Effect of Blackberry in Management of Dry Eye in Experimental Animal Model.

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Abstract

Objective: The present study was performed to investigate the protective effect of blackberry as polyphenolic compounds for managing dry eye in experimental animal model.

Materials and Methods: The Blackberry was dried in air oven. Dry eye induced by atropine sulphate 1% drops (2 drops in each eye daily) for 2 months, blackberry was taken as 20 % of the main diet. Thirty two rabbits (1000-1300g) were divided into four groups: G1: healthy control, G2: dry eye, G3: normal fed on blackberry, G4: dry eye feed on blackberry. Poly phenolic compounds of blackberry were analyzed by using High Performance Liquid Chromatography (HPLC). Tear production measured using tear break up time (TBUT) and schirmer test. Tears were collected day after day from all groups using 5-μL silanated microcapillary pipettes. At the end of experiments rabbits were fasted overnight and blood was withdrawn. The levels of reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and interleukin-1beta (IL-1β) were estimated in the tears and blood. In addition catalase (CAT) and superoxide dismutase (SOD) were determined in the blood. Cornea excised and examined by light microscope.

Results: Significant improvements were noticed in tear breakup time and schirmer I score of dry eye rabbits fed on 20% dried blackberry compared to dry eye group. There was a significant (p < 0.05) decrease in serum and tears of IL-1β, NO and a statistically increase in the blood GSH, plasma CAT in dry eye rabbits fed on 20% dried blackberry compared to dry eye rabbit group.

In conclusion: Supplementation with dried blackberry is effective against the dry eye syndrome by decreasing ocular inflammation and increasing antioxidant contents in tears of experimental animal model.

Key words: dry eye, polyphenolic compounds, atropine, blackberry, oxidative stress, antioxidants.

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Introduction

Dry Eye Disease (DED) is a disorder of tear film that results in ocular surface damage and ocular discomfort. Dry eye is a very common disease, associated with high osmolarity of the tear film and mild inflammation at the surface of eye (Martínez-Carrasco et al., 2017). Dry eye is associated with a decrease in tear aqueous production. Any stress to the surface of eye like change in environmental conditions, genetic factors, antigens, infection and endogenous stress is associated with the pathogenesis of dry eyes (Funari et al., 2013). Dry eye syndrome in which eyes does not produce sufficient tears is also known as aqueous deficient dry eye disorder. Hypervaporative dry eye disorder is usually associated with infections, infrequent blinking and other factors (De Clerck et al., 2015). Pro-inflammatory cytokines activate the pro-inflammatory helper T cells which infiltrate the lacrimal gland and cause damage to the surface of eye (McLaughlin et al., 2010; Lv et al., 2014).

Oxidative stress and damage have been recognized to be involved in a variety of inflammatory diseases, including ocular surface disease or dry eye syndromes. The mitochondria-induced oxidative damage has a strong relationship with lacrimal gland inflammation and dysfunction, resulting in dry eye disease in a conditional transgenic mouse model (Uchino et al., 2012a). Blades et al. (2001) found that oral antioxidants improved both tear stability and conjunctival health.

Blackberries (family: Rosaceae, genus Rubus, common cultivated variety: Rubus fruticosus) have a similar morphology to raspberries; it is an aggregate fruit consisting of many drupelets. The best dietary sources of bioactive compounds. They have delicious taste and flavor, have economic importance, and they are of great interest also for nutritionists and food technologists due to the opportunity to use as functional foods ingredients. The bioactive compounds in berries contain mainly phenolic compounds {phenolic acids, flavonoids, (such as anthocyanins and flavonols) and tannins} and ascorbic acid. These compounds, either individually or combination, are responsible for various health benefits of berries, such as prevention of inflammatory disorders, prevention cardiovascular diseases, or protective effects to lower the risk of various cancers (Skrovankova et al., 2015).

The estimated number of people experiencing the ocular discomfort due to dry eye disorder ranges from 25 till 30 million around the world (Patel and McGhee, 2013). It is more common in women especially over 50 years of age due to autoimmune diseases (Ostrovski et al., 2015). The frequency of dry eye disorder is more prevalent in older age groups (Edwards et al., 2016).

Chronic dry eye has been demonstrated to cause inflammation, evidenced by an increase in pro-inflammatory cytokines in the tear fluid (Baudouin et al., 2013).
The present study aims to investigate the protective effect of blackberry as polyphenolic compounds for managing dry eye in experimental animal model.

