Inulin activity against Ehrlich Ascites Carcinoma

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ABSTRACT

Keywords: Inulin, prepiotic, SCFAs, antitumor, antioxidant, NK lymphocytes.
Abbreviations
SCFAs : Short Chain Fatty Acids. NK lymphocytes : Natural Killer lymphocytes.

Background: Inulin is a natural storage carbohydrate has benefits effect largely related to its chemical structure as it has antitumor and antioxidant activity. Objectives: This study aims to investigate the antitumor & antioxidant activities of inulin against Ehrlich Ascites Carcinoma (EAC) in female mice, also study the effects of inulin on liver and kidney tissues. Materials & Methods: forty female Swiss albino mice were divided into four groups each group include ten mice: Group 1 “negative control group” mice were injected with sterile saline, group 2 “Positive control group” mice injected with EAC, group 3 “Therapeutic group” mice injected with EAC then inulin, and group 4 “Preventive group” in which mice were injected with inulin then EAC. The most effective dose of inulin was determined, viability test and life span were performed. Also, antioxidants, liver and kidney functions were measured. Results: inulin was safe compound with the most effective dose (5 mg/kg), inulin treatment showed a significant inhibitory on EAC count and volume in both preventive and therapeutic groups as well as, it recorded a significant reduction in anti-oxidants (malondialdehyde, total antioxidant capacity, superoxide dismutase, catalase, and reduced glutathione), with slightly changes in liver & kidney tissues. Conclusion: Inulin has strong effect against EAC because of its chemical structure that responsible for its antioxidant activity.

Introduction:
Cancer is considered one of the major causes of mortality in the world. It is estimated that by 2020 there will be 16 million new cancer cases every year (¹). Treatments may be used alone or in combination depending on the type and stage of cancer; tumor characteristics; and the patient’s age, and preferences. Supportive therapies to reduce side effects and address other patient and family quality of life concerns may also be used. (²). The Inulin chemical structure is glucose and repeating units of fructose GFₙ or Fₙ, N characterizes the number of fructose (2-60) units.

Inulin has been successfully considered in targeted anticancer therapy (³). Where, Inulin can be characterized according to the molecular size of the chains, the total number of fructose units forming the molecule and is used in the treatment of cancer (⁴). It has been suggested that the consumption of inulin supplementation exerts the anti-oxidative effects (⁵). So, the aim of
study to investigate the antitumor & antioxidant effects of Inulin.

Material and Methods:

Materials
Chicory Inulin was obtained from Sigma Company, Melting point is about 158–165 °C, and dissolve in water.

Animals
Forty female Swiss albino mice weigh (20-25 g) purchased from veterinary medicine faculty, Zagazig university and housed in animal house faculty of science, Zagazig University. Before one week they maintained for normal diet and ad libitum.

Tumors
Ehrlich ascites carcinoma cells were initially supplied from the National Cancer Institute, Cairo, Egypt (only for the first transplantation), and maintained in female Swiss albino mice through serial intraperitoneal (I.P.) inoculation at 8 or 10 day intervals in our laboratory in an ascites form.

Experimental design
The forty female mice were divided into 4 groups (ten mice in each one) as follows: Group 1: Negative Control: This group comprised mice were injected I.P. with sterile saline for 10 days. Group 2: Positive Control: This group comprised mice were injected I.P. with Ehrlich ascites carcinoma (EAC), (2×10^6 cells/ 0.3 ml/mouse). Group 3: Preventive Group: (Inulin + EAC): The mice were injected I.P. with Ehrlich ascites carcinoma (EAC), (2×10^6 cells/ 0.3 ml/mouse). Group 4: Therapeutic Group: (EAC + Inulin): This group mice were injected I.P. with Inulin (5 mg/Kg) one day after EAC injection; followed by Inulin at 1, 3, 5, 7, 9 days of EAC injection for 10 days.

Sample Collection
At the end of the experiment, the blood samples were collected from the retro-orbital venous plexus under light ether anesthesia. Serum was prepared by centrifuging blood at 4000 r.p.m. for 10 minutes. Serum samples were aliquoted and stored at -20 °C until biochemical analysis, while the plasma were collected and stored to use in antioxidant analysis. EAC cells were harvest from each mouse in centrifuge tube containing heparinized saline and recorded the volume of ascetic fluid in each mouse in each group.

