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Medicinal effects of saffron and chamomile on diabetes mellitus and associated hyperlipidemia and memory impairment

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Abstract: Diabetes mellitus is a multifactorial metabolic syndrome, in which FDA approved treatments have their own limitations and drawbacks. Therefore, herbal alternatives have recently garnered attention for their effectiveness against diabetes. Herbs saffron and chamomile are renowned for their potent benefits. The present study was designed to observe their effects separately as well as in combination on blood glucose, lipids and antioxidant profile along with memory in diabetic animal model. Fifty male Sprague-dawley rats of 200g ± 20g weight were divided into healthy and diabetic controls while the test groups received methanolic extract of saffron (10 mg/kg), chamomile (30 mg/kg) and combined extract of saffron and chamomile (5mg/kg and 15mg/kg, respectively) over a period of two weeks. It was observed that there were considerable anti-diabetic and anti-hyperlipidemic effects in all treatment groups with modulation of body weight especially in combined saffron and chamomile administered group. Likewise, the antioxidant profile showed significantly decreased MDA levels with reduced SOD activity in all test groups particularly in the combined group with significant improvement in cognition. The considerable results of combined group showed herbal synergy at half dose and bring forth a cost-effective treatment for diabetes and associated disorders than treatment with a single herb.

Keywords: Chamomile, saffron, diabetes mellitus, hyperlipidemia, cognitive impairment, oxidative stress.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to insufficient secretion or abnormal action of insulin (Zierath, 2019). Although the etiology of the disease is not well defined, diabetic patients are more prone to oxidative stress, reactive oxygen species (ROS) production and reduced antioxidant protection. This further progresses the disease pathogenesis and related complications such as, hypercholesterolemia, hypertension and psychoneurological deficits (Dal and Sigrist, 2016, Zilliox *et al*., 2016, Papatheodorou *et al*., 2018, Bellou *et al*., 2018). In the modern medical society, different drugs and regimens have been developed to treat diabetes mellitus but they are not devoid of adverse effects (Pickering *et al*., 2018, Tangvarasittichai, 2015). It is believed that natural foods are mostly safe with treatment superiorities. World Health Organization (WHO) has also recommended ethno-botanical search for identifying anti-diabetic herbs and their use in managing diabetes and its complications (Jacob and Narendhirakannan, 2019). Traditionally, herbs have been used for the treatment of diabetes mellitus (Pang *et al*., 2019). Their chief benefits lie in their efficacy, low incidence of side effects, low cost and human inclination towards the use of natural foods for treatment (Choudhury *et al*., 2018). Several herbs including turmeric, ginger, fenugreek, ginkgo biloba and galega (a source of anti-diabetic drug metformin) have been reported to produce antidiabetic effects (Odeyemi and Bradley, 2018). Some of these herbs are expensive and cost cutting is generally achieved through their combined use in reduced amounts to allow action on different target sites individually or synergistically in body (Sotoudeh *et al*., 2019, Zhou *et al*., 2016).

Saffron (Saf) and chamomile (Cham) are previously documented of their benefits in alleviation of inflammation, skin ailments, gout, neuralgia and rheumatic pain (Balogun *et al*., 2016, Meyer-Rochow, 2017, Rafraf *et al*., 2015). Saffron is collected as stigmas of *Crocus sativus L* flower and mainly contains active compounds crocin, crocetin and safranal. These are known to possess antioxidant activities and modulate enzymes activity of catalase, glutathione peroxidase, and superoxide dismutase (Khorasany and Hosseinzadeh, 2016). Cham comes from dried flowers of *Matricaria chamomile* L., with major active ingredients bisabolol,

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farnesene, sesquiterpenes, coumarins and flavonoids that are documented to influence various enzymatic activities, however, the exact mechanism of their action is still unknown (Ghasemi *et al*., 2016, Miguel *et al*., 2015, Zemestani M *et al*., 2016). Therefore, these two herbs were selected for the present study to examine their potential efficacy in treatment of diabetes and related complications of hyperlipidemia and memory loss in diabetic rats.

