REPORT

Antioxidant and hypoglycemic activity of strawberry fruit extracts against alloxan induced diabetes in rats

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Abstract: The strawberries (Fragaria x ananassa) of Rosaceae family are an accomplished source of bioactive compounds such as ascorbic acid and diverse range of polyphenols including anthocyanins, phenolic acids, flavonols, ellagitannins etc. These phenolic compounds classify strawberry as an important health promoting food. Strawberries are proved to have potent antioxidant capacity in various in vitro assay systems. The in vivo beneficial effects are getting explored against various ailments including cancer, metabolic syndrome, and cardiovascular diseases. The present research study was designed to analyze the effect of strawberry fruit extracts (water and methanol) against alloxan induced hyperglycemia in albino rats of Wister strain. Upon alloxan (150mg/kg body weight) induction, the diabetic animals showed marked increase in the values of plasma glucose, urea, uric acid, creatinine and concomitant decrease in body weight and plasma insulin level. The oral administration of strawberry extracts for 45 days in diabetic animals reversed the biochemical changes significantly (P < 0.05) to near normal. Furthermore, the restoration of body weight loss was also observed. The results suggest that the strawberry extract has effective hypoglycemic activity against alloxan diabetes. The poly phenolic antioxidant contents of the strawberry fruit extracts are responsible for the observed biological effect.

Keywords: Alloxan, methanolic extract, free radicals, diabetes, antioxidants, DPPH assay.

INTRODUCTION

Diabetes mellitus (DM) is the most prevalent metabolic disorder among the global population. Insulin insufficiency and hyperglycemia are the associated features of diabetes that affects almost all metabolic pathways (Norris and Wolfsdorf, 2005). According to 2012 diabetes atlas of the International Diabetes Federation (IDF), more than 371 million people around the globe are having diabetes and it is expected to reach 552 million by 2030 (IDF Diabetes Atlas 2012). The occurrence of diabetes is rapidly increasing among developing nations and epidemic in recently industrialized countries. Free radical and ROS mediated oxidative stress is a contributing key factor in the mechanism of several diseases including diabetes (Baynes 1999). Diabetes management with conventional treatment is not possible without adverse effect and high economic input (Srivastava et al., 1993). Thus a suitable antioxidant therapy would benefit in diabetes apart from traditional antibiotic treatment. Plant materials are considered as an alternative resource for finding new leads for hypoglycemic agents. The research in the recent past has focused in the quest of plant-derived novel antioxidants for treating diabetes and related complications (Fabricant and Farnsworth 2001; Pari and Venkateswaran 2003).

Fruits and vegetables are fundamental components of a well-balanced, healthy diet. Strong literature evidence claims that vegetables, seeds, fruits, and fruit peels are rich source of phenolic phytochemicals with dietary fiber and are shown to possess hypoglycemic properties (Scalzo et al., 2005). The strawberry (Fragaria x ananassa) is one of such source due to its bioactive compounds attributed by high levels of vitamin C, folate, and phenolic constituents. Many researchers have demonstrated the relevant antioxidant potential of strawberry both in vitro and in vivo conditions (Wang and Lin 2000; Scalzo et al., 2005; Tulipani, et al., 2009). In recent years the beneficial role of strawberry is being explored against various ailments including cancer, aging, obesity, cardiovascular diseases and metabolic syndrome. Despite numerous reports revealing the in vitro antioxidant activity of strawberry and antidiabetic effect of strawberry leaves (Vahid Rostamian et al. 2011), there is no conclusive study examined the antidiabetic potential of strawberry fruit extract against experimentally induced diabetes in vivo. Thus, the present study explores the antioxidant and hypoglycemic effect of water (WES) and methanolic extract (MES) of garden strawberry (Fragaria x ananassa) in alloxan induced diabetic rats.

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MATERIALS AND METHODS

Chemicals
1. 1-diphenyl-2-picrylhydrazyl (DPPH) and alloxan monohydrate were purchased from Sigma Chemical Corporation (USA). 2. 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and all other analytical grade solvents, salts, acids, and bases used in the present study were purchased from SD Fine Chem. Ltd., Sisco Research Lab. Pvt. Ltd., and CDH division of Glaxo Laboratories India or otherwise mentioned in the text. The in vitro antioxidant analysis was done in Shaqra University Research Laboratory, Shaqra, Saudi Arabia and in vivo animal experiments were carried out in the Biotechnology Research Laboratory of K. S. Ranagasamy College of Engineering and Technology, Tamil Nadu, India.