Methods
i. Determination median lethal dose (LD₅₀) the acute median lethal dose of Inulin was determined according to Meier and Theakston. ii. Dose response curve of Inulin in mice was determined according to method Crump et al.,. iii. Life span prolongation was carried out according to the method described by Mazumdar et al.,. iv. The viability of EAC cells was determined by the Trypan Blue Exclusion Method described by McLiman et al.,. v. Determination of antioxidants: Lipid-peroxidation (Malondialdehyde, MDA) were estimated according to Satoh, as well as Catalase were estimated According to method of Aebi, while Superoxide dismutase (SOD) were estimated according to Nishikimi et al., also Reduced Glutathione (GSH) level was determined by using the method of Beutler et al., and Total Antioxidant Capacity (TAC) level was determined by...
vi. **Biochemical determination of liver functions:** liver functions (total protein, albumin levels, Alanine aminotransferase "ALT", Aspartate aminotransferase "AST", and bilirubin) were assayed according to methods described by Doumas et al., (17), Doumas, et al. (18); Schumann et al., (19) and Karmen et al., (20); and Dacie & Lewis (21), respectively.

vii. **Biochemical determination of kidney functions:** urea and creatinine were estimated by Chaney, et al, (22), and Murray, (23); respectively.

viii. **Histological study:** for liver and kidney were studied according to Lillie (24).

ix. **Statistical Analysis:** All statistical analyses were done by a statistical for social science package "SPSS" 14.0 for Microsoft Windows, SPSS Inc Levesque (25).

**Results**

The median lethal dose of Inulin was found to be up to 1 g/kg and there was no mortality observed in mice so, this compound was safe.

The most effective dose of inulin was found to be 5mg/kg.

The mean life span prolongation in the positive control group was found to be 16 days. Preventive and therapeutic treated groups showed a significant increase in the life span prolongation to 60 days by 252.9% (T/C ratio is 352.9), and to 27 days by 58.8% (T/C ratio is 158.8); respectively, compared to positive control group.

**Effect of Inulin on tumor volume and count in EAC studied groups:**

The mean volume of EAC in the positive control group was found to be 3.31 ± 0.53 (ml) as reported by Zahran et al., (26). This value was highly significantly decrease to 0.0 (ml) by -100% and to 0.87 ± 0.33 (ml) by -73.71% in preventive and therapeutic groups; compared to the positive control group, respectively (Fig 1).

Also, the mean count of EAC cell in the positive control group was found to be 179.8 ± 23.57 (×10^6 cells/ml) which decreased significantly to zero (No EAC) by -100%, and to 80.8 ± 12.15(×10^6 cells/ml) by -55.06% (p<0.0001) in preventive and therapeutic groups; respectively, compared to positive control group (Fig 2).

**Determination of antioxidant**

The mean MDA levels were found to be increased significantly from 12.18 ± 0.35 (nmol/ml) in negative control group to 20.4 ± 0.74 (nmol/ml) in the positive control group (EAC group) by 46% as compared to the negative control group. Meanwhile, Inulin treatments showed a very highly significant decrease in MDA levels reached to 10.97 ± 0.57 by 46% in preventive group, and to 15.41 ± 0.49 (nmol/ml) by 24% in therapeutic group; respectively, compared to the positive control group, Fig (3).

Also, the mean values of TAC levels in negative control group were found to be 0.427 ± 0.05 (mM/L), these levels were significantly decreased to 0.198 ± 0.04 (mM/L) by 53.% (p<0.0001), compared to negative control. Furthermore, the treatments with Inulin showed a very highly significant increase in TAC levels to 3.73 ± 0.49 by 1784%, and to 2.39 ± 0.28 (mM/L) by 1107%, 1784% in both preventive and therapeutic groups; respectively when compared to positive group, Fig. (4).

Moreover, the mean activities of SOD were very highly significant decreased from 167.76 ± 24.27 in negative control group to 84.95 ± 9.58 (U/ml) by 247% (p<0.0001),
compared to negative control group. Furthermore, preventive and therapeutic groups, Inulin showed a very highly significant increase in SOD activities to 295.24 ± 45.50 by 247%, and to 142.16 ± 13.68 (U/ml) by 67% respectively when compared to positive group, Fig. (5).

Catalase (CAT) activities were significantly decreased in positive control group from 233.82 ± 16.14 to 140.55 ± 8.34 (U/ml) by 210% compared to negative control group. Furthermore, the treatments with Inulin showed a very highly significant increase in CAT levels 436.51 ± 31.29 , and 339.14 ± 27.55 (U/ml) by 141%, 211% in both preventive and therapeutic groups, respectively when compared to positive group, Fig. (6).