MATERIALS AND METHODS

Medicinal plant collection

Dried packed floral parts of Cham, known as "Baboona" herb in native Urdu language and stamens of Saf with native Urdu name "Zafaran" were purchased from a local supermarket in the suburbia of city Karachi, Pakistan. A sample of these plant materials were assigned the herbarium-voucher number MC-FL-08-18-05 for Cham and CS-ST-08-18-05 for Saf, so as to be preserved in the Natural Products Research Division, Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi.

Preparation of herbal extracts

The herbs, after cleaned of adulterants, weighed approximately 950 g for Cham and 40g Saf, were immersed in 2 L of aqueous methanol (containing 30% water and 70% methanol) for a total of 3 days and subjected to intermittent shaking every day. The next step was filtration of the plant material for which a muslin cloth was used. The filtrate obtained was again filtered through a "Whatman qualitative grade-1 filter paper." After repeating the process twice, all the filtrate was combined and concentrated in a rotary evaporator with the temperature maintained at 35–40^{o}C and the pressure was subnormal. A paste-like, dark brown crude extract (Wt.Cr) with a yield of 13% (wt/wt) was obtained. This was completely soluble in normal saline and in distilled water.

Sample size calculation

Sample size was calculated using the method of mean difference using open epi sample size calculator version 3.01 by inserting mean and standard deviation of curcumin-treated rats (15.26±2.85) and aspirin treated rats (11.6±0.909) reported previously (Haider *et al*., 2013). At 95% confidence interval with 80% power of study and 5% margin of error we get *n*= 6 sample size per group of five study groups. However, to increase the sampling accuracy and reduce the sampling error, 10 animals per group were taken.

Experimental animals

Fifty Sprague-dawley rats weighing 200g ± 20g were purchased from Aga Khan University animal house, Karachi and housed individually in the plastic cages under 12 h light dark cycle at room temperature (22 ± 2^{o}C) with free excess to standard rodent diet and clean tap water. All experiments were conducted according to a protocol approved by Ethical Committee of Animal Care and Use vide number 68 ECACU-BBS-17. The animals were randomly divided into five groups (*n*=10 rats/group), comprising of two control groups as healthy control (HC), diabetic control (DC) and three test groups given individually methanolic extracts of Saf (10 mg/kg), Cham (30 mg/kg) and combined extract of Saf and Cham (5mg/kg and 15mg/kg). The respective treatment was given through oral administration once a day over a period of two weeks. After two weeks of treatment, the rats were subjected to behavioral test for assessment of cognitive ability by novel object recognition task (NOR). The next day after completion of behavioral test, all rats were euthanized through cardiac puncture and blood samples were collected for analysis of lipid and antioxidant profile parameters. The serum was stored at -20^{o}C.

Induction of diabetes in rats and estimation of glucose

For the induction of diabetes in the test and DC rats, streptozotocin (STZ) in citrate buffer 0.1 M (pH 4.5) was freshly prepared every day and injected intraperitoneally for three days at the dose of 60 mg/kg. Four days after the administration of STZ, fasting blood sugar levels were monitored and sugar level above 200 mg/dl in rats was marked as diagnostic of the diabetes onset. At the end of experiment, blood was drawn from tail after overnight fasting for estimation of fasting blood glucose levels (mg/dl) through the Accu-Chek Performa glucometer.

Measurement of bodyweight

All animals were weighing 200g ± 20g at the time of acclimatization. Body weight of all animals was again measured at the end of the experiment.

Estimation of insulin

The estimation of plasma insulin level was performed using Ultra-Sensitive Rat Insulin ELISA Kit (Crsytal Chem, USA).

Estimation of blood lipid profile

The estimation of blood cholesterol, triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins (LDL) were performed using the Roche kits Cobas c 111(Roche, Pakistan).

