pear contains a fruit known as cactus pear (\textit{Opuntia ficus-indica}) and the plant is referred to as nopale (pad). Prickly pear contains pectin, carotenes, betalains, ascorbic acid, quercetina and quercetin derivatives all of which have antioxidant activity (Knishtinsky, 1971). In Chinese medicine, prickly pear fruit is considered a weak poison and used as medicine for treatment of inflammation and pain (Wang, 1988) and (Nanjing Pharmacologic College, 1976). It has also been used as a detoxification agent for snake bite (Wang, 1988 and Nanjing Pharmacologic College, 1976).

The main aim of this study was to evaluate the effect of prickly pear seeds oil supplemented diet on rats on feed intake and body weight, feed efficiency ratio and protein efficiency ratio of rats were determined were measured during 60 days of treatment and to find a natural product that can be used in chemoprevention of cancer.

**MATERIALS AND METHODS**

**Oil extraction**

**Seeds Pre- treatment**

The prickly pear fruits cut into thin slices and dried by solar drying system using forced circulation in the solar energy department of National Research Centre. Giza, Egypt. Afterward, dried seeds were removed manually from the flesh and get rid of the foreign materials. The seeds were washed with distilled water several times and external dried into solar drying system then grounded to fine powder with a crusher. The seeds powder was exposed to extraction processes.

The prickly pear seeds oil was extracted from the seeds powder with hexane in a Soxhlet extractor for 9 hrs. The organic phase was then removed using a rotary evaporator under reduced pressure and the pure yellow oil was flushed with a stream of nitrogen (Monia et al., 2006). Collect the demoded quantities of oil and stored at -20°C in sealed dark glass bottle.

**In vivo biological experiment**

**Principle and Housing**

To achieve the major objective of this work to evaluate the effect of the hexanic extract prickly pear seeds oil as oil supplemented diet on rats. Biological experiment lasted for two months using 18- male albino rats weighing about 130±10g each, were divided into two groups, and nine rats of each group were housed in cages, three cages for each group. The rats were three housed in stainless steel cages and maintained at 22-24°C with relative humidity 45-55%. Diet and water were freshly provided \textit{ad-libitum}. Adaptation time took three days using barley as the sole diet.

**Diets**

Two types of diets (A and B) were freshly prepared as follows: The control rats group was fed a standard diet (A) formulated according to (NRC, 1995) as illustrated in Figure 1 and the treated rats group(B) was given the lipid enriched diet with 50 g/kg diet more than (A) with hexanic extract of prickly pear seeds oil as fat source to prepare high fat diet. All diets were analyzed for moisture, crude protein, fiber, fat and ash according to (AOAC, 2000). Table 1.

**Experimental design**

To avoid the stress on rats, blood samples were withdrawn without heparin by decapitation in the beginning of the experiment (zero time), after 30- days and the end of experiment 60- days. Blood samples were then centrifuged at 3000 rpm for 10 min and kept at -20°C until analysis. Liver samples were also taken and stored at -20°C until analysis of glycogen contents by the method of (Caroll et al., 1956). Serum glucose concentration was determined according to the method of (Trinder, 1969). Triglycerides and cholesterol (Guder and Zawta, 2001), HDL (Friedewald et al., 1972) and LDL (Levy, 1981).
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Figure 1: illustrates composition of basal diet in pie form as consider that the total size of the pie is 100; sucrose is 50, casein is 20, corn starch is 15, corn oil is 5, fiber is 5, mineral mixture is 3.5, vitamin mixture is 1, DL- methionine is 0.3 and finally, choline bitartarate is 0.2.

Table 1. Chemical composition of the experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Control diet%</th>
<th>Fatty diet%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>5.6</td>
<td>7.8</td>
</tr>
<tr>
<td>Ash</td>
<td>3.2</td>
<td>3.6</td>
</tr>
<tr>
<td>Protein</td>
<td>21.4</td>
<td>21.8</td>
</tr>
<tr>
<td>Fat</td>
<td>05.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Fiber</td>
<td>06.8</td>
<td>06.6</td>
</tr>
<tr>
<td>Nitrogen free extract*</td>
<td>57.2</td>
<td>48.0</td>
</tr>
</tbody>
</table>

*Nitrogen free extract = 100 - (sum of percentages of protein, lipids, fiber, ash and moisture).

