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Selçuk Üniversitesi
Selçuk Tarım ve Gıda Bilimleri Dergisi
23 (47): (2009) 77-88
ISSN: 1309-0550

FARELERE UYGULANAN STREPTOZOTOZİN (STZ) VE CCl_4'ÜN TOKSİK ETKİSİNE KARŞI AROMATİK BİTKİ KARIŞIMI İNFÜZYONLARININ ANTİOKSİDAN VE KORUYUCU ETKİSİ

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ÖZET

Sağlık açısından faydalı ve lezzetli olan bitki infüzyonları dünya çapında yaygın olarak tüketilmektedir. Çeşitli farmakolojik etkileri bulunmakla birlikte, bu etkilerinin bileşimlerinde bulunan uçucu ve fenolik maddelerden kaynaklandığına atfedilir. Bu çalışmanın amacı beş aromatik bitkinin karışımından kabul edilebilir lezzette doğal ve sağlığı destekleyici bir içecek hazırlamaktır. Bitki infüzyonlarının uçucu bileşenleri izole edilerek GC ve GC/MS ile belirlenmiştir. Toplam fenolik maddeleri ve in vitro antioksidan aktiviteleri belirlenmiştir. STZ ile beslenen farelerin bazı organlarında gulukoz seviyesi, böbrek fonksiyonları, süperoksit dimustaz (SOD)'ın antioksidan enzim aktivitesi, glutation redüktaz (GR), glutation redüktaz (GPx), gulukoz -6- fosfat dehidrogenaz (G-6-PhDH) ve malondialdehid (MDA) seviyesi belirlenmiştir. Ayrıca, plazmada üre, kreatinin, lipid profile ve kan hemoglobin seviyeleri belirlenmiştir. CCl_4-'ün oluşturduğu toksik etkiye karşı aromatik bitki karışımının koruyucu etkisini değerlendirmek amacıyla transaminaz aktivitesi, alkali fosfataz, γ-glutamiltransferaz, laktat dehidrogenaz, toplam protein seviyesi ve toplam bilirubin belirlenmiştir. Sonuç olarak, aromatik bitki karışımı streptozotozin (STZ) ve CCl_4'ün toksik etkisini azaltmış ve farede antioksidan etki göstermesiyle, karaciğer, böbrek ve pankreas korunmuştur.

***Anahtar kelimeler**: Aromatik bitkiler, Antioksidanlar, GC-MS, Karbon tetraklorid ve streptozotozin*

THE ANTIOXIDANT AND PROTECTIVE ACTIVITY OF AROMATIC PLANTS BLEND INFUSION ON STREPTOZOTOZINE (STZ) AND CCl_4 INDUCED TOXICITY IN RATS

The consumption of plant infusions as a healthy tasty drink is a worldwide practice. Various pharmacological activities inherent to aromatic plants have been attributed to their volatiles and phenolic compounds. The present study aimed to prepare a natural healthy drink from blend of five aromatic plants possessing an acceptable flavour and good taste. Plants infusion volatiles were isolated and analyzed using GC and GC/MS, the total phenolic content and in vitro antioxidant activity were determined. The effect of aromatic plants infusion on glucose level, kidney function and antioxidant enzyme activities of superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and glucose -6- phosphate dehydrogenase (G-6-PhDH) as well as the level of malondialdehyde (MDA) in different organs of streptozotozine (STZ) diabetic rats were determined. Also, plasma urea, creatinine, lipid profile and blood hemoglobin were tested. Activities of transaminases, alkaline phosphatase, γ-glutamyltransferase and lactate dehydrogenase and level of total protein and total bilirubin were determined to evaluate the hepatoprotective activity of blend infusion in CCl_4- induced toxicity. The results showed that supplementation with aromatic plants blend infusion significantly attenuated toxic effects induced by STZ and CCl_4 and protecting liver, kidney, pancreas and maintain the antioxidant status in rats.

***Key words**: Aromatic plants, Antioxidants, GC-MS, Carbon tetrachloride and Streptozotosine*

INTRODUCTION

Herbs and spices, which are important part of the human diet, have been used for thousands of years to enhance flavour, colour and aroma of food. In addition, they are also known for their preservative, antimicrobial, antioxidative (Shobana and Naidu, 2000) and various other medicinal values (Wood1, et al., 2001). Free-radicals are generated continuously in the body due to metabolism and disease. In order to protect themselves against free radicals, organisms are endowed with endogenous and exogenous antioxidant defenses; yet these defense systems are not sufficient in critical situations (oxidative stress, contamination, UV exposure, etc.) where the production of free radicals significantly increases.