Estimation of superoxide dismutase (SOD)

The activity of SOD was determined using protocol by Misra H.P (Misra and Fridovich, 1972). The superoxide anion O_2 causes the auto-oxidation of epinephrine to adenochrome at alkaline pH, in competition with this reaction, the SOD reduces the adenochrome formation. 0.15 ml of ice cold chloroform and 0.75 ml of ethanol were then mixed together in 0.1 ml of serum and centrifuged at 3000 rpm for 15 minutes. Afterwards 1.0

ml of 0.1 M carbonate-bicarbonate buffer (pH 10.2), 0.5 ml of EDTA (0.6 mM) and epinephrine (1.8 mM) were added. Changes in the absorbance were recorded at 480 nm.

Estimation of malondialdehyde concentration (MDA)

Serum MDA was measured by lipid peroxidation intermediate cleavage which released MDA to react with thiobarbituric acid (TBA). 0.20 ml serum was put in a test tube containing 3.0 ml of glacial acetic acid and 3.0 ml of 1% TBA in 2% NaOH solution. The mixture was placed in boiling water for 15 min and absorbance was read at 532 nm after cooling (Gwarzo *et al*., 2014).

Behavioral test

Novel object recognition (NOR) test

This test was used to analyze cognition in rats. A 40×40×42 cm apparatus as an open arena was used for the test. The test was comprised of habituation, familiarization and test sessions. Rat was allowed to habituate in the arena for 10 min followed by familiarization phase after 24 h with two identical wooden blocks placed in horizontal plane of the arena for 5 min. After 20 min, test session was conducted during which one of the objects was replaced by a new object with different texture, color and size. Time to sniff both objects was recorded during test phase. A recognition index was calculated by the ratio TB/(TA + TB) where TA = time spent exploring the familiar object; and TB = time spent exploring the novel object (Batool *et al*., 2016).

STATISTICAL ANALYSIS

The statistical analysis of the study groups was done by one way ANOVA using SPSS version 22.0 followed by post hoc Tukey's test. Values of $p \leq 0.05$ were considered significant.

RESULTS

Effects on fasting blood sugar (FBS) and insulin level

Effects of methanolic extract of Saf and Cham and their co-administration on FBS and insulin level in animal model of diabetes are shown in fig. 1a and 1b. Data was analyzed by one-way ANOVA which revealed a significant effect of treatment on FBS ($F_{4,45}$ = 11.96, $p<0.01$) and insulin ($F_{4,45}$ =11.916, $p<0.01$). *Post-hoc* analysis by Tukey's test showed a significant increase in FBS level and decrease in insulin in DC group as compared to healthy controls ($p<0.01$). There was a tendency of decreased FBS levels and significantly increased insulin level in Saf and Cham treated groups as compared to DC group. However, Saf+Cham group exhibited significantly decreased sugar levels and increased insulin when to compared to DC group ($p<0.01$) and to the groups treated with single herb.

Effects on body weight

Effects of treatment of methanolic extract on body weight are shown in fig. 2. Statistical analysis by one-way ANOVA revealed significant effects on body weight ($F_{4,45}$ =144.528, $p<0.01$). Tukey's *post-hoc* test showed a significant decrease in body weight in DC rats as compared to healthy controls ($p<0.01$). This decrease in body weight was significantly modulated ($p<0.01$) by the treatment of methanolic extract of Saf, Cham and their combination when compared to that of diabetic rats. However, the effects of Saf+Cham were shown more significant ($p<0.05$) as compared to single herbs.

Effects on blood cholesterol level

Effects of treatment of methanolic extract on cholesterol levels are shown in fig. 3. Statistical analysis by one-way ANOVA revealed significant effects on cholesterol levels ($F_{4,45}$ = 7.14, $p<0.01$). Tukey's *post-hoc* test showed a significant increase in cholesterol levels in DC rats as compared to healthy controls ($p<0.01$). This increase in cholesterol level was significantly decreased ($p<0.01$) by the treatment of methanolic extract of Saf, Cham and their combination when compared to that of diabetic rats.

Effects on blood TG level

One-way ANOVA showed significant effects of treatment on TG levels ($F_{4,45}$ = 12.29, $p<0.01$). Tukey's *post-hoc* test showed significantly increased levels of TG in diabetic rats as compared to controls ($p<0.01$). Treatment with Cham significantly decreased TG levels when compared to that of DC group ($p<0.01$). However, Saf and Saf+Cham treatment did not affect the TG levels (fig. 3).