**Material and Methods:**

**Martials:**

**Chemicals**

Atropine sulfate 1% eye drops was obtained from pharmacy (Orchidia Pharma, Egypt). Glutathione, pyrogallol, thiobarbituric acid, 5, 5' dithio-bis-2-nitrobenzoic acid, tris HCl buffer were used (Sigma company). All chemicals were of analytical grade. Kits were purchased from Bio-Diagnostic Company El-Dokki, Giza, Egypt.

**Biological experiment:**

**Diet:**

**Commercial diet**

A commercial diet purchased from the animal house of the Research Institute of Ophthalmology was used as basal diet. The commercial diet consists mainly of not more than 64% carbohydrates, not less than 21% protein, not less than 6% fat, not less than 3% fiber, and not less than 6% of vitamins and minerals mix, methionine and choline chloride (National Research Council, 1995).

**Preparation of dried blackberry:**

Blackberry was procured from the Ministry of agriculture and gently washed in cold water just before drying to remove dirt, bacteria, and insects. The Blackberry was dried in air oven at 60°C. After 2 to 3 three hours, lower the dryer temperature to 45°C till complete dryness, ground and used. Dried blackberry was used as 20% of commercial diet and served as experimental diet (Doymaz, 2011).

**Experimental animals design:**

**Experimental animals:**

Thirty two rabbits New Zealand male white rabbits weighing (1000 -1300 g) supplied from the Research Institute of Ophthalmology, Giza, Egypt. At the start of the experiment, rabbits were housed randomized individually in a polyethylene cages and served as groups. Animals were maintained in a controlled environment (25±2°C, 50-60% relative humidity and 12 hours light/dark cycle). All animals’ research protocols were conducted in accordance with the association for research in vision and ophthalmology (AROV) statement for us of animals in ophthalmic.

The rabbits randomly divided into four groups of eight rabbits for each. Group one: received commercial diet and served as normal control group; group two: dry eye (topical administration of atropine sulphate 1% twice daily for along two months) and rabbits fed on commercial diet; group three: healthy rabbits received commercial diet containing 20% blackberry; group four: topical administration of atropine sulphate 1% twice daily for along two months and rabbits fed on commercial diet containing 20%
dried blackberry. The progression of dry eye was under observation till the end of the experiment (two months).

**Induction of dry eye:**

The rabbits in groups (2, 4) were topically administrated of atropine sulphate 1% eye drops. Twice daily (2 drops in each eye) for two month as recommended by El-shazly et al., (2008).

**Dry eye examination:**

The rabbits’ eyes were examined weekly with naked eyes and by direct ophthalmoscope.

Schirmer test and Tear break-up time used for assist of dry eye as the following:

1. **Schirmer test** (without anaesthesia) was done to measure tear secretion in response to both conjunctival stimulation. It was performed using Schirmer strips (SEPO, Egypt) which are made of filter paper of 0.5 mm width and 3.5 mm length. The strip was inserted over the inferior lid margin towards the lateral canthus for five minutes. Eyes were left to blink normally. The wet area was measured in millimeters cho and yap, (1993).

2. **Tear break-up time:** TBUT is an indicator of the tear film stability over the ocular surface. TBUT was measured with a hand held tear scope-plus (2413-P-2003, Keeler, UK). In this test, fluorescein drops of fluorescein sodium 1% eye drops (CID, Egypt) are instilled without any topical anaesthesia and checked with slit lamp with a cobalt blue filter. After one complete blink of eye, the time is noted for the first tear film break-up Norn (1969).

**Sampling**

**Tear samples**

Tear samples collected day after day from each rabbit and stored in epindorf then frozen in refrigerator until used.

**Blood samples**

At the end of the experimental period, all rabbits were fasted overnight and the blood samples were collected from jugular vein using two separated tubes. The first tube contained ethylene diamine tetraacetic acid (EDTA) to determine glutathione content (GSH) and superoxide dismutase activity (SOD) and whole blood is then centrifuged at 3500 r.p.m for 10 minutes to separate plasma for determined catalase (CAT) activity. The second tube was used to separate serum to determine malondialdehyde (MDA), nitric oxide (NO) and interlukein 1β (IL-1β). All samples were kept in a deep freezer under -70°C until used.