It is generally assumed that the active dietary constituents contributing to these protective effects are the antioxidants (vitamins, carotenoids, polyphenos and sterols) (Yeum, et al., 2003). The intake, in the human diet, of antioxidant compounds, or compounds that ameliorate or enhance the biological antioxidant mechanisms, can prevent and in some cases help in treatment of some oxidative- related disorders and carcinogenic events (Havsteen, 2002). Natural plant products have been used empirically for this purpose since ancient times and tendency is emerging today for their increased used.

Liver, an important organ actively involved in metabolic functions, is a frequent target of a number of toxicants. The principal cause of carbon tetrachloride (CCl_4) induced hepatic damage is lipid peroxidation and decreasing activities of antioxidant enzymes and generation of free radicals. Also, resulting in leakage of cellular enzymes into the blood stream and centrilobular necrosis (Poli, 1993). Presently, the use of herbal medicines for prevention and control of chronic liver diseases is in the focus of attention for the physicians, pharmaceutical manufacturers and patients; the

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reasons for such shift towards the use of herbals include the expensive cost of conventional drugs, adverse drug reactions, and their inefficacy.

Diabetes, as one of the most common global diseases, affects approximately 200 million individuals worldwide. Type 2 diabetes (insulin independent diabetes mellitus) is the most common form of diabetes accounting for 90% of cases worldwide (not dependent to use insulin) (Thompson and Godin, 1995). Besides all the medical treatments for diabetes, people still need to use traditional remedies prepared from herbs and plants. Approximately 800 plants worldwide have been documented to support antidiabetic effects, however a few comprehensive studies on traditional antidiabetic plants have been carried out (Alarcon-Aguilara, et al., 1998 and Chhetri, et al., 2005). Different aromatic plants with antioxidant, hypoglycemic, hypolipidimic, renal and hepatoprotective activities provide important sources for the development of new drugs in the treatment of many diseases (Cemek, et al., 2008).

Herbal tea, which is generally a polyherbal formulation made up of different aromaic plants, is also considered as a source of antioxidants. These antioxidants found in herbal tea play an important role as a part of a healthy diet (Babenko and Shakhova, 2006). Herbal teas are reported to contain natural antioxidants such as vitamin A, B_6, C, polyphenols, co-enzyme Q10, carotenoids, selenium, zinc and phytochemicals (Atoui, et al., 2005). Many therapeutic properties such as neuroprotective, cardioprotective, chemoprotective, anticarcinogenic, hepatoprotctive, hypoglycemic and anti- inflammatory have been attributed to herbal preparations (Shahidi and Naczk, 1995; Hollman and Katan, 1997; Parr and Bolwell, 2000; Visioli, et al., 2000; Campanella, et al., 2003; Trouillasa, et al., 2003; Luczaj and Skrzydlewska, 2005).

Water extract (infusion) of different aromatic plants was found to be richer in polar phenols and therefore more effective in retarding lipid oxidation and in scavenging of free radicals than methanol, ethanol and acetone extracts of the same plant materials (Triantphyllou, et al., 2001).

Psidium guajava Linn, belonging to the family of Myrtaceae, has been used as a health tea. Its leaf contains copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body, and therefore, can be expected to prevent various chronic diseases such as diabetes, cancer and heart-disease. It was reported that the leaves of *P. guajava Linn* contain an essential oil rich in cineol, tannins and triterpenes (Ramadan, et al. 2008).

Corn silk (*Zea mays* L.) refers to the stigmas from the female flowers of maize. Corn silk has been used as a remedy for various diseases such as inflammation of the bladder and prostate as well as treatment for irritation in the urinary system. The hepatoprotective activity of corn silk studied on an acute hepatitis model induced by tetrachloromethane (Katikova, et al., 2002; Maksinovic, et al., 2004). Recently, the volatile extract (more than 99% of it terpinoids) a well known chemicals used in flavour and fragrance ingredients and non-volatile extracts obtained from Egyptian corn silk were found to possess strong antioxidant activities (El-Ghorab, et al., 2007).

Ginger (*Zingiber officinale*; Zingiberacae), is one of the oldest herbs known to the people and is one of the earliest spices to be known in the east. Ginger of the commerce consists of thick scaly rhizomes. The essential oil and oleoresins extracted from ginger rhizomes are very valuable products responsible for characteristic ginger flavour and pungency, are used in many food items, soft drinks, beverages and many types of medicinal substances (Singh, et al., 2008).