Effects on blood LDL/HDL ratio

LDL/HDL ratio was also calculated after the serum estimation of LDL and HDL levels (fig. 3). One-way ANOVA revealed significant effects of treatment on LDL/HDL levels ($F_{4,45}$ = 14.16, $p<0.01$). Post-hoc analysis by Tukey's *post-hoc* test showed significantly increased ratio of LDL/HDL in DC group as compared to controls. However, treatment with Saf, Cham and Saf+Cham resulted in significantly reduced LDL/HDL ratio as compared to DC ($p<0.01$) as well as HC groups ($p<0.01$).

Effects on blood SOD and MDA levels

Statistical analysis of SOD data showed significant effects of treatment on SOD ($F_{4,45}$ = 187.58, $p<0.01$) and MDA levels ($F_{4,45}$ = 62.64, $p<0.01$). Tukey's *post-hoc* test showed significantly increased levels of SOD and MDA in DC group as compared to control group. Treatment with Saf, Cham and their combination significantly reduced SOD ($p<0.01$) and MDA ($p<0.01$) levels when compared with that of DC group. On the other hand, the levels of SOD and MDA were still higher in Saf and Cham groups as compared to healthy control group (fig. 4). However, the estimated levels of MDA were

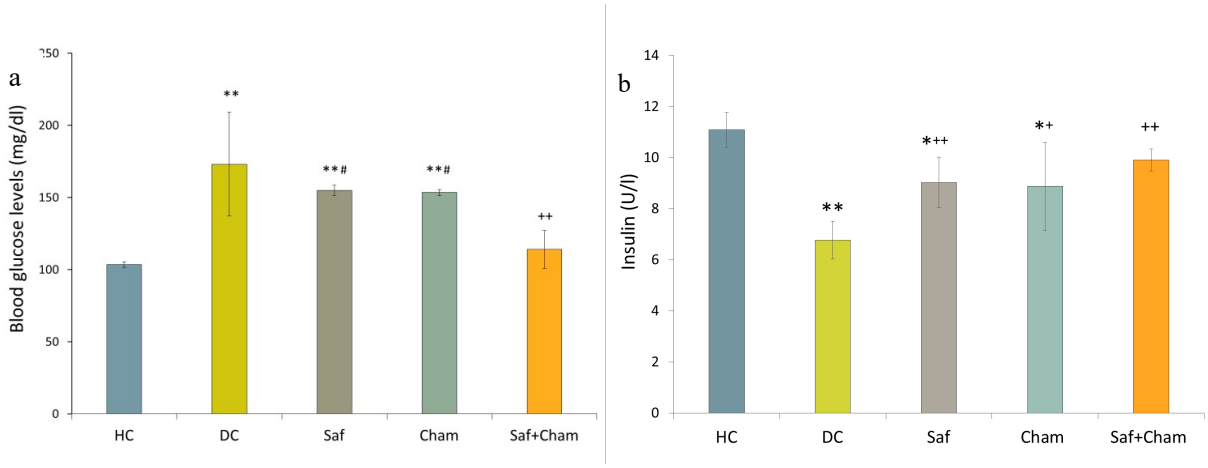

Fig. 1: Effects of two week administration of methanolic extract of saffron, chamomile and there combination on blood glucose levels (a) and insulin levels (b) in streptozotocin-induced rat model of diabetes. Values are presented as mean±SD (n=10). Data was analyzed by one-ANOVA followed by Tukey's post-hoc test. **p<0.01 as compared to healthy control (HC); ++p<0.01 as compared to disease control (DC); #p<0.05 as compared to saffron and chamomile (Saf+Cham) combine group.

