Ameliorative effect of blackberry cultivated from taif city on serum and liver tissue in hyperlipidemic rats

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Abstract: Blackberry is an edible fruit produced by many species (family *Rosaceae*).It's contain number of phytochemicals associated with heath benefit for many diseases. In this study ameliorative effect of blackberry cultivated from taif city in K.S.A on serum liver tissue in hyperlipidemic rats. Duration of this study was 65 days, included 50 Meals Wister Albino Rats were divided into five groups: control group **G1**, rats were fed high-fat diet **G2** (5g/kg/days), rats were fed blackberry containing diet **G3** (15g/kg/day), rats were fed blackberry containing diet (15g/kg/day) along with high fat diet **G4** (5g/kg/day) and rats were fed blackberry containing diet **G5** (15g/kg/day) after 45 days from high fat diet to complete 65 days. After 45 and 65 days, rats were weighted and anesthetized by ether, and collection of blood from the eyes and separate to get serum for used the measurement of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and glucose. liver tissue was stained by hematoxyline and eosin (H&E) for histopathological examination. Analyses using one-way ANOVA by SPSS. We demonstrated that, G3 and G4 respectively was successfully to decrease of AST, ALT, ALP, glucose and protect effect on liver tissue from damage that caused by HFD. While G5 was significant decrease in AST, ALT, ALP, and glucose after 65 days when compare to HFD but similar effect on liver tissue as G2. Overall, G3 and G4 was beneficial effect hypolipidemic and hypoglycemic. The results of the current study may suggest that prophylactic treatment with the tested combination of blackberry was ameliorative effect in attenuating hyperglycemia and liver damage induced in rat liver in response to the effects of HFD.
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1. Introduction

Abnormally rise concentration of fats or lipids in the blood due to hyperlipidemia. It is dangerous because it leads to be hardening of the arteries in the heart specifically. Also known as dyslipidemia which is caused by a defect in lipoprotein metabolism and it is the major risk factor for liver disorder (Rohilla et al., 2012), which lead to increases serum liver function enzymes including alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphotase (ALP) (Tim and Franciscue, 2010). Also coronary artery disease can result in angina or a heart attack. Section of the heart muscle no oxygen receives because in the heart arteries of systemic circulation are blocked by plaque that leads to heart attack. Also break off the plaque from an artery wall and flow in the body, lead to stroke or atherosclerosis (Alan, 2011). Impaired glucose tolerance results from high fat diets lead to defect in transfer of glucose through cell membrane and cell ability for utilization of glucose and defect of activity of glycogen synthase due to defect of glucose storage as glycogen (Rask-Madsen and Kahn, 2012).

Blackberry In Greeks used as a cure for gout, and made a tea from the leaves of the blackberry plant to treat various illnesses in Romans. Blackberry cultivated a traditional and contain bioactive compounds, possessing important role of biological activities such as phenolics compound (galic, p-coumaric, caffeic and ferulic), flavonoids (anthocyanin) is the active component, vitamins (vitamin A, B complex, C and E), some minerals (Ca, Mg, Fe, P, K, Na, Zn, Mn and Cu), ellagic acids and tannin (Kaume et al., 2011).

The antioxidant capacity of blackberry as flavonoids (anthocyanin), phenolic compound and rich sources of Zn and vitamin C, E in fresh blackberry has a benefit effect on the body and protect cells, also keeping some enzymes and internal components of cells from being destructed. Agha et al., (2012) they said these contents they can amend immunity, play an antagonistic role as protective agents from toxic source. Liver is the central organ responsible for metabolism, removing toxic substance, and secretory functions in the body and vary different metabolic role in the body.

Liver injury is related with the deformation of these metabolic functions (Uboh et al., 2012). The battery of liver enzymes includes alanine and aspartate transaminase (ALT and AST), alkaline phosphatase

(ALP). The aminotransferases stimulate the reversible conversion of α-ketoacids into amino acids. Their serum levels reflect the amount of hepatocellular injury and death on a day-by-day basis. Transaminase (and predominantly AST) are not only found in hepatocytes but also in other tissues. Damage to one gram of liver tissue results in increase in the serum ALT activity (Verslype, 2010).