Preparation of crude extracts
The frozen strawberries were purchased from Al-Othaim super market, Shaqra, Kingdom of Saudi Arabia. The fruit specimen was identified and authenticated by a botanist A.M. Sadaby Ph.D., Professor, College of Applied Medical Sciences, Shaqra University and a voucher specimen (FA-SU01/13) was deposited in the department herbarium for future reference.100g of strawberry fruits were cut into small pieces and were dried under shade and powdered. One litre of 95% methanol was mixed with strawberry powder and stirred with a magnetic stirrer over night at room temperature. The residues were removed through filtration with Whatman No.1 filter paper. Rotary evaporator at a temperature<30°C was used to concentrate the filtrate under vacuum conditions. The procedure was continued until the total evaporation of methanol, to yield 8.2g of crude extract (8.2%). Similarly, water extract of strawberry powder was also prepared. These extracts were stored at room temperature and used in the study.

In vitro antioxidant assays
Antioxidant activity of fruit extracts were analyzed by in vitro ABTS and DPPH assay systems as described by Pellegrini et al. (2002) and Burits and Bucar (2000) respectively.

Animals for the study
The experimental animals (male albino rats of Wister strain) with a body weight range of 160 to 200g were selected for the study. All the animals were exposed to 12 hour light and dark cycle throughout the experimental period and housed in polypropylene cages over husk bedding. Rats were supplied water ad libitum and fed with a commercial pelleted rat chew diet of standard quality. Animal handling and experiments were performed in strict adherence to the ethical guidelines approved by the Institutional Animal Ethics Committee (IAEC) for the laboratory animal usage.

Toxicity assessment of fruit extracts
To evaluate the side effects of fruit extracts, the animals were divided into three groups (Control, WES treated and MES treated) of 6 animals in each group. The WES and MES treated group of animals were received with a standard dose of (50mg/kg body weight) fruit extract for 15 days. Every alternate day, body weight was measured and the average was noted. After 15 days of extract administration, the blood was collected and examined for the side effects on normal physiological functions. Activity of alkaline phosphatase (ALP) and the levels of creatinine and urea were measured using standard kit reagents. The RBC and WBC counts were also performed as described by Huxtable (1990) and Raghuramulu et al. (1983) respectively.

Diabetes induction
Intraperitoneal injection of alloxan monohydrate in normal saline (150mg/kg body weight) was given to animals as a single dose (Nagappa et al., 2003). After alloxan injection, animals with hyperglycemia and glycosuria were selected for experiments. Two days after alloxan injection, strawberry extract (WES and MES) treatment was started.

Experimental design
The experiments were conducted in 5 groups of animals. All the five groups contained equal number (six in each group) of experimental rats.

Group 1: Normal animals, served with standard diet and water;
Group 2: Alloxan (150mg/kg body weight) induced diabetic control;
Group 3: Alloxan induced diabetic animals treated for 45 days with water extract (WES) of garden strawberry (Fragaria x ananassa) 50mg/kg body weight.
Group 4: Alloxan induced diabetic animals treated for 45 days with water extract (MES) of garden strawberry (Fragaria x ananassa) 50mg/kg body weight.
Group 5: Alloxan induced diabetic animals treated with 600 µg of glibenclamide/kg body weight as recommended by Pari and Uma Maheswari2000.
The entire treatment regimen was carried out for 45 days through an oral route.

Collection of samples for analysis
After stipulated experimental duration, the animals were anesthetized and sacrificed after a single shot of 30 mg pentobarbital sodium/kg body weight via intraperitoneal route. The blood samples from the animals were collected in two different test tubes for separating serum and plasma.

Evaluation of diabetic and renal function markers
The levels of plasma glucose, uric acid, urea and creatinine were estimated by adopting the procedures of the standard diagnostic kits supplied by Coral clinical
systems, Goa and Sigma Diagnostics (I) Pvt. Ltd., Baroda, India. Plasma insulin assay was assayed by enzyme linked immunosorbent assay kit (ELISA, Boerhriger Mannheim, Germany). Body weight of all experimental animals was recorded using a digital weighing scale.

**Statistical description**

All the data are represented as a means ±SEM for 6 animals. Multivariate analysis of variance (MANOVA) and Dunnett test for in vivo studies was done to reveal the significant statistic difference. MANOVA and Least Significant Difference (LSD) test were performed for in vitro analysis using the statistical software SPSS Version 17.