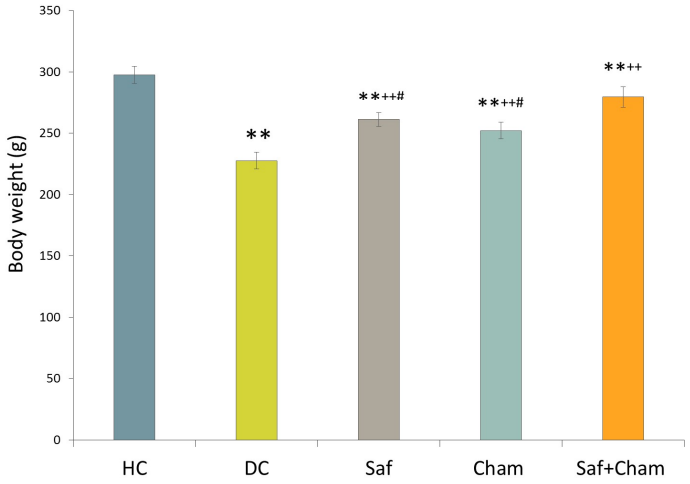

Fig. 2. Effects of two week administration of methanolic extract of saffron, chamomile and their combination on body weight in streptozotocin-induced rat model of diabetes at the end of experiment. Values are presented as mean±SD (n=10). Data was analyzed by one-ANOVA followed by Tukey's post-hoc test. **p<0.01 as compared to healthy control (HC); ++p<0.01 as compared to disease control (DC); #p<0.05 as compared to saffron and chamomile (Saf+Cham) combine group.

significantly reduced by the combination of Saf and Cham when compared to the groups treated with individual herb (p<0.01).

Effects on novel object recognition (NOR) test

Recognition ability was also monitored at the end of treatment to analyze brain function in disease and treated animals. Data analyzed by one-way ANOVA showed significant effects of treatment on recognition index ($F_{4,45}$= 463.66, p<0.01). *Post-hoc* analysis revealed a marked decrease in recognition index in diabetic rats as compared to controls (p<0.01). Treatment with Saf, Cham and Saf+Cham resulted in significantly increased recognition index when compared to that of DC group (fig. 5).

DISCUSSION

Diabetes is an incurable global pandemic with limited treatment options that are costly and cause many side effects (Marín-Peñalver *et al*., 2016, Samarghandian *et al*., 2017). There is a need for easy availability, cheaper with better compliance of anti-diabetic drugs. In this regard, alternative traditional antihyperglycemic medicine has a greater role to play. The present study showed that the single and combined treatment of herbs Saf and Cham

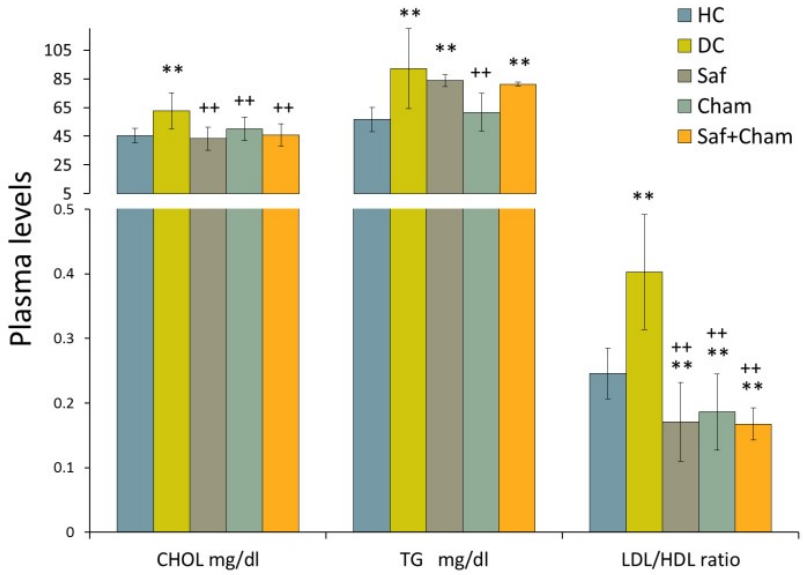

Fig. 3: Effects of two week administration of methanolic extract of saffron, chamomile and there combination on plasma levels of cholesterol (CHOL), triglycerides (TG) and LDL/HDL ratio in streptozotocin-induced rat model of diabetes. Values are presented as mean±SD (n=10). Data was analyzed by one-ANOVA followed by Tukey's post-hoc test. **p<0.01 as compared to healthy control (HC); ++p<0.01 as compared to disease control (DC).