The essential oil and oleoresins of ginger possesses antioxidative, hypoglycaemic, hypocholesterolaemic and hypolipidaemic potential. Additionally, raw ginger is effective in reversing the diabetic proteinuria observed in the diabetic rats. Thus, ginger may be of great value in managing the effects of diabetic complications in human cases (Al-Amin, et al., 2006).

Chamomile (*Matricaria chamomilla*), is one of the most popular cultivated aromatic plant allover the world and well documented herbal medicine whose flower-heads are used both internally and externally to alleviate or even to cure a vast list of ailments particularly those related to inflammation conditions (Blumenthal, 2000; Mills and Bone, 2000; Hernández-Ceruelos, et al., 2002; Srivastava and Gupta, 2007). Chamomile is mostly consumed as infusion for sedative and anxiolytic purposes as a digestive aid to treat gastrointestinal disturbances, specially in babies and small children (Weizman, et al., 1993; De la Motte, et al., 1997; Madisch, et al., 2001). The biologically active substances in chamomile essential oil are α-bisabolol, bisabolol oxides, chamazulene, and enyndicycloethers (Grgesina, et al., 1995).

The Tiliaceae plant *Tilia argentea* (linden), is commonly called silver linden flowers, have been widely used in herbal teas and as a diuretic, stomachic, antineuralgic, and sedative in European countries. Aqueous extracts or infusions obtained from the flowers of Tilia species are widely used for the treatment of anxiety, to relieve sleeplessness, headache, and nervous excitement in folk medicine (Herrera-Ruiz, et al., 2008). Water extracts of Tilia species are able to show statistically significant antioxidant and hepatoprotective effect (Yildirim, et al., 2000; Matsuda, et al., 2002; Manuele, et al., 2008).

Many hepatoprotective herbal preparations have been recommended in alternative systems of medicine for the treatment of hepatic disorders. No systematic study has been done on protective efficacy of the blend infusion under study to treat hepatic diseases. Therefore, the protective action of the blend infusion was evaluated in an animal model of hepatotoxicity induced by carbon tetrachloride.

The present study aimed to use some aromatic plants namely ginger, guava leaves, linden, corn silk and chamomile, which are known to possess antioxidant activities, as ingredients in blend that gives an acceptable flavour after reconstitution in hot water beside its chemopreventive activity. Chemopreventive effectiveness of the aromatic plants blend infusion was tested by subjection to biological evaluation concerning its antioxidant, hypoglycemic, hypolipidimic, renal and hepatoprotective activities.

MATERIALS AND METHODS

Plant materials and *Preparation of blend infusion*

Dry Egyptian guava leaves, corn silk, linden flowers, chamomile and ginger root were purchased from local market. The aromatic plants under investigation were separately grounded and blended at variable concentrations (45% guava leaves, 35% linden, 10% ginger, 5% corn silk and 5% chamomile). One gram of grounded aromatic plants blend was infused with 100 ml freshly boiled water for 5 min. followed by filtration. The infusion filtrate was subjected to further studies.

Sensory evaluation

The different sensory attributes (odour, colour, taste and appearance) of blend infusion under investigation was estimated and scored by 15 assessors (Chemistry of Flavour and Aroma Dept., NRC).The grading system was based on a total score of 100 of which 35% was awarded for odour, 35% for taste, 15 % for colour and 15% for appearance. This grading system is commonly used to evaluate tea quality in China (Liang, et al., 2003).