Anti- Cancer test

Cell culture

Colo-205 cell line and Hepatocellular carcinoma cell line (HepG2) were obtained from Nawah Scientific Inc, (Mokatam, Cairo, Egypt). Cells were maintained in RPMI media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO₂ atmosphere at 37 °C.

Cytotoxicity assay

Cell viability was assessed by SRB assay. Aliquots of 100 µL cell suspension (5x10³ cells) were in 96-well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 100 µL media containing subjected extracted oil at various concentrations ranging from (0.01, 0.1, 1, 10, 100 µm). After 72 h of extracted oil exposure, cells were fixed by replacing media with 150µL of 10% TCA and incubated at 4°C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. A liquots of 70µL SRB solution (0.4%w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then,150µL of TRIS (10 mM) was added to dissolve protein-bound SRB stain (Figures 2 and 3); the absorbance was measured at 540 nm using a BMG LABTECH® FLUO star Omega micro plate reader (Ortenberg, Germany) according to (Skehan et al., 1990 and Allam et al., 2018) methods.

Statistical analysis

Data were analyzed using the (SAS, 2004) software. An ANOVA was carried out to determine differences between control group and high lipid diet at the significant
Table 2. Body weight gain, feed intake, feed efficiency ratio and protein efficiency ratio in rats fed diets containing prickly pear seed soil for 30 and 60 days.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control rats group</th>
<th>Fatty rats group</th>
<th>±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>After 30 d</td>
<td>126.00</td>
</tr>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total gain, g</td>
<td>--</td>
<td>49.00 c</td>
<td>25.32</td>
</tr>
<tr>
<td>Average feed intake (g/day)</td>
<td>--</td>
<td>21.33 a</td>
<td>22.00 a</td>
</tr>
<tr>
<td>Feed efficiency ratio (FER)</td>
<td>--</td>
<td>10.95 b</td>
<td>11.11 b</td>
</tr>
<tr>
<td>Protein efficiency ratio (PER)</td>
<td>--</td>
<td>2.80 a</td>
<td>2.34 b</td>
</tr>
</tbody>
</table>

a-b and c Mean values within the same row bearing different superscripts differ significantly P<0.05).
NS non-significant
* significant

RESULTS AND DISCUSSION

Diet analysis

Body weight and feed intake of rats during the first 30 days and 60 days of treatment, a linear increase in body weight of rats fed a high lipid diet was observed compared to the control (Table 2). A small variation between the two groups occurred after these periods (30 and 60 days). The lack of a weight difference between control and high lipid fed rats might be explained by decreased feed intake of the high lipid diet in comparison with the control animals. Also, fatty rats group was more efficient than control group in feed efficiency ratio (FER) and protein efficiency ratio (PER). This finding is supported by (Monia et al., 2006) who fed rats a high lipid diet 25 g per kg of prickly pear seed oil for 63 days. (Rodríguez-Rodríguez et al., 2015) pointed that, mice fed high-fat diet-induced obesity supplemented with Opuntia ficus-indica extract for 12 weeks gained less body weight when compared to mice fed high-fat diet alone. Thus, these results provided many clues to the potential effects of Opuntia extracts in terms of energy metabolism, gene regulation, and insulin and glucose pathways regulation, suggesting that prickly pears, given in different ways in the diet, could be efficient in human treatment of obesity.

Serum Lipid Profile of Rats

The results of serum lipid profile are summarized in (Table 3). The two rat groups (control and fatty rats group) resulted significant difference (P<0.05) in triglyceride (TG) value ranging from 5.12-14.30 mg/dl at zero time to after 60 days. However, fatty rat group after 60 days treatments recorded lowest TG values compared to control diet (5.12 vs. 14.10 mg/dl). There was significant difference (P<0.05) in total cholesterol content between all rat groups which recorded values ranging from 30.13-56.78 mg/dl. This diet based fat fed to rats has affect total cholesterol content in their blood serum. On the other hand, there was no significant difference (P>0.05) in HDL-cholesterol content between treatments. (Osorio-Esquível et al., 2012) mentioned that, mice fed a hypercholesterolemic diet of methanolic extract from Opuntia joconostle seeds for six days exhibited significantly higher plasma lipid levels than mice fed a normal diet.