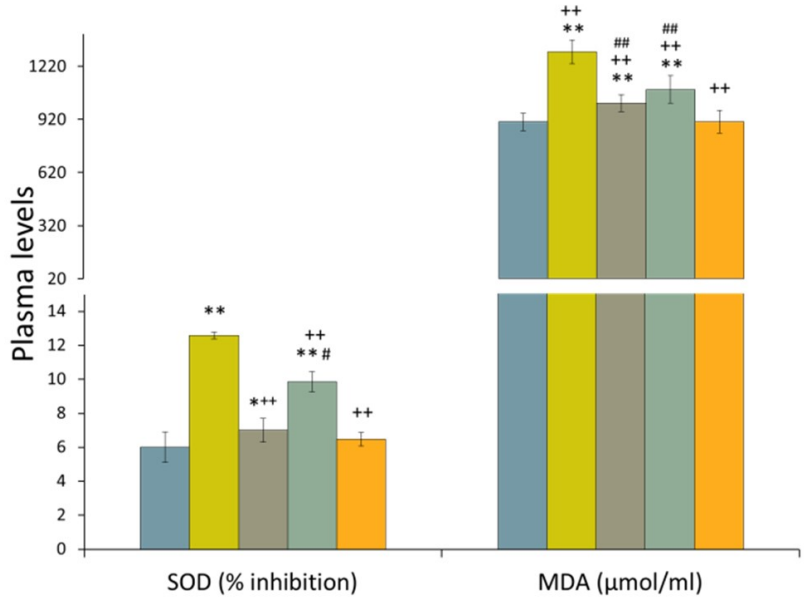

Fig. 4: Effects of two week administration of methanolic extract of saffron, chamomile and there combination on plasma levels of superoxide dismutase (SOD) and malondialdehyde (MDA) in streptozotocin-induced rat model of diabetes. Values are presented as mean±SD (n=10). Data was analyzed by one-ANOVA followed by Tukey's post-hoc test. **p<0.01, *p<0.05 as compared to healthy control (HC); ++p<0.01 as compared to disease control (DC); ##p<0.01, #p<0.05 as compared to saffron and chamomile (Saf+Cham) combine group.

facilitated significant reduction in the blood sugar level and lipid profile while enhanced the antioxidant profile with cognitive improvement in NOR. However, treatment with combined herbal group at reduced doses for a period of two weeks, validated and supported the synergistic therapeutic effects of these herbs.

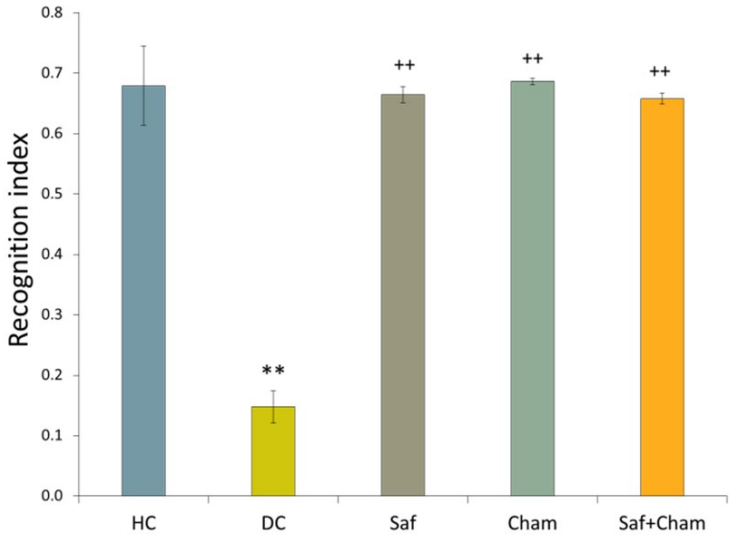

Fig. 5: Effects of two week administration of methanolic extract of saffron, chamomile and there combination on novel object recognition (NOR) index in streptozotocin-induced rat model of diabetes. Values are presented as mean±SD (n=10). Data was analyzed by one-ANOVA followed by Tukey's post-hoc test. **p<0.01 as compared to healthy control (HC); ++p<0.01 as compared to disease control (DC).