As well as, the mean values of GSH in positive control group were very highly significant decreased from 11.12 ± 0.95 to 5.75 ± 0.73 (U/ml) by 192% compared to that of negative control group. Furthermore, the treatments with Inulin showed a very highly significant increase in GSH levels 16.81 ± 0.99, and 9.35 ± 0.54 (U/ml) by 63%, 1928% in both preventive and therapeutic groups respectively when compared to positive group, Fig. (7).

**Routine liver and Kidney functions tests**

Table (1) summarized routine liver function tests in all studied groups: on hand; the mean AST and ALT activities were increased from 79.95 ± 7.28 (U/L), and 43.59 ± 6.44 (U/L) respectively in negative control group to 241.28 ± 14.17, and to 126.29 ± 8.43 in positive control group. While preventive and therapeutic groups showed a significant decrease in AST activity to 138.79 ± 5.63, and 154.59 ± 5.09 (U/L) by 42% and 35%; respectively. As well as, the ALT activities were significantly decreased to 59.23 ± 3.33 , and 74.14 ± 4.81 (U/L) by 53%, 41% in preventive and therapeutic groups respectively; compared to the positive control group.

On the other hand, total protein, and albumin were decreased from 7.02 ± 0.61 (g/dl), 3.29 ± 0.43 (g/dl), and 0.338 ± 0.06 (mg/dl) in negative control group to 5.12 ± 0.58, 2.1 ± 0.33, and 0.753 ± 0.09; in positive control group; respectively compared to negative control group. Meanwhile, preventive and therapeutic groups showed an elevation in total proteins to 6.78 ± 0.86 , and 6.03 ± 0.45 by 32%, 17%, respectively. Also, albumin levels were elevated to 2.89 ± 0.64 , and 3.2 ± 0.46 by 37% , and 52% in preventive and therapeutic groups; respectively compared to positive one.

Moreover, the bilirubin levels were significantly elevated from 0.338 ± 0.06 (mg/dl) in negative control group to 0.753 ± 0.09 in positive control group. While, the treatments with Inulin showed a very highly significant increase in bilirubin levels showed an elevation in these levels to 0.344 ± 0.06 , and 0.402 ± 0.04 by 54%, 46% in preventive and therapeutic groups respectively; compared to the positive control group.

Table (2) illustrated the mean urea and creatinine levels in all studied groups. The urea and creatinine levels were recorded a significant increase to 70.95 ± 0.70 (mg/dl), and 0.964 ± 0.11 (mg/dl) in positive control group compared to negative control groups 22.33 ± 2.74 and 0.374 ± 0.10; respectively. Inulin treatments showed a significant reduction in these levels in both preventive and therapeutic groups to 37.28 ± 1.67 , and 45.65 ± 2.33 by 47%, 35.6% in urea levels; respectively, to 0.366 ± 0.07 , and 0.57 ± 0.06 by 62%, 41% in creatinine levels; respectively.
Histological Studies:

Liver of negative control mice showing the normal histological structure of hepatic lobule with normal central vein and normal sinusoids. (H & E X 400) Fig (8). Positive control mice showing different alterations in liver tissues included focal hepatic necrosis associated with inflammatory cells infiltration as well as cytomegalic vacuolated hepatocytes[arrows] (H & E X 400). Fig. (9). But, inulin treatments ameliorated liver tissues in both Preventive mice showing necrotic hepatocytes in The hepatic capsule (H & E X 400) Fig (10), and in therapeutic mice showing focal hepatic necrosis associated With inflammatory cells infiltration (H & E X 400), fig (11).

Kidney tissues of negative control mice showing the normal histological Structure of renal parenchyma (H & E X 400), Fig (12). while, EAC bearing mice Kidney of positive control mice in positive control group showed perivascular infiltration of Ehrlich tumor cells and vacuolation in the epithelial lining Renal tubules (H & E X 400), Fig. (13). But inulin treatments recorded an improvement in Kidney tissues of Preventive mice that showed slight congestion of glomerular tuft and pyknosis of some nuclei of renal epithelium (H & E X 400), Fig. (14), and focal necrosis of renal tubules associated with inflammatory cells infiltration in therapeutic groups (H & E X 400), Fig. (15).