**Methods:**

Quantitative chemical analysis of phenolic compounds for dried blackberry was determined by high performance liquid chromatography (HPLC), according to method of Gourpy et al. (1999).
Biochemical assessment:

In whole blood analysis for all samples, the activity of superoxide dismutase was determined by monitoring the inhibition of the autoxidation of pyrogallol according to the method described by Marklund and Marklund (1974). The determination of reduced glutathione was carried out in tears and blood according to the method described by Beutler et al., (1963). Plasma catalase activity was determined according to the method described by Aebi (1984), using the kits obtained from Bio-diagnostic Company. The concentration of IL-1β in the serum and tears were determined according the method of eliza interlukin 1 β (cloud-clone-corp) Malondialdehyde in serum and tears was determined according to the method described by Ohkawa et al., (1979). Nitrite was determined in serum and tears according to the method described by Moshage et al., (1995).

Histological Studies:

At the end of the experiment, the rabbit’s eyes were enucleated after being sacrificed. Specimens were taken from central corneas. Tissues were fixed in 10% formalin and processed to obtain paraffin blocks. Sections of 4-mm thickness were obtained and stained with haematoxylin and eosin stains. Sections were examined by light microscopy to assess the histopathological changes of dryness and inflammation. Histological preparation of the cornea was done according the method described by Glauert (1965).

Statistical analysis:

SPSS package (version 10) was used for data analysis. Data were analyzed using one way ANOVA, mean and standard deviation were descriptive measures of data. Least significant difference (LSD) multiple comparison tests were carried out. Propriety level (P values) were significant if <0.05.

Results:

Quantitative identification of polyphenolic compounds in blackberry by high performance liquid chromatography (HPLC)

The data of blackberry represent the high amount of phenolic compounds such as Syringic, Pyrogallol, Protocatechuic, Catechein, Epicatechein, Caffeic, Cholrogenic, Catechol, and Benzoic. Also, medium amount of phenolic compounds such as Salycillic, Ellagicm, P.hydroxybenzoic, Caffeine, Vanillic, (p-Coumaric, Gallic, Ferulic, Iso-ferulic, and α-Coumaric. In addition to low amount of phenolic compound such as 3,4,5.Methoxy Cinnamic. The lowest poly phenolic compounds Coimarin and Cinnamic.
Table.1 Quantitative identification of polyphenolic compounds in blackberry by high performance liquid chromatography (HPLC)

<table>
<thead>
<tr>
<th>Phenolic compounds in blackberry</th>
<th>(µg/100gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Syringic</td>
<td>204218.71</td>
</tr>
<tr>
<td>2. Gallic</td>
<td>725.67</td>
</tr>
<tr>
<td>3. Pyrogallol</td>
<td>14506.71</td>
</tr>
<tr>
<td>4. 4-Aminobenzoic</td>
<td>416.21</td>
</tr>
<tr>
<td>5. Protocatechuic</td>
<td>14246.84</td>
</tr>
<tr>
<td>6. Catechein</td>
<td>8945.33</td>
</tr>
<tr>
<td>7. Cholrogenic</td>
<td>5797.68</td>
</tr>
<tr>
<td>8. Catechol</td>
<td>5569.95</td>
</tr>
<tr>
<td>9. Epicatechein</td>
<td>6503.19</td>
</tr>
<tr>
<td>10. Caffeine</td>
<td>1375.25</td>
</tr>
<tr>
<td>11. P.OH.benzoic</td>
<td>1791.45</td>
</tr>
<tr>
<td>12. Caffeic</td>
<td>6178.03</td>
</tr>
<tr>
<td>13. Vanillic</td>
<td>1366.84</td>
</tr>
<tr>
<td>14. p-Coumaric</td>
<td>880.33</td>
</tr>
<tr>
<td>15. Ferulic</td>
<td>649.67</td>
</tr>
<tr>
<td>16. Iso-ferulic</td>
<td>572.06</td>
</tr>
<tr>
<td>17. Ellagic</td>
<td>2003.20</td>
</tr>
<tr>
<td>18. α –Coumaric</td>
<td>327.61</td>
</tr>
<tr>
<td>19. Benzoic</td>
<td>5439.71</td>
</tr>
<tr>
<td>20. Salycillic</td>
<td>1974.62</td>
</tr>
<tr>
<td>21. Coimarin</td>
<td>105.47</td>
</tr>
<tr>
<td>22. 3,4,5.Methoxy Cinnamic</td>
<td>194.36</td>
</tr>
<tr>
<td>23. Cinnamic</td>
<td>6.32</td>
</tr>
</tbody>
</table>

Biochemical assessment

A- In blood

Table (1) shown the mean values±SD of blood reduced glutathione (GHS), blood superoxide dismutase (SOD), plasma catalase (CAT), serum malondialdehyde (MDA), serum nitric oxide and serum interlukin 1β in different experimental groups.