Some antioxidants are obviously found in the body, such as antioxidant vitamins, micronutrients in the diet play an important role in improve the toxicity effects of ROS by chemical agents in biological systems. Vitamins C and E are known to be strong antioxidants (Verslype, 2010) that protect of liver from injury. Anthocyanins rise the resistance of hepatocytes to oxidation, activate liver enzymes (AST, ALT and LDH), normalize the activity of liver enzymes (AST, ALT) and lower the reduced glutathione concentration in the liver. A protective effect of anthocyanins on liver cells has also been observed by (Kowalczyk et al., 2011). In this study ameliorative effect of blackberry cultivated from taif city in K.S.A on serum liver tissue in hyperlipidemic rats

2. Materials and methods

Chemicals:

Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and glucose were purchased from Dimension (DAD BEHRING Company, USA). Coconut oil from (Abazer-KSA). Deoxycholic acid and cholesterol were purchased from Sigma Chemicals Company (Saint.Louis, U.S.A).

Animals and treatments:

Sixty male Wister Albino Rats weighing 100–120 g were supplied by King Fahad Medical Research Center, Jeddah. The rats were performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Committee of the King Abdel-Aziz University. Animals were maintained in special cages and consumed ad libitum and fed with standard pelleted diet.

The rats were divided into 5 groups, each of 10 rats

G1: Serves as normal control received normal diet for 65 days.

G2: Rats was fed high-fat diet (5g/kg/days) 65 consecutive days.

G3: Normal rats were fed blackberry containing diet (15g/kg/day) for 65 consecutive days.

G4: Rats was fed blackberry containing diet (15g/kg/day) along with high fat diet (5 g/kg/day) for 65 consecutive days.

G5: Rats were fed blackberry containing diet (15g/kg/day) after 45 days from high-fat diet to complete 65 days.

Blood samples from all groups were collected after 45 days and 65 days respectively from eyes, in gel separator tube and centrifuge at 3000 r.p.m for 12 minutes to separate serum which then was divided into several aliquots and kept at -35 until analysis was performed. Serum was used for the determination of liver function test, aspartate aminotransfer enzyme (AST) (U/L), alanine aminotransfer enzyme (ALT) (U/L), alakaline phosphate enzyme (ALP) (U/L) and glucose (mmol/L). Samples of the liver from all animals were fixed in 10% neutral formalin and embedded in paraffin wax. Sections (4-6 µm thickness) were stained with hematoxylin and eosin (H&E) for histological examination (Robinson and Gray, 1996).

Preparation of Blackberry (BB), high fat diet (HFD) and both

In summer season, blackberry was collected from Kingdom of Saudi Arabia, Western Region, Taif City, and was Freeze-dried device then by an electrical blender crushed and storage at -18C° to be used in the study. The diet pellets, prepare by 980 g of powder standard rats diet were mixed with 20 g of blackberry powder then added water to mixture by homogenous and was designed to small pellets and left to dry at room temperature for three days (Rodriguez-Mateos et al., 2012).

Feeding the rats hyperlipidemia was induced by prepare with cholesterol rich high fat diet for 45 days. Deoxycholic acid (5g) was mixed thoroughly with powdered standard rats diet (700g). Simultaneously cholesterol 5g was dissolved in 290g warm coconut oil. The oil and cholesterol mixture was added slowly into the powdered mixture to obtain a soft homogenous cake. This cholesterol rich HFD was designed into pellets of about 5g to each rat (Kumar et al., 2008).

Five grams of deoxycholic acid, 5g of cholesterol, 20 g of BB and 700 g of powdered standard rats die was mixed in an electrical blender. Then 920 ml of water was added to obtain a soft homogenous cake. The mixture was divided into pellets and complete dried in the presence of a good source of ventilation in the room temperature. After four days, 270 g of warm coconut oil was added to the pellets and mixed and used to feed the rats.

Statistical analysis:

Data were analyzed by comparing values for control and HFD groups with the values for other groups. Results are expressed as mean ± SE. Significant differences among values were analyzed using one-way test (ANOVA).