Even though the treatment with fatty rat groups recorded lowest content in LDL cholesterol (18.60 mg/dl), after 60 days of experiment, this was significantly different (P<0.05) compared to other treatments except for control treatment.
The control treatment recorded the highest value of LDL cholesterol (35.31 mg/dl) compared to other treatments. The addition of lipid enriched diet with 100g/kg diet included 50% hexanic extract of prickly pear seeds oil as fat source in this experiment resulted in the decrease of serum total cholesterol and LDL-cholesterol with no effect on HDL-cholesterol concentrations (Table 3). This is probably due to the wealth of seed oil in phytosterols, especially in β-sitosterol (6 g kg⁻¹ of seed oil), according to (Ramadan and Morsel, 2003) and (Monia et al., 2006). Same results were obtained by (Wolfram et al., 2002); (Linarés, 2007) and (Osorio et al., 2012) on Opuntia spp. seed oil. Numerous studies have noted that phytosterols induce a decrease in lipoprotein cholesterol levels in total plasma (Ikedia et al., 1988; Moghadasian and Frohlich, 1999), but the mode of their action is not fully understood.

It has been hypothesized that these compounds provoke a decrease in cholesterol solubility and its absorption across the intestinal barrier, inducing consequently low serum cholesterol levels (Heinemann et al.,1993; Wasan et al., 2001). Specifically, phytosterols have been shown to decrease LDL-cholesterol levels in animal models and in humans (Weststrate and Meijer, 1998; Moghadasian and Frohlich, 1999; Moghadasian et al., 1997). It has been demonstrated that these compounds prevent or delay the development of atherosclerotic lesions (Moghadasian et al., 1997; Jones et al., 1999). Other compounds, such as beta-carotenes and vitamin E, are present in prickly pear seed oil (0.047 and 0.403 g kg⁻¹ of oil respectively), and could prevent the structural alteration of lipoproteins (Ramadan and Morsel, 2003). Recent investigations were divided concerning the protective role of these vitamins. Some studies indicate a protective role of vitamin E in the development of atherosclerosis (Kartal et al., 2003), but these effects have not been confirmed by others (Clarke and Armitage, 2002; Torres et al., 2003).

Mice fed with the high-fat diet supplemented with O. ficus-indica extract for 12 weeks gained less body weight and exhibited significantly lower circulating cholesterol, LDL cholesterol, and HDL cholesterol, when compared to mice fed with the high-fat diet alone. These observations may be due to, the O. ficus-indica extract prevented the development of metabolic abnormalities associated with diet-induced obesity (Rodríguez-Rodríguez et al., 2015). Thus, the use of different animal models provided many clues to the potential effects of Opuntia extracts in terms of energy metabolism, gene regulation, and insulin and glucose pathways regulation, suggesting that prickly pears, given in different ways in the diet, could be efficient in human treatment of obesity. Also, when the mice were supplemented with a methanolic extract from O. joconostle (polyphenol enriched) seeds, they exhibited a marked reduction in circulating LDL cholesterol and triglyceride levels, by comparison with animals fed with a placebo (Osorio- Esquivel et al., 2012). These results pointed that prickly pears seeds extract of O. robusta lower the cholesterol levels in hyperlipemic non-diabetic human patients.

(Osorio-Esquivel et al., 2012) reported that, supplementation diet of methanolic extract from Opuntia joconostle seeds for six days at doses of 1, 2, and 5 g/kg body weight of mice was significantly prevented the increase in total cholesterol, low-density lipoprotein cholesterol, triglycerides level. Similar concentrations of the HDL cholesterol were observed in both treated and control groups. A significant dose-dependent reduction in lipid levels was noted for treated groups compared to the hypercholesterolemic group. This result may be attributed to the seeds' phenolic composition, This methanolic extract has potential to be included in short-term hypercholesterolemia treatment regimens as it exhibits hypolipidemic activity with no apparent toxic manifestations.