DISCUSSION

The cancer is an abnormal growth of cells, which tend to proliferate in an uncontrolled way and, in some cases, to metastasize. Cancer is a group of more than 100 different and distinctive diseases (27). Inulin has anti-carcinogenic effect as shown in our result of the volume and cell count of EAC. The antioxidant effect of Inulin gives a very highly significant as in MAD, TAC, SOD, CAT, GSH plasma level compared to positive control group. The liver and kidney function also are affected by Inulin treatments. As, Inulin is a mixture of polysaccharides composed of fructose chains linked by β(2-1) bonds with a terminal unit of glucose and fructose chains linked by β(2-1) without a terminal unit of glucose (28), where its chemical structure is GFₙ or Fₙ (4).

Inulin is an FOS fructose-oligosaccharide approved biopolymer endowed with a combination of relevant properties making it eligible to design a variety of targeted anticancer delivery systems. First of all, it contains primary and secondary hydroxyl groups homogeneously distributed along the main chain which confer it a structural versatility enabling to finely tune its functionalization with a wide array of functional groups such as targeting agents, anticancer drugs, environment sensitive spacers and long-chain lipophilic moieties. The last but not the least, it intrinsically displays an anticancer prebiotic effect which equips the potential carrier with a synergistic anticancer strategy (29).

Among possible mechanisms of protection against chemical carcinogenesis could be anti-oxidant dependent induction of detoxifying enzymes (30). Oxidative stress is potentially harmful to cells and reactive oxygen species (ROS) are implicated in the etiology and progression of many diseases including cancer. Under conditions of excessive oxidative stress, however antioxidants are depleted and ROS can damage cellular components and interfere with critical cellular activities (31).

Also, mechanism that may have contributed to the antioxidant indices is
the lowering of the formation of advanced glycation end products (AGEs). Increased blood glucose levels could be due to oxidative stress and this would result in the formation of AGE products. It is suggested that the dietary oligosaccharides may reduce the oxidative stress by reducing the formation of these AGE products (32). The possible mechanism may be due to the ability of prebiotics to modify gene expression of antioxidant enzymes. It is reported that the consumption of chicory reduces oxidative stress, restores GSH levels and induces gene expression, which results in the over expression of the activity of the antioxidant enzyme catalase and in turn, up-regulating the endogenous antioxidant defense system (33). It has been suggested that the consumption of inulin supplementation exerts these same systemic anti-oxidative effects in the colon. It is known that enhanced concentrations of butyrate in colonic cells results in reduced colonic myeloperoxidase activity and restored GSH concentration (34). Also, butyrate has been effective in controlling the enhancement of ROS levels (5).

The possible anti-carcinogenic activity of prebiotics is not known clearly (35). Being indigestible, they have been linked with better bowel functions and metabolisms of the distal colon, including a reduced risk of colon cancer. It has been observed that longer the chains of non-digestible carbohydrates, slower are their rate of fermentation that allows the stimulation of bacterial metabolism in a more distal part of the colon. The short chains are readily fermented in the proximal part of the colon. As has been indicated, prebiotics may stimulate probiotic bacteria not only to grow but also to produce compounds beneficial to the host. The anaerobic breakdown of prebiotic substrates enhances the growth of LAB, and formation of short chain fatty acids (SCFAs) and lactic acid as fermentation products. Depending on the nature, quantity and ferment ability of indigestible polysaccharides reaching the colon, the amount of the SCFAs like acetate, propionate and butyrate can vary (36). Lactic acid bacteria have SOD and in vitro studies have shown that lactic acid and the fermentations of FOS by different strains of bifid bacteria lead to the elimination of free radicals (37). Also, it may be that lactobacilli resident in gut lyses and release their intracellular anti-oxidative constituents that in turn help to decrease the MDA (34).

Supplementation of the probiotic or and prebiotic for 3 months decreased AST and ALT serum levels in the patients with NAFLD (nonalcoholic fatty liver disease) (35). Moreover, supplementation with probiotic, with or without prebiotic, significantly recovered the grade of fatty liver in NAFLD patients. Studies evaluated the effects of pre/probiotics on liver function tests in patients with NAFLD. reported that supplementation with Lactobacillus bulgaricus decreased serum levels of AST, ALT, and GGT after 3 months of intervention, yet, after supplementation with Streptococcus thermophilus, no changes were observed in any factor of liver function (38).