3. Results

Effect of blackberry on serum of liver function test after 45 and 65 days of hyperlipidemic rats

Effect on serum activity of liver enzymes

Figure 1, 2 and 3 respectively, shows the serum activities of liver enzymes AST (U/L), ALT (U/L) and ALP (U/L). There were no significant differences in serum activity of AST in G2, G3, G4 and G5. A significant increase (P≤0.01) in serum activity of ALT was found in G2, and no significant changes of ALT level in G3, G4 and G5. On the other hand, a significant increase in serum activity of ALP was registered in G2 and G4 (P≤0.05), while no significant change was noticed in serum activity of ALP in G3 and G5 **as compared to control group (G1) after 45 days**. A highly significant increase was found in serum activity of AST in G2 (P≤0.001) and no significant difference was noticed in serum activity of AST in G3, G4 and G5. A highly significant increase (P≤0.01) in serum activity of ALT in G2, while no significant changes in serum activity of ALT in G3, G4 and G5. A very highly significant increase in serum activity of ALP in G2 and G4 (P=0.000) as compared to control group, and a highly significant decrease in serum activity of ALP in G3 and G5 (P≤0.001) was found **as compared to control group (G1) after 65 days**.

When compare to HFD group in 45 days, the serum activity of AST in G3, G4 and G5 was no significant differences. Highly significant decrease in serum activity of ALT in G3, G5 (P≤0.001), and no significant change in G4. The serum activity of ALP was highly significant decrease in G3 and G5 (P≤0.005) and no significant change registered in G4. The serum activity of AST was found very highly significant decrease noticed in G4 (P=0.000) and significant decrease in G3 and G5 (P≤0.01). A very highly significant decrease noticed in serum activity of ALT in G3, G5 (P=0.000) and significant decrease was found in G4 (P≤0.05). The serum activity of ALP was a very highly significant decrease in G3, G5 (P=0.000) and no significant change in G4 **as compare to HFD group (G2) in 65 days**,

Effect on serum level of glucose

Figure 4 presents serum level of glucose (mmol/L). There was significant increase in serum level of glucose in G2 (P≤0.01) and no significant difference in G3, G4 and G5 **as compared to control group (G1) after 45 days**. There was low significant (P≤0.01) in serum level of glucose in G3 and no significant difference in G2, G4 and G5 **as compared to control group (G1) after 65 days**. The serum level of glucose shows significant decrease in G3, G4 and G5 (P≤0.01) **as compare to HFD group (G2) after 45 days**. A highly significant decrease in serum level of glucose in G3 (P≤0.001), and significant decrease of serum level of glucose in G5 (P≤0.05). No significant difference in G4 **as compare to HFD group (G2) after 65 days**.

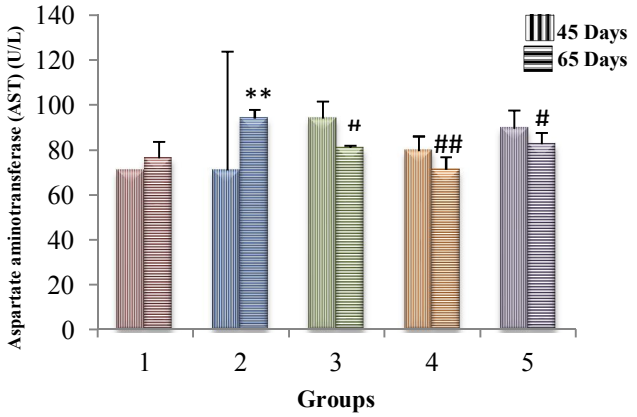

Figure 1. Effect of blackberry on serum activity of aspartate transaminase (AST) after 45 and 65 days of hyperlipidemic rats. Mean±SE: stander error. G1 (negative control group), G2 (high fat diet group HFD), G3 (blackberry group BB), G4 (high fat diet group HFD with blackberry group BB) and G5 (high fat diet HFD then treated blackberry BB). *P≤0.05 significant, **P≤0.001 highly significant and ***P=0.000 very highly significant when compared with G1. $^{\#}$P≤0.05 significant, $^{\#\#}$P≤0.001 highly significant and $^{\#\#\#}$P=0.000 very highly significant when compared with G2.