It is known that oleic and linoleic acids have beneficial health effects including alleviating numerous diseases and it is an essential fatty acid and a precursor of arachidonic acid biosynthesis, the substrate for eicosanoid synthesis. It has long been accepted as having hypcholesterolemic effects.

The addition of 50 g kg⁻¹ seed oil of prickly pear to the diet of rats resulted in the decrease of plasma total cholesterol and LDL (VLDL) cholesterol with no effect on HDL-

<table>
<thead>
<tr>
<th>Table 3. Triglycerides and cholesterol profile in rats blood serum fed diet containing high level of prickly pear oil seed.</th>
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<tbody>
<tr>
<td>Item</td>
</tr>
<tr>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Control rat group:</td>
</tr>
<tr>
<td>Zero time</td>
</tr>
<tr>
<td>After 30 days</td>
</tr>
<tr>
<td>After 60 days</td>
</tr>
<tr>
<td>Fatty rats group:</td>
</tr>
<tr>
<td>Zero time</td>
</tr>
<tr>
<td>After 30 days</td>
</tr>
<tr>
<td>After 60 days</td>
</tr>
<tr>
<td>±SE</td>
</tr>
</tbody>
</table>

*+a, b, c and d Mean values within the same column bearing different superscripts differ significantly P<0.05. NS non-significant. *significant
Table 4. Serum glucose levels and Glycogen levels in liver of rats fed diet containing high level of prickly pear oil seed.

<table>
<thead>
<tr>
<th>Item</th>
<th>Serum glucose levels (mg/dl)</th>
<th>Glycogen levels in liver (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero time</td>
<td>93.24a</td>
<td>6.72a</td>
</tr>
<tr>
<td>After 30 days</td>
<td>101.44a</td>
<td>5.22b</td>
</tr>
<tr>
<td>After 60 days</td>
<td>117.78a</td>
<td>4.10b</td>
</tr>
<tr>
<td>Fatty rats group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero time</td>
<td>93.22a</td>
<td>6.73a</td>
</tr>
<tr>
<td>After 30 days</td>
<td>88.67b</td>
<td>10.36a</td>
</tr>
<tr>
<td>After 60 days</td>
<td>82.56b</td>
<td>15.20a</td>
</tr>
<tr>
<td>±SE</td>
<td>8.21*</td>
<td>2.54*</td>
</tr>
</tbody>
</table>

a-c Mean values within the same column bearing different superscripts differ significantly P<0.05).
NS non-significant
* significant

Cholesterol concentrations (Table 3). This is probably due to the wealth of seed oil in phytosterols, especially in β-sitosterol, according to (Ramadan and Morsel 2003). Numerous studies have noted that phytosterols induce a decrease in lipoprotein cholesterol levels in total plasma (Ikeda et al., 1988; Moghadasian and Frohlich, 1999 and Monia et al., 2006). It has been hypothesized that these compounds provoke a decrease in cholesterol solubility and its absorption across the intestinal barrier, inducing consequently low plasma cholesterol levels (Heinemann et al., 1993; Wasan et al., 2001). Specifically, phytosterols have been shown to decrease LDL-cholesterol levels in animal models and in humans (Weststrate and Meijer, 1998; Moghadasian and Frohlich, 1999; Moghadasian et al., 1999). It has been demonstrated that these compounds prevent or delay the development of atherosclerotic lesions (Moghadasian et al., 1997; Jones et al., 1999). Other compounds, such as β-carotenes and vitamin E, are present in prickly pear seed oil and could prevent the structural alteration of lipoproteins (Ramadan and Morsel, 2003). Recent investigations were divided concerning the protective role of these vitamins. Some studies indicate a protective role of vitamin E in the development of atherosclerosis (Kartal et al., 2003), but these effects have not been confirmed by others (Clarke and Armitage, 2002 and Torres et al., 2003).