Our study includes the histological studies to liver and kidney tissue, which illustrate the effect of Inulin on preventive and therapeutic group on liver and kidney by apoptosis as well as necrosis. This result of histological tested tissues simulates our result to effect of Inulin on tumor volume and count in EAC studied groups, also the Known effect of Inulin on colorectal cancer as it has antioxidant effect (especially MAD).
There are no researches on the histological studies to Inulin effect on tissue. But the study on numerous scientists have noticed that bacteria in the colon produce many different types of compounds that maintain both positive and negative effects on gut physiology, as well as other systemic influences. As an example, SCFAs are produced by the fermentation of bacteria, when the bacteria in the colon metabolize proteins and complex carbohydrates. It is known that inulin, particularly in its long-chain form, stimulates the human immune system by binding to the specific lectin-like receptors on the leucocyte membrane and stimulating the proliferation of macrophages. It has also been shown in mice that feeding with inulin increases both the number of NK lymphocytes and the kinetic response of the macrophages. It has likewise been shown that the insoluble gamma form of inulin is capable of triggering the presence of C3 fraction complement receptors on the surface of macrophages (39).

**Conclusion**

Inulin has antitumor and antioxidant effect on EAC model, moreover anti-apoptotic, anti-necrotic effect. More studied are needed on gene expression of Inulin effect. Therefore, Inulin effect on human studied is recommended.

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**REFERENCES:**


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![Chart](image)

**Fig. (1) Effect of Inulin on the Volume (ml) of EAC in mice studied groups**
Fig. (2) Effect of Inulin on the Count of EAC ($\times 10^6$ cells/ml) in mice studied groups.

Fig. (3) Effect of Inulin on the MDA level (nmol/ml) in mice studied groups.

Fig. (4) Effect of Inulin on the TAC level (mM/L) in mice studied groups.
Fig. (5) Effect of Inulin on the SOD activity (U/ml) in mice studied groups.

Fig. (6) Effect of Inulin on the Catalase activity (U/ml) in mice studied groups.

Fig. (7) Effect of Inulin on the GSH level (U/ml) in mice studied groups.
Table 1. Effect of Inulin on the Liver Parameters

<table>
<thead>
<tr>
<th></th>
<th>AST U/L mean ± std</th>
<th>ALT U/L mean ± std</th>
<th>TP g/dl mean ± std</th>
<th>ALB g/dl mean ± std</th>
<th>BILI Mg/dl mean ± std</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative Group</strong></td>
<td>79.95 ± 7.28</td>
<td>43.59 ± 6.44</td>
<td>7.02 ± 0.61</td>
<td>3.29 ± 0.43</td>
<td>0.338 ± 0.06</td>
</tr>
<tr>
<td><strong>Positive Group</strong></td>
<td>241.28 ± 14.17</td>
<td>126.29 ± 8.43</td>
<td>5.12 ± 0.58</td>
<td>2.1 ± 0.33</td>
<td>0.753 ± 0.09</td>
</tr>
<tr>
<td><strong>Preventive Group</strong></td>
<td>138.79 ± 5.63</td>
<td>59.23 ± 3.33</td>
<td>6.78 ± 0.86</td>
<td>2.89 ± 0.64</td>
<td>0.344 ± 0.06</td>
</tr>
<tr>
<td><strong>Therapeutic Group</strong></td>
<td>154.59 ± 5.09</td>
<td>74.14 ± 4.81</td>
<td>6.03 ± 0.45</td>
<td>3.2 ± 0.46</td>
<td>0.402 ± 0.04</td>
</tr>
</tbody>
</table>

Highly significant at P<0.00001

Table 2. The Effect of Inuline on the Kidney Parameters

<table>
<thead>
<tr>
<th></th>
<th>Urea mg/dl mean ± std</th>
<th>Creat. mg/dl mean ± std</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative Group</strong></td>
<td>22.33 ± 2.74</td>
<td>0.374 ± 0.10</td>
</tr>
<tr>
<td><strong>Positive Group</strong></td>
<td>70.95 ± 0.70</td>
<td>0.964 ± 0.11</td>
</tr>
<tr>
<td><strong>Preventive Group</strong></td>
<td>37.28 ± 1.67</td>
<td>0.366 ± 0.07</td>
</tr>
<tr>
<td><strong>Therapeutic Group</strong></td>
<td>45.65 ± 2.33</td>
<td>0.57 ± 0.06</td>
</tr>
</tbody>
</table>

Highly significant at P<0.00001
Fig 10. Liver of Preventive Group

Fig 11. Liver of Therapeutic Group

Fig 12. Kidney of Negative Control

Fig 13. Kidney of Positive Control
Fig 14. Kidney of Preventive Group