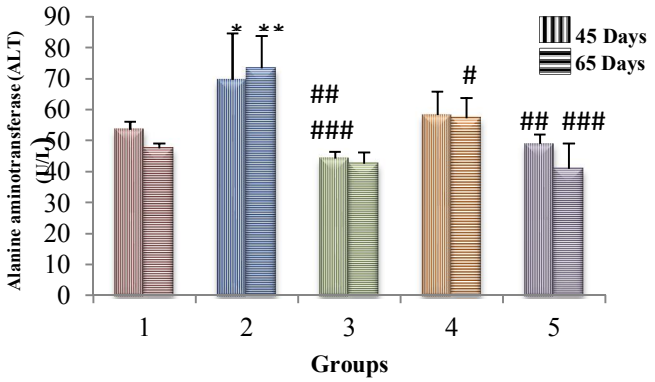

Figure 2. Effect of blackberry on serum activity of alanine transaminase (ALT) after 45 and 65 days of hyperlipidemic rats. Mean±SE: stander error. G1 (negative control group), G2 (high fat diet group HFD), G3 (blackberry group BB), G4 (high fat diet group HFD with blackberry group BB) and G5 (high fat diet HFD then treated blackberry BB). *P≤0.05 significant, **P≤0.001 highly significant and ***P=0.000 very highly significant when compared with G1. $^{\#}$P≤0.05 significant, $^{\#\#}$P≤0.001 highly significant and $^{\#\#\#}$P=0.000 very highly significant when compared with G2

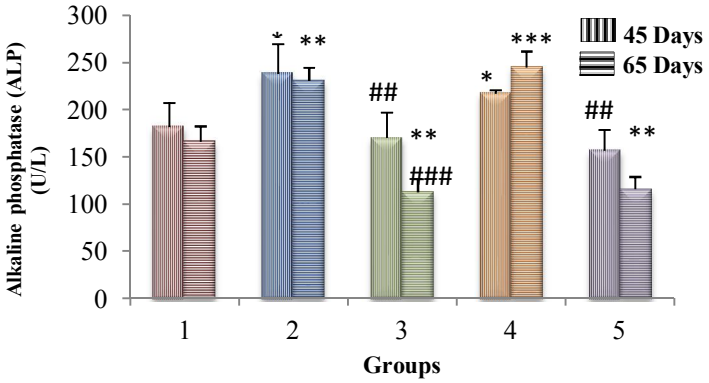

Figure 3. Effect of blackberry on serum activity of alkaline phosphatase (ALP) after 45 and 65 days of hyperlipidemic rats. Mean±SE: stander error. G1 (negative control group), G2 (high fat diet group HFD), G3 (blackberry group BB), G4 (high fat diet group HFD with blackberry group BB) and G5 (high fat diet HFD then treated blackberry BB). *P≤0.05 significant, **P≤0.001 highly significant and ***P=0.000 very highly significant when compared with G1. $^{\#}$P≤0.05 significant, $^{\#\#}$P≤0.001 highly significant and $^{\#\#\#}$P=0.000 very highly significant when compared with G2.

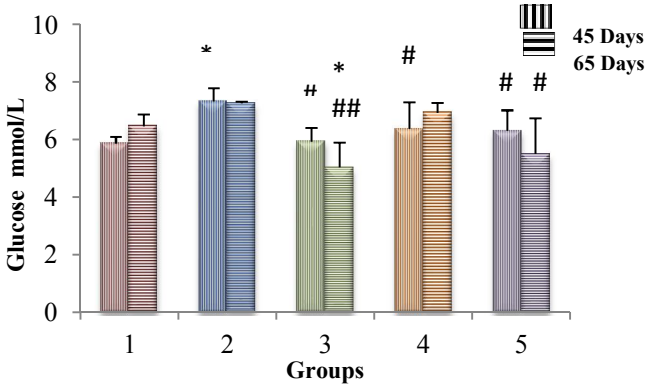

Figure 4. Effect of blackberry on serum level of glucose after 45 and 65 days of hyperlipidemic rats. Mean±SE: stander error. G1 (negative control group), G2 (high fat diet group HFD), G3 (blackberry group BB), G4 (high fat diet group HFD with blackberry group BB) and G5 (high fat diet HFD then treated blackberry BB).*P≤0.05 significant, **P≤0.001 highly significant and ***P=0.000 very highly significant when compared with G1. $^{\#}$P≤0.05 significant, $^{\#\#}$P≤0.001 highly significant and $^{\#\#\#}$P=0.000 very highly significant when compared with G2.