The results showed significantly (p < 0.05) decrease in GSH, SOD and CAT activities and significant(p < 0.05) increase in the levels of MDA, NO and IL-1β in the dry eye group compared to control group. On the other hand, in the dry eye group fed on 20%
dried blackberry, significantly \((p < 0.05)\) decreased in nitric oxide, malondialdehyde and interleukin \(1\beta\) levels were noticed comparing to dry eye group.

**Table 2. Mean ±S.D and percentage change of blood reduced glutathione (GSH), blood superoxide dismutase activity (SOD), plasma catalase activity (CAT), serum malondialdehyde (MDA), serum nitric oxide (NO) and serum interleukin 1\(\beta\) (IL-1\(\beta\)) for normal and dry eye groups with and without blackberry feeding.**

Values are expressed as mean ±SD.

The mean difference is significant at the .05 level

Different litters at the same column means significant.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>GSH mg/dl</th>
<th>SOD U/ml</th>
<th>CAT U/L</th>
<th>MDA nmol/ml</th>
<th>NO µmol/l</th>
<th>IL-1(\beta) Pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Mean ±S.D</td>
<td>67.05</td>
<td>88.83</td>
<td>9.39</td>
<td>1.57</td>
<td>22.00</td>
<td>41.97</td>
</tr>
<tr>
<td>Dry eye</td>
<td>Mean ±S.D</td>
<td>50.85</td>
<td>51.51</td>
<td>6.67</td>
<td>2.90</td>
<td>26.17</td>
<td>195.29</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>-24.1 b</td>
<td>-42.0 b</td>
<td>-28.96</td>
<td>84.71</td>
<td>365.3</td>
<td></td>
</tr>
<tr>
<td>Normal fed on blackberry</td>
<td>Mean ±S.D</td>
<td>61.50</td>
<td>72.75</td>
<td>9.59</td>
<td>1.32</td>
<td>19.67</td>
<td>48.17</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>-8.27 a</td>
<td>-18.10 a</td>
<td>-15.92 a</td>
<td>1.91</td>
<td>-3.24 c</td>
<td>14.77</td>
</tr>
<tr>
<td>Dry eye fed on blackberry</td>
<td>Mean ±S.D</td>
<td>66.35</td>
<td>73.66</td>
<td>9.57</td>
<td>1.75</td>
<td>18.67</td>
<td>96.29</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>-1.04 a</td>
<td>-17.07 a</td>
<td>-39.65 a</td>
<td>11.46</td>
<td>-8.16 b</td>
<td>129.4</td>
</tr>
<tr>
<td></td>
<td>% Change*</td>
<td>30.48 b</td>
<td>43.47 b</td>
<td>11.55 b</td>
<td>70.86 b</td>
<td>-50.69 b</td>
<td></td>
</tr>
</tbody>
</table>

% Change: Percentage change from normal control.

% Change*: Percentage change from dry eye.