**RESULTS**

**DPPH and ABTS radical scavenging potential of WES and MES**

Fig. 1 and 2 depict the antioxidant potential of water and methanolic extract of *Fragaria x ananassa* compared with α-tocopherol and BHT. Both the extracts are exhibiting better radical inhibition p<0.05 than the α-tocopherol and BHT, however the methanolic extract showing highest scavenging activity among the tested extracts.

**Side effect evaluation of WES and MES**

The side effect evaluation of fruits extracts has been presented in table 1. The body weight, ALP activity, urea, and creatinine levels of water and methanolic extract fed rats did not vary significantly from their control counterparts. The blood cell counts (RBC and WBC) of water and methanol derived fruit extracts were also found normal with that of control animals.

**Effect of Strawberry extracts (WES and MES) on diabetic markers**

The drastic changes observed in blood glucose, insulin, and bodyweight of the animals are exhibited in table 2. The alloxan induced group 2 animals demonstrated rapid swift in the level of blood glucose. The insulin level and bodyweight of the group 2 animals were found to be decreased in comparison with that of control group 1 rats. The observed changes were found to be reverted to their near normal values in the animals treated the WES (group 3), MES (group 4) and glibenclamide (Group 5). The MES (group 4) fed rats showed better recovery (P<0.01) than the WES (group 3) rats.

**Effect of WES and MES on the levels of renal markers**

The levels of renal markers such as urea, uric acid, and creatinine are presented in table 3. Group 2 animals were recorded with increased values of all these parameters in comparison with normal control group 1 animals. Even though both WES (group 3) MES (group 4) treated animals exhibiting recouptment towards the control, the group 4 animals exhibited close reversal (p<0.01) of these parameters than the group 3 rats.

**DISCUSSION**

Plant based natural antioxidants have been the current focus of research in the area of disease prevention (Lijubunic *et al.*, 2005; Yang *et al.*, 2006; Khalil *et al.*, 2007). Different part of plants such as seeds, roots, barks, vegetables, fruits and plant derived products are proved to have significant health benefits in different animal models. The antioxidant contents are responsible for their biomedical effect. These natural antioxidants taken through diet can neutralize the oxidative stress, and cellular oxidation reaction under appropriate conditions. In the present investigation, the antioxidant and hypoglycemic properties of water and methanolic crude (WES and MES) of strawberry fruits were analyzed. Both the fruit extracts effectively scavenged the free radicals generated in the in vitro DPPH and ABTS radical system. The strawberry fruits are rich source for ascorbic acid, and contain a diverse range of polyphenols including anthocyanins, phenolic acids, flavonols, flavanols, ellagittamins or proanthocyanidins (Aaby *et al.*, 2007; Buendia *et al.*, 2010). The in vitro free radical scavenging activity of the fruit extract is due to its polyphenolic content mainly contributed by anthocyanins (Wang *et al.*, 1996; Wang and Lin, 2000). Toxicity evaluation of strawberry fruit extracts does not reveal adverse effect and warrants the safety. Different investigators had used the strawberry fruit extract in human subjects (Tulipani *et al.*, 2009) and in various animal models (Somasagara *et al.*, 2012). Similar to the present observation none of them have reported any adverse effects.

Alloxan-induced hyperglycemia is a most accomplished animal model for the preliminary screening of plant and plant derived extracts against diabetes (Ramkumar *et al.*, 2009). This model is characterized by severe reduction of body weight (Odetola *et al.*, 2006), attributed by various metabolic changes arising from gluconeogenesis, catabolism of proteins and fats, increased muscle wasting, loss of tissue and structural proteins. Alloxan induction resulted in an elevated glucose (hyperglycemia) and significant drop of plasma insulin level. The oral administration strawberry fruit extracts and the drug glibenclamide was halting the sustained hyperglycemia and reverting back the body weight values and insulin levels to that of control animals. Maximum control was observed with MES treated group. This hypoglycemic property of strawberry extracts could be ascertained to its antioxidant components (Marques *et al.*, 2010). The possible hypoglycemnic mechanism of *Fragaria x ananassa* extracts would be due to the facilitation of insulin mediated glucose uptake by the cells. The extracts either may directly or indirectly enhance the section of insulin by existing pancreatic beta cells. The strawberry
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The extracts would be restoring structural integrity of beta cells and mobilizing synergetic action along with insulin to potentiate the reversal of hyperglycemic condition. The extracts would have also facilitated the secretion of insulin in its bound form to reverse the low insulin levels.