Histopathological examination

Liver

Figure 5 (A, B, C, D and E) show liver tissues of different experimental groups after 65 days. **Liver tissue in control group (G1) (A)** showing hepatic cell around the central vein (C.V), nucleus (n) and blood sinusoid (B.S), **liver tissue in HFD group (G2) (B)** showing increase the number and size of nucleus also found binucleated hepatic cell and necrotic of nucleus (N.N) and necrotic foci (N.F) and fatty change in hepatocytes (F.C), **liver tissue in BB group (G3) (C)** showing increase in number of kupffer cell (K.C), hemorrhage in the cell (H), necrotic of nucleus (N.N) and necrotic foci (N.F), **liver tissue in HFD+BB group (G4) (D)** showing hepatic cell around the central vein (C.V), blood sinusoid (B.S), binucleated and mononucleated, many kupffer cell (K.C) and hemorrhage in the cell (H) and fatty change in hepatocytes (F.C), and **liver tissue in HFD then treated with BB group (G5) (E)** showing many hepatocytes was binucleated accompanid by granular degeneration and fatty change (F.C) activation of kupffer cell (K.C).

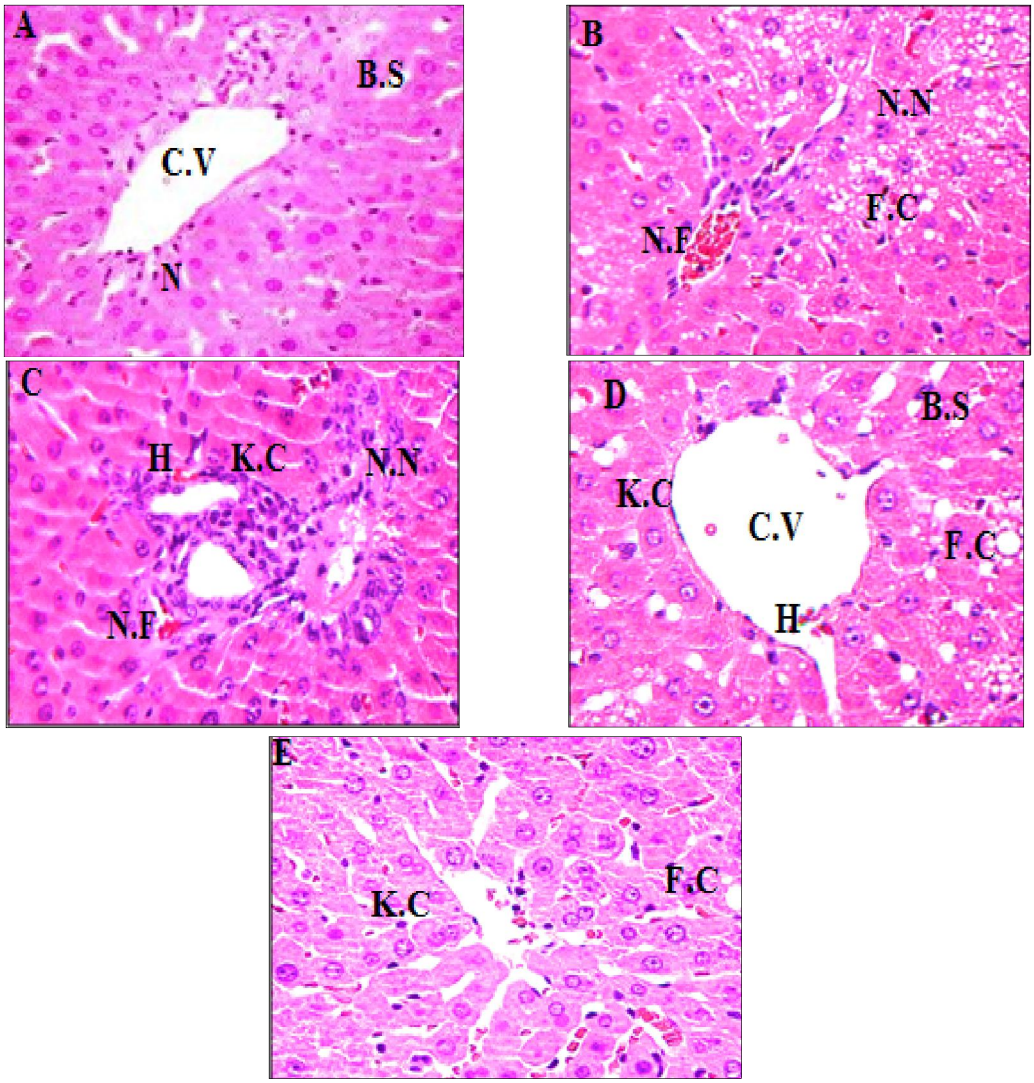

Figure 5. **(A)** light micrograph of liver tissue in control group (G1) showing hepatic cell around the central vein (C.V), nucleus (N) and blood sinusoid (B.S). **(B)** light micrograph of liver tissue in HFD group (G2) showing increase the number and size of nucleus also found binucleated hepatic cell and necrotic of nucleus (N.N) and necrotic foci (N.F) and fatty change in hepatocytes (F.C). **(C)** light micrograph of liver tissue in BB group (G3) showing increase in number of kupffer cell (K.C), hemorrhage in the cell (H), necrotic of nucleus (N.N) and necrotic foci (N.F). **(D)** light micrograph of liver tissue in HFD+BB group (G4) showing hepatic cell around the central vein (C.V), blood sinusoid (B.S), binucleated and mononucleated, many kupffer cell (K.C) and hemorrhage