**B-In the tears**

The levels of reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and interleukin-1\(\beta\) (IL-1\(\beta\)) in the tears of normal control group, dry eye, normal control fed on 20% dried blackberry, dry eye fed on 20% dried blackberry were presented in table 2. The result showed significantly \((p < 0.05)\) decrease of GSH activities in the dry eye group, while the levels of MDA, NO and IL-1\(\beta\) showed highly significant \((p < 0.05)\) increased compared to control group. On the other hand, in the dry eye group fed on dried blackberry, significantly \((p < 0.05)\) decreased in nitric oxide, malondialdehyde and interleukin \(1\beta\) levels were noticed comparing to dry eye group.
Table 3. Mean ±S.D and percentage change of tear reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and interleukin 1-β (IL-1β) for normal and dry eye groups with and without blackberry feeding.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>GSH mg/dl</th>
<th>MDA nmol/ml</th>
<th>NO µmol/l</th>
<th>L1β Pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mean ±S.D</td>
<td>2.94</td>
<td>1.68</td>
<td>17.77</td>
<td>7.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.41</td>
<td>0.59</td>
<td>13.03</td>
<td>2.83</td>
</tr>
<tr>
<td>Dry eye</td>
<td>Mean ±S.D</td>
<td>1.98</td>
<td>8.34</td>
<td>58.00</td>
<td>98.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.90</td>
<td>3.09</td>
<td>13.90</td>
<td>53.71</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>-32.65</td>
<td>396.42</td>
<td>226.9</td>
<td>1214.62</td>
</tr>
<tr>
<td>Normal fed on blackberry</td>
<td>Mean ±S.D</td>
<td>2.64</td>
<td>1.26</td>
<td>16.5</td>
<td>8.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
<td>1.03</td>
<td>5.86</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>-10.20</td>
<td>-25</td>
<td>-7.14</td>
<td>18.48</td>
</tr>
<tr>
<td>Dry eye fed on blackberry</td>
<td>Mean ±S.D</td>
<td>2.68</td>
<td>3.42</td>
<td>40.5</td>
<td>58.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.77</td>
<td>2.63</td>
<td>29.56</td>
<td>6.33</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>-8.84</td>
<td>103.57</td>
<td>127.9</td>
<td>675.13</td>
</tr>
<tr>
<td></td>
<td>% Change*</td>
<td>35.40</td>
<td>58.99</td>
<td>-30.1</td>
<td>-41.03</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD. The mean difference is significant at the .05 level. Different litters at the same column means significant. % Change: Percentage change from normal control. % Change*: Percentage change from dry eye.

**Dry eye examination:**

1. **Tear break up time (TBUT):**

Data of TBUT in control, dry eye, dry eye fed on 20% dried blackberry groups was shown in the tables (4). The time of TBUT in normal control group was 10 seconds. As shown in the table, there was decrease in TBUT (sec) in the dry eye group and time record 4 seconds. On the other hand, there was increase in TBUT (sec) in the group of dry eye fed on 20% dried blackberry, since the data of TBUT (sec) in the group dry eye fed on 20% dried blackberry was 8 seconds.

**Table 4. Mean ±S.D and percentage change for TBUT in control, dry eye and dry eye fed on blackberry groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±S.D</th>
<th>seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.0±0.76</td>
<td>a</td>
</tr>
<tr>
<td>Dry eye</td>
<td>4.12±0.83</td>
<td>b</td>
</tr>
<tr>
<td>Dry eye fed on blackberry</td>
<td>8.0±0.76</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>-60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>94.17</td>
<td></td>
</tr>
</tbody>
</table>
Values are expressed as mean ±SD.
The mean difference is significant at the .05 level
Different litters at the same column means significant.
% Change: Percentage change from normal control.
% Change*: Percentage change from dry eye.

2. Schirmer test

The results of schirmer test values were observed in tables (5). As shown in this table data revealed non-significant different of schirmer test in normal control group and normal groups fed on 20% dried blackberry through whole experiment period. There was gradual significant (p < 0.05) decrease in tear volume in 2 weeks, 1 month and 2 months in dry eye group. Schirmer test values were 11.34±0.77mm, 7.79±0.23mm and 6.18±1.12 mm respectively comparing to normal control group. While in the dry eye groups fed on 20% dried blackberry, data of schirmer test showed significant improvement in 2 weeks, 1 month and 2 months 14.0±0.89 mm, 14.7±0.40 mm, and 14.93±0.92 mm comparing to dry eye group.

Table 5. Mean±S.D and percentage change of schirmer test in normal control and dry eye groups with and without 20% dried blackberry feeding.