Fig. 1: ABTS radical inhibition of *Fragaria x ananassa* fruit extracts (WES and MES). The values are expressed as ±SEM

The hyperglycemia is endorsed with marked elevation in the levels of renal function markers such as creatinine, urea, and uric acid etc. These parameters highlight the renal dysfunction and reduced glomerular filtration capacity (Mauer et al., 1981). Elevated urea level and urea nitrogen build up is due to excessive degradation plasma and liver proteins. Increased catabolism of proteins and higher muscle waste during alloxan-induced diabetes not only results in the loss of body weight but also tends to accumulate urea and associated uric acid elevation (Anwar and Meki 2003). Mostly creatinine elevation is associated with the concomitant increase in blood urea nitrogen (Travlos et al., 1996; Saravanan et al., 2009). This observation of present study is in consent with the earlier reports. Oral administration of strawberry fruit extracts and glibenclamide for 45 days resulted in the reversal of above said parameters to near normal value. The synergistic antioxidant action of extract and endogenous urate antioxidant system would have complemented each other to combat the free radical intimidated attack during alloxan induced diabetes and would have brought the reversal. Thus, the antioxidant potential of strawberry extract is in par with earlier findings reported by different authors (Kang et al., 2006; Eidi et al., 2009) with different extracts (Murugan and Pari, 2007; Poongothai et al., 2011). These attributes of

<table>
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<tr>
<th>Table 1: Toxicity evaluation of <em>Fragaria x ananassa</em> fruit extracts on general physiological indicators</th>
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<tr>
<td>15th day serum test</td>
</tr>
<tr>
<td>Body weight (gm)</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
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<tr>
<td>RBC (10^6 µL)</td>
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<tr>
<td>WBC (10^3 µL)</td>
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Values are mean ± SEM, n = 6.

<table>
<thead>
<tr>
<th>Table 2: The effect of <em>Fragaria x ananassa</em> fruit extracts on body weight, blood glucose and serum insulin levels of normal, control and experimental groups of rats</th>
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<tr>
<td>Groups</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Diabetic Control</td>
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<tr>
<td>Diabetic + Water extract</td>
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<tr>
<td>Diabetic + Methanol extract</td>
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<td>Diabetic + Glibenclamide</td>
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Values are mean ± SEM, n=6 aSignificantly different from normal, b,c,dSignificantly different from diabetic control. *p<0.05, **p<0.01, ***p<0.001

Table 3: The effect of *Fragaria x ananassa* fruit extracts on urea, uric acid, creatinine (mg%)levels of normal, control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea</th>
<th>Uric acid</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>34.56±1.23</td>
<td>1.39±0.17</td>
<td>0.83±0.14</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>67.80±1.56k**</td>
<td>1.82±0.14k*</td>
<td>1.98±0.10k*</td>
</tr>
<tr>
<td>Diabetic + Water extract</td>
<td>49.62±1.23k**</td>
<td>1.77±0.11k*</td>
<td>1.91±0.08k*</td>
</tr>
<tr>
<td>Diabetic + Methanol extract</td>
<td>39.46±1.33k**</td>
<td>1.57±0.13k*</td>
<td>1.43±0.09k*</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide</td>
<td>35.69±1.43k***</td>
<td>1.44±0.12k**</td>
<td>1.04±0.07k**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n=6 aSignificantly different from normal, b,c,dSignificantly different from diabetic control. *p<0.05, **p<0.01, ***p<0.001
strawberry extracts could be due to its total antioxidant capacity mainly contributed by total vitamin C and total phenolic content represented by anthocyanins and ellagitannins (Shin et al. 2008; Wang and Millner 2009).

![Fig. 2: DPPH radical inhibition of *Fragaria xanana* fruit extracts (WES and MES). The values are expressed as ±SEM.](image)

**CONCLUSION**

Based on the available literature, the present investigation is forefront to address the hypoglycemic effect of strawberry extracts in animal model. The comprehensive analysis made in our investigations revealed that the ability of strawberry fruit extract to check and/or to revert the hallmarks of diabetic complications mainly through its antioxidant composition contributed by vitamin C and various phenolic components. The strawberry extract holds the promise against diabetes; however more detailed and in vivo studies are necessary to analyze the mechanism and role of these individual compounds to develop strawberry contents as potential therapeutic agent.

**REFERENCES**


Pellegrini N, Re R, Yang M and Rice-Evans C (2002). Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2′-