in the cell (H) and fatty change in hepatocytes (F.C). **(E)** light micrograph of liver tissue in HFD then treated with BB (G5) showing many hepatocytes was binucleated accompanied by granular degeneration and fatty change (F.C) activation of kupffer cell (K.C). **Haematoxylin & Eosin stain. X400.**

4. Discussion

Our finding suggests AST, ALT and ALP that in Figure 1, 2, 3 respectively. There was no significant in activity serum of AST in G3, G4 and G5 except G2 w see highly significant increase when compared with control group G1. Among the results shows no significant in G3, G4 and G5 when compared with G2 in 45 days. A very highly significant and significant decrease when compared with HFD show G3, G4 and G5 respectively in 65 days. From the results in serum activity of ALT is significant increase in G2 but no significant in other groups in 45 days and highly significant increase in G2 but no significant in other groups in 65 days when compared with control group G1. While highly significant decrease in G3 and G5 but no significant in G4 in 45 days and very highly significant and significant decrease in G3, G5 and G4 when G2 in 65 days. The finding provide evidence that significant increase of ALP serum activity in G2 and G4 but no significant in G3 and G5 when compared to control group G1 in 45 days, while in 65 days very highly significant increase in G2, G4 but highly significant decrease in G3and G5 respectively, in 65 days. AST in liver cells contain more than ALT, but ALT in cytoplasm concentration is higher than that of AST. In injury or infective case: a relatively more increase in serum ALT than AST because the membrane sustains the major hurt and leak of cytoplasmic contents. In infiltrative disorders, a proportionally greater increase in AST activity than ALT in which there is harm to both mitochondrial and cytoplasmic membranes (Hassoun and Stohs, 1995; Akila et al., 1998).