<table>
<thead>
<tr>
<th>Group Parameter</th>
<th>2 weeks mm</th>
<th>1 month mm</th>
<th>2 months mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Mean ±S.D</td>
<td>19.5 ±0.45a</td>
<td>19.42 ±0.47a</td>
<td>19.77 ±0.39a</td>
</tr>
<tr>
<td>Dry eye Mean ±S.D</td>
<td>11.34 ±0.77c</td>
<td>7.79 ±0.23c</td>
<td>6.18 ±1.12c</td>
</tr>
<tr>
<td>% change</td>
<td>-41.84</td>
<td>-59.88</td>
<td>-68.74</td>
</tr>
<tr>
<td>Normal Mean ±S.D</td>
<td>18.71 ±0.56a</td>
<td>19.0 ±0.89a</td>
<td>18.67 ±1.2a</td>
</tr>
<tr>
<td>% change</td>
<td>4.05</td>
<td>-2.16</td>
<td>-5.56</td>
</tr>
<tr>
<td>Dry eye Mean ±S.D</td>
<td>14.0 ±0.89b</td>
<td>14.7 ±0.40b</td>
<td>14.93 ±0.92b</td>
</tr>
<tr>
<td>% change</td>
<td>-28.20</td>
<td>-24.30</td>
<td>-24.48</td>
</tr>
<tr>
<td>% Change*</td>
<td>23.45</td>
<td>88.70</td>
<td>123.84</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD.
The mean difference is significant at the .05 level
Different litters at the same column means significant.
% Change: Percentage change from normal control.
% Change*: Percentage change from dry eye.
Result of histopathological examination:

Fig.1: Light micrograph of rabbit’s cornea fed on control diet showing its normal layers. Stratified squamous non-keratinized epithelium (EP), Bowmen’s layer (B), Stroma (S), Descemet’s membrane (D) and endothelium (E). Toluidine (blue ×500).

Fig.2: Light micrograph of rabbit’s cornea fed normal diet + atropine sulphate 1% drops showing lack of columnar appearance basal cell appearance (*), keratinization of superficial epithelium (arrow), irregularity Bowmen’s layer (B) and vascularization of endothelial cytoplasm (V). Toluidin (blue ×500).

Fig.3: A light micrograph of rabbit’s cornea fed with normal diet and 20% blackberry showing its five layers from outside inwards. Corneal epithelium, regularity Bowmen’s layer (B), stroma (S) Descemet’s membrane (D) endothelium (E). Toluidin (blue ×500).

Fig.4: Light microscopic examination of dry eye rabbit’s cornea fed on normal diet and 20% blackberry showing normal appearance of epithelial cells (EP), irregularity Bowmen’s layer (B).

Results of light microscopic examination of control rabbit’s cornea showed its normal layers. The stratified squamous non-keratinized epithelium (three to five layers), Bowmen’s layer, stroma with regular collagen, Descemet’s membrane and single layer
of simple squamous to cuboidal endothelium fig (1). In dry eye group, light microscopic examination of the cornea showed thinner of the corneal epithelium with loss of normal arctecture, flattened and keratinized cells in the apical portion and irregularity of basement membrane (fig.2) In addition, differences in the stroma and Descemet’s membrane were unremarkable. However, the endothelium showed few vascules in the cytoplasm. No significant microscopic alterations were seen in the cornea of rabbits fed on normal diet and 20 % of blackberry with exception of irregularity of Bowmen’s layer fig (3). Light microscopic examination of rabbit’s cornea fed on normal diet and 20 % blackberry showing normal appearace of epithelial cells arrangement, irregularity Bowmen’s layer (B) normal stroma (S), However the endothelium showed cytoplasmic vascular degenerative change (fig. 4).

Discussion

The development of a simple dry eye model in the rabbit was induced by two times daily repeated instillations of 1 % atropine sulphate. The evolution of the dry eye syndrome in the animals was assessed by the Schirmer I test and examination of the cornea after fluorescein staining. The disorder was assisted by TBUT. Schirmer test may reflect the severity of dry eye disease (Choi et al., 2016), also, Yokoi et al. (2017) suggesting that TBUT and schirmer test are the most significant tests to examine dry eye disease among all other available investigations.

Data in the present study showed that most rabbits of dry eye group had abnormal schirmer test and TBUT values. Gradual significant decrease in the percentage and the value of schirmer test (mm) and tear volume was record through 2 weeks, 1 month and 2 months in dry eye group, also TBUT value decreased comparing to normal control groups shown in tables 4 &5. Kaido et al. (2016) found that short TBUT-type dry eye had increased the pain was higher in the short TBUT than to the normal controls. Shujaat et al. (2017) found that disturbance in the basal secretions of lacrimal gland, lead to development of dry eye disease.