Previous studies revealed the effectiveness of Saf and Cham in management of blood glycemic index (Marín-Peñalver *et al*., 2016, Samarghandian *et al*., 2017, Samarghandian *et al*., 2015). It has been reported that Saf extract as well as its individual components, crocin and safranal; possess significant antioxidant property through radical scavenging actions (Samarghandian *et al*., 2015). In addition, it also exhibits regenerating properties, preserving the architecture of pancreas in diabetes (Miraj and Alesaeidi, 2016). Like Saf extract, Cham extract is also known to lower blood glucose levels, through augmentation of peripheral glucose uptake, inhibition of hepatic glucose synthesis, escalation of liver glycogen storage, and induction of strong antioxidant activities (Hosseini *et al*., 2015). This is achieved by activation of aldose reductase and sorbitol dehydrogenase enzymatic activities (Yan *et al*., 2017). Moreover, individual Cham constituents such as chlorogenic acid reduces carbohydrate absorption through intestine; esculetin and quercetin, inhibit intestinal α-Glucosidase activity and prevent carbohydrates digestion (Bhandarkar *et al*., 2019), while luteolin and quercetin inhibit hepatic glycogen phosphorylase lowering liver glycogen synthesis (Leonidas *et al*., 2017, Vinayagam and Xu, 2015, Bhandarkar *et al*., 2019). In addition, the antidiabetic molecular mechanism of Cham is found to be through interaction with liver peroxisome proliferator-activated receptors, namely PPARγ and PPARα. Activation of PPARγ systemically improve insulin sensitivity, whereas activation of PPARα reduces both plasma cholesterol levels and insulin resistance in the body (Leonidas *et al*., 2017). Furthermore, administration of whole Saf or its single constituent crocin, leads to reduction in serum total cholesterol, TG, LDL with augmentation of HDL levels through inhibition of pancreatic lipase activity (Altinoz *et al*., 2015). The molecular mechanism of lipid lowering effects of Cham are also suggested to be similar to activation of PPARs in liver (Miraj and Alesaeidi, 2016; Samarghandian *et al*., 2015). Studies show that Saf improves cognition which is due to blockade of diabetes related oxidative stress in brain hippocampus (Fathimoghadam *et al*., 2019; Ghaffari *et al*., 2015). Safranal, provides neuroprotection through elevation of total sulfhydryl contents, antioxidant capacity and decreasing MDA content in brain (Samarghandian *et al*., 2017). In fact, elevations of serum SOD and MDA activities in diabetic control rats suggest a possible stress-induced increase in reactive oxygen species production with accompanying compensatory adaption of the free-radical scavenging superoxide dismutase enzyme activity (Samarghandian *et al*., 2015; Samarghandian *et al*., 2017; Feidantsis *et al*., 2018). The present study has also shown that both herbs alone as well as in combination decreased the activity of the enzyme and MDA content in rats.

The present study showed the effects of herbs Saf and Cham against diabetes and its associated complications preferably in combined extract. The reduction in the quantity also reduced the cost of the treatment. The limitation of the study is being done only on animal model, however, the future implications will be human translational studies that will be based on the positive results attained from the present study. That will enable the co-administration of these herbs in reduced doses for better and cost effective treatment alternative for diabetes and associated complications.

CONCLUSIONS

Saffron and Chamomile have been used separately as herbal remedies since ancient times and this study has shown that the co-administration of both herbs in reduced doses lead to better and cost effective management alternative for diabetes and related complications such as hyperlipidemia, oxidative profile and cognitive disorders.

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