Our results showed that HFD indeed increased serum ALT and AST, ALP activates, revealing that HFD induced liver damage in rat fed with HFD. Generally HFD increases through the stimulation of oxidative stress in the liver. Histpathological examination showed HFD group rats has increased number of fatty change (F.C) in the liver tissue as compared to BB groups Figures 5. (B and C). This demonstrated that BB might target energy homeostasis in the liver and reduced the hepatic lipid droplet accumulation. The results from the liver tissue quantification discovered that the increase of fat in HFD group rat demonstrating large of necrotic foci (N.F) in the liver tissue as compared to BB rat Figure 6. (D). Current biochemical analysis results are also in agreement with the histological study which shows that BB can decrease the fatty change and control the progression of HFD. This result is in agreement with the study done by Al-Rawi and Ali (2010). In addition, HFD cause a imperfect secretion of bile by the liver was reflected by an increase activity of ALP (Rajesh and Latha, 2004 ; Yanardag and Tunali, 2006). Whereas, BB treated rats showed significantly decreased levels of these serum liver enzyme as compared to HFD group. The administration of polyphenols content of BB cause significant decrease in the activities of these enzymes and were similar to the control group. Those levels of these markers tend toward normal in rats treated with the flavonoid extract as a hepatoprotective effects (Tapas et al., 2008; Kumar, 2012). Hassan and Yousef (2009) revealed that anthocyanin, phenolics compound causes decrease activity of AST and ALT and has a protective hepatotoxicity by antagonizing the free radicals and improvement of the antioxidant protection. So, also avoidance of hepatic disruption, anthocyanin possibly a impending for protective that.

Our result suggests that in Figure 4, presents serum level of glucose. A significant increase in G2 but no significant in G3, G4 and G5 when compared to control group G1 after 45 days and significant decrease in G3, no significant in G2, G4, and G5 as compared to control group G1 after 65 days. When compared to HFD group, serum level of glucose shows significant decrease in G3, G4 and G5 as compare to HFD group G2 after 45 days. A highly significant, significant decrease in G3, G5 and no significant in G4 as compare to HFD group G2 after 65 days. This finding highlight of insulin and its receptors, a phosphorylate cascade, and transferring of glucose transporter type-4 (GLUT-4), controlled fully of enter glucose inside the cell which are fixed to the membrane. The liver takes up glucose and creates free fatty acids, releasing glucose when needed and free fatty acids as TAG and very low density lipoprotein (VLDL), which also can be reused as energy sources (Akila et al., 1998).

HFD due to fatty liver which rises glucose level and VLDL creation and, in turn, promotes the dyslipidemia characteristic of the metabolic syndrome. Evidence that a HFD may alter GLUT4 intrinsic activity has been provided by Hansen et al., (1998) observed that the GLUT4 in vesicles of plasma membrane prepared from muscles of HFD rats had a lower transport capacity than GLUT4 in vesicles prepared from chow-fed controls. HFD have been demonstrated to cause metabolic dysfunction with several abnormalities including glucose intolerance (Gollisch et al., 2009). Vareda et al., (2014) determined that circulating glucose increased after one week on HFD and remained elevated during 12-month study period in rats. Insulin resistance induces by HFD reduce of glucose metabolism and causes excess in fat oxidation (Kim et al., 2000). Tsuda et al., (2004) also reported that anthocyanins normalized glucose, insulin and leptin levels increased by the HFD. Even though the antidiabetic properties of flavonoids can be described by different mechanisms (Kato et al., 2008; Pinent et al., 2008), pancreatic cells exhibited

particular sensitivity to oxidative stress (Lapidot et al., 2002) but antioxidants, flavonoids due to repair the injured of pancreatic cells or activate secretion of insulin by β–cells of the pancreas (Seetharam et al., 2002) by prevent the progressive impairment of pancreatic β-cells function (Coskun et al., 2005; Song et al., 2005).

Effect of flavonoid due to enter the glucose into cells, stimulation of glycolytic enzymes and glycogenic enzymes, reduction of gluconeogenic enzymes or inhibiting the glucose -6-phosphatase in the liver, later reducing the release of glucose in the blood (Naik et al., 1991). In liver damage causes disturbed of hepatocytes transport, leakage of plasma membrane, thereby causing an higher liver enzyme activities, glucose, lipid profile levels in HFD group.

Conclusion:

Findings of this study may suggest that prophylactic treatment with the tested combination of blackberry was beneficial in attenuating hyperglycemia and liver damage induced in rat liver in response to the effects of HFD.

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