High Reactive oxygen species (ROS) levels within the mitochondria lead to oxidative stress and potential organelle damage. ROS outside the mitochondria may be involved in inflammation, a primary mechanism of dry eye disease (Wakamatsu et al., 2008). An abundant amount of reactive oxygen species and nitric oxide lead to the formation of toxic reactive products contributing in tissue damage. Thus, the accumulated oxidative damage may cause the functional decline of the lacrimal gland and subsequently induce dry eye disease (Uchino et al., 2012b).

Antioxidants such as reduced glutathione or enzymes such as superoxide dismutase provide electrons to convert ROS into less-reactive forms. Reduced glutathione deficit in tears inhibits proliferation of conjunctival cells especially goblet cells, which are the main source of mucin responsible for tear film stability (Hao et al., 2013). Over production of superoxide anions from the mitochondria may directly and/or indirectly affect oxidative damage and inflammation in the lacrimal gland. Behndig et
al. (1998) reported that sclera and cornea had less SOD activity than the retina and the tears contain little SOD activity.

The current study revealed, decreased activity of blood SOD, GSH, and plasma CAT, whereas the levels of serum malondialdehyde, nitric oxide and IL-1β were increased in the dry eye group compared to control group. These findings may explain imbalance between oxidative stress and antioxidant. These results proved previously by Čejková and Čejka, (2015).

These results showed marked increases of inflammatory markers in tears and the presence of oxidative damage to the ocular surface. The results showed significant elevation of malondialdehyde and nitric oxide in tears and not significant in serum in dry eye group. In dry eye disease, the expression of malondialdehyde and nitric oxide (a marker of oxidative stress) appeared in tears and not appeared in serum of dry eye group may be due to local effect of atropine sulphate to induce dry eye but not effect on the blood. This is in contrast to healthy rabbit groups represented in the current study in which normal levels of malondialdehyde and nitric oxide. Increased levels of malondialdehyde were found at the ocular surface in patients with dry eye syndrome was previously reported by Augustin et al. (1995) and Čejková et al. (2007).

The results showed in the dry eye group tht the level of IL-1β was elevated in tears and serum samples, indicating that increased inflammation in dry eye. The present results agreed with the result of Solomon et al. (2001) who suggested that diseased conjunctival cells of dry eye might be the source of the increased levels of pro-inflammatory cytokines (interleukin-1ß) in the tear fluid. Furthermore, significant improvement were observed in the level of IL-1β in serum and tear fluids obtained from dry eye group fed on 20% dried blackberry compared to those of normal groups. Numerous epidemiological studies have shown an inverse association between fruit and vegetable consumption and eye diseases (Abd El-Razek et al., 2012; Andrea et al., 2017). Therefore, interest in the health benefits of fruit and vegetable consumption is important.

Dry eye is a highly prevalent disease among several common ocular problems. It can initiate and magnify many ocular diseases leading to several corneal complications which then eventually lead to blindness. Examination and treatment of dry eye disease are not only meant to determine dry eye disease but to reduce almost all ocular morbidities leading to severe corneal complications. The conjunctival epithelial cells of patients with severe dry eye disease revealed an increased expression of nitric oxide synthases that generate nitric oxide. Our results of histopathological examination show that, a vasculuation of endothelial cytoplasm in epithelium cells in dry eye group as shown in fig. 2. In addition to this, the results of the blood reveal a decrease in the activity of superoxide dismutase and increase in the level of nitric oxide in tears. The data confirmed with the result of Čejková et al. (2007) who suggested that nitric oxide synthase expressions in dry eye disease is highly involved in injuries of the ocular surface and pronounced symptoms of dryness, perhaps through the formation of peroxynitrite.
Flavonoids have gained prominence in the pharmaceutical arena by virtue of their therapeutically beneficial properties. Bioflavonoids possess antioxidant, anti-angiogenic, and/or anti-inflammatory activities and are also capable of reducing fluid retention and strengthening capillary walls. Interestingly, the etiology of most ocular diseases involve free radical mediated oxidative damage, hypoxia, decreased blood supply to ocular tissues and, in certain conditions, angiogenesis, increased vascular permeability and leakage of vascular contents. Thus, select bioflavonoids may be effective in the prevention or treatment of ocular diseases (e.g. diabetic retinopathy, macular degeneration, and cataract) that lead to vision loss if left untreated (Erickson et al., 2007).