Serum glucose concentration and glycogen

The treated rats diet with 50 g kg⁻¹ seed oil exhibited a significant decrease in serum glucose concentration by 4.88 and 11.43% after 30- and 60- days, respectively, and a significant increase in liver glycogen levels by 53.94 and 125.85% as compared to the control group (P<0.05) (Table 4). In this respect, (Monia et al., 2006) reported that, the addition of 25 g kg⁻¹ seed oil to the rats diet for 30 days resulted, exhibited a significant decrease in serum glucose concentration (22%) and a significant increase in liver glycogen levels (270.73%) as compared to the control group. This increase could be explained by the increase in insulin secretion, which stimulates glucose incorporation into glycogen in skeletal muscles and liver for the regulation of blood glucose. Also, same results were obtained by (Wolfram et al., 2002) with Opuntia spp. seed oil on rats.

Growth inhibitory effect of prickly pear solution on liver and colon cell lines

Prickly pear extracts were used at different concentrations to compare the inhibitory effect on a growth of two different types of cancer cells (Colo-205) cell line and Hepatocellular carcinoma cell line (HepG2) in monolayer cultures. The sensitivity of cancer cells to prickly pear treatment differed among cell types. IC50 is the concentration of drug required to inhibit the growth of 50% of the cells. IC50 <1uM means that the compound is very potent while IC50 10-100uM means that the compound is active but not potent and the current study demonstrated that the hexane extract of prickly pear seeds oil has potent activity (IC50 = 0.052µM) toward Hepatocellular carcinoma cell line (HepG2) as is evident in (Figure 4); while this extract exhibited active but not potent activity (IC50= 29.5µM) against Colorectal cancer (Colo-205) cell line as shown in (Figure 5). In this respect, (Zou et al., 2005) mentioned that ovarian and cervical epithelial cells, as well as ovarian, cervical, and bladder cancer cells exposed to prickly pear extracts (at six concentrations 0, 0.5, 1, 5, 10 or 25%) had a significant increase in apoptosis and growth inhibition in both immortalized epithelial cells and cancer cells in a dose- and time-dependent manner. However, they showed that Opuntia pear aqueous extracts suppressed tumor growth in nude mice, in an extent similar to the one these authors observed with the synthetic retinoid N-(4-hydroxyphenyl) retinamide (4-HPR) used as a chemopreventive model compound. Also, (Naselliet al., 2014) studied the effect of O. ficus-indica fruit aqueous extract and its betalain pigment indicaxanthin on the proliferation of the human colon cancer cell line Caco-2. These authors showed a dose-dependent apoptotic effect on proliferating cells, while no effect was reported on...
Figure 4: Viability (%) of hepatocellular carcinoma cells at different concentrations of prickly pear oil seed during the experiment.

Figure 5: Viability (%) of carcinoma colon cells at different concentrations of prickly pear oil seed during the experiment.

differentiated cells. All these studies show that *Opuntia* spp., oil seeds, could provide an interesting anticancer strategy. Serra et al. (2013) showed that polyphenol-rich juice concentrates of various Opuntia were cytotoxic to HT-29 colon cancer cell lines, but not to Caco-2, while natural extracts from juice residues (peels and seeds) were
reported to be more effective than juice concentrates to induce a cell-cycle arrest in the same cells. Interestingly, this effect paralleled an increase of ROS in the cells, which suggests a ROS-induced cell death probably due to the pro-oxidant effects of the extracts.

CONCLUSION

The supplementation of animal diets with 50 g kg\(^{-1}\) of \textit{O. ficus-indica} seed oil had no significant effect on body weight gain, but caused a decrease in feed conversion efficiency, cholesterol, LDL and serum glucose levels. Also, this oil had inhibitory effect on a growth of two different types of cancer cells (Colo-205 cell line and Hepatocellular carcinoma cell line (HepG2)). The findings of this trial highlight the beneficial effect of \textit{O. ficus-indica} seed oil on animal health. Further work that may provide information on the effect of supplementation diet with nano oil seeds of prickly pear as a natural prevention of cancercells.

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Conflicts of interest

The authors declare no conflict of interest.

REFERENCES