Oxidative damage to the corneal epithelium may be involved in dry eye disease. Quercetin, epigallocatechin gallate (EGCG), n-propyl gallate, and gallic acid and xanthine oxidase were effective at quenching ROS in human corneal limbal epithelial cells, indicating that they are bioavailable and might be effective in protecting the corneal epithelium from oxidative damage (Stoddard et al., 2013). Blackberry polyphenol extract strongly inhibits NO production (Dai et al., 2007). Our results agreed with this study and suggested that, the level of NO in tears and serum was decreased in the dry eye group treated with 20% dried blackberry. This study suggested that blackberry is a botanical therapeutic fruit which can provide health benefits.

For these reasons, the current study ensured that eye protection is very important to avoid different eye diseases. It is important to provide food with active components, rich in polyphenolic compounds such as blackberry. Interestingly, we found that low malondialdehyde, nitric oxide and interleukin-1β; In addition to high reduced glutathione, catalase, and superoxide dismutase were more pronounced in the dry eye group treated with 20% dried blackberry than that in dry eye group, which was statistically significant(p < 0.05). It is quite surprising that blackberry, which is well known for its preventive effect in inflammatory diseases, may acts to prevent of dry eye disease in animal model.

Blackberry acts as anti-inflammatory, antimicrobial, prebiotic, antioxidant, and estrogenic, this can be attributed to the high amounts of acylated anthocyanins and cyanidin 3-glucoside in blackberries (Kaume et al., 2012). Hodges, et al., (1999) found that polyphenolic compounds such as Epigallocatechin, epigallocatein gallate, myricetin and Tannic acid lowered Intraocular pressure (IOP) below control levels.

**Conclusion:**
The others conclude that dry eye disease in animal model could be diagnosed by physical and biochemical study. Through Schirmer test and TBUT and through detection of biochemical marker in tears nd blood samples as well. Prevention of dry eye disease in study rabbits was proved by blackberry oral intake.
References


الملخص باللغة العربية

تأثير التوت الأسود في تحسين حالة جفاف العين المستحذثة في حيوانات التجارب.

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الهدف من هذا البحث هو دراسة تأثير التوت الأسود المجفف المعطى كمذيب يغذى للفئران

• لتحسين حالة جفاف العين في حيوانات التجارب.
• الجفاف في العين، تم تحضير الوجبات بنسبة 20% من مسحوق التوت المجفف.
• تم استخدام 23 ذكر وزن (100-200 جم) وتم تقسيم المجموعات إلى 4 مجموعات: 1- مجموعة أصحاء يتناولون غذا من الطرق المتواضعة، و2- مجموعة تم إعطاؤها جفاف العين باستخدام قطرة مادة سلفات 1% وتم تغذيتها على الغذاء المجفف، و3- مجموعة أصحاء تغذى غذاء متوافقتشيحتي على 60% من مسحوق التوت الأسود المجفف، و4- مجموعة اقتصادات يغذى جفاف العين باستخدام قطرة مادة سلفات 1% وتم تغذيتها على غذاء متوافقتشيحتي على 60% من مسحوق التوت الأسود المجفف.

• تم قياس حالة جفاف العين باستخدام اختبار شيرمير و زمن انقشاع طبقة الدموع.
• تم تحضير المركبات حسب اللزوين باستخدام تقنية الترصيع اللوني للسائل ذات القدرة العالية.
• التقديرات اليبوكيتومية: الجلولاتوين المختزل وميالواده ونيتريك أكسيد وانتروكين 1 بتا في كلا من بد ودمو بالإضافة إلى ذلك تم عمل الكتالاز والسويلوكسيدسيميترز في الدم، وتم فحص القرنية بالميكروسكوب الضوئي.

وأوضح النتائج تحسين ملحوظ في قيم كلا من اختبار شيرمير و زمن انقشاع طبقة الدموع من التوت الأسود المجفف، بالمقارنة بالمجموعة المصابة.

وجد ان انخفاض في عينات الدم والدموع في كلا من الانتروكين 1 بيتا و النيتريك أكسيد وزيادته في الجلولاتوين المختزل وسيروم الكتالاز في المجموعة المصابة بجفاف العين و تناولت 20% من التوت الأسود المجفف بمقارنة بالمجموعة المصابة.

الخلاصة:
• تناول التوت الأسود المجفف له تأثير ضد جفاف العين عن طريق تقليل الالتهابات التي تصيب العين، وزيادة كمية مضادات الأكسدة المولدودة بالدموع في حيوانات التجارب.

45