Isolation and analysis of the blend volatiles

Briefly, 100g of powdered material was boiled in water (1:10 w/v) for 4 h. The water extract was filtered through Whatman No. 1 filter paper and then extracted with 100 ml of dichloromethane using a liquid- liquid continuous extractor for 6 h. After that, the volatile extract was dried over anhydrous sodium sulfate and the solvent was evaporated under vacuum at 40 °C followed by nitrogen stream until the volume was reduced to 0.5 ml. Volatile compounds in the blend aqueous extract obtained by three replicate experiments were identified by comparison with the Kovats gas chromatographic retention indices (Kovats, 1965) and by the mass spectral fragmentation pattern of each GC component compared with those of authentic compounds and/or NIST/EPA/NIH Mass Spectral Library. An Agilent model 6890 gas chromatograph equipped with a 30 m × 0.25 mm (inside diameter) (df 0.25 µm) bonded phase DB-5 fused silica) capillary column (Agilent, Folsom, CA) and a flame ionization detector (FID) was used to obtain the Kovats index, which was also compared with published data (Adams, 1995). The oven temperature was increased from 35 to 220 °C at a rate of 3 °C/min and held for 40 min. The linear helium carrier gas flow rate was 29 cm/s. The injector temperature was 200 °C, and the detector temperature was 250 °C. An Agilent model 6890 gas chromatograph interfaced with an Agilent 5791A mass selective detector (GC–MS) was used for mass spectral analysis of the GC components at a MS ionization voltage of 70 eV. A 30 m × 0.25mm (inside diameter) (df 0.25 µm) DB-5 bonded phase fused silica) capillary column (Agilent) was used for GC. The linear velocity of the helium carrier gas was 30 cm/s. The injector and the detector temperatures were 250 °C. The oven temperature was increased from 35 to 220 °C at a rate of 3 °C/min and held for 40 min.

Determination of total phenolic content

It was determined in the blend infusion with Folin-Ciocalteu reagent using gallic acid as the standard (Kahkonen, et al., 1999).

Animals and diets

Forty eight male Swiss albino rats with initial weights ranging from 150 to 170g were used as experimental animals for the biochemical studies. Animals were provided from the breeding unit of the National Research Center (Cairo). The animals were maintained under laboratory condition for an acclimatization period before performing experiment. Throughout the experimental period, the rats were fed on standard pellets prepared by Cairo Company of Oil & Soap, Egypt, for experimental animals. The pellets contain 23% protein, 6.5% fat, 4% fibers, 8% ash, 2.5% added minerals and 56% carbohydrates. Rats were provided with food and water *ad libitum*.

Hypoglycemic activity

A total of 24 rats were used. The rats were divided into four groups (six rats each). Group I (control group): non-treated normal rats were fed commercial standard diet and tap water *ad libitum*. Group II (supplemented group): normal rats were fed commercial standard diet and supplemented daily with freshly prepared blend infusion (1g/ 100 mL) as a drink for four weeks and rats of this group were used to examine the safety of the blend infusion. Group III (STZ diabetic control): The rats were injected intraperitoneally (i.p.) with streptozotocin (STZ) dissolved in sterile normal saline at a dose of 52 mg/kg body weight (b.wt). Group IV (protected group): rats were maintained on standard diet and blend infusion (instead of water) for two weeks, followed by a single injection of STZ. Diabetic rats were continuously supplemented with blend infusion for another two weeks.

Hepatoprotective activity

Hepatic injury in rats was induced separately by intraperitoneal administration of CCl_4 (l.195 mL/kg b.wt.; 3 times a week) for two weeks as described by

Mac Sween, et al. (1994). The animals were divided into four groups (six rats each). Group I and II as described later. Group III (CCl_4 intoxicated group; rats were injected intraperitonealy with CCl_4 3 times a week for two weeks. Group IV (protected group): rats were maintained on standard diet and blend infusion instead of water for four weeks and at the1st day in the third week CCl_4 was injected as in group III.

Blood sampling

At the end of experimental period, rats were lightly anesthetized with diethyl ether and blood samples were collected from sinus orbital puncture in heparinized tubes then centrifuged for 15 min at 3000 r.p.m and the separated plasma was divided into small aliquots to avoid freezing and thawing. Aliquots were then stored at -20°C for biochemical measurements. The sediment contains red cells was washed several times with ice cold saline solution and the packed RBCs were stored at -20°C for determination of antioxidant enzymes.

Tissue sampling and processing

Rats were euthanized by decapitation under ether anesthesia. A portion of liver was excised immediately thereafter, and a section was placed in 10% formalin for later preparation of histopathological and morphometrical examinations. An adjacent portion of liver as well as kidney, spleen, heart and lung were removed and rinsed with cold saline, blotted dry and weighed then stored at -20°C for malondialdehyde (MDA) determination.

Biochemical methods

Plasma glucose was estimated by glucose oxidase method (Trinder, 1969). Haemoglobin was determined by using cyanomethemoglobin method (International committee for standardization in hematology of the European society of hematology, 1965). Triglycerides (TG), total cholesterol and HDL cholesterol levels in plasma were carried out according to the methods of Wahlefeld (1974); Allain, et al. (1974) and Finley, et al. (1978), respectively. Plasma samples were analyzed for urea (Tabacco, et al., 1979) and creatinine (Bartel, et al., 1972). The activities of glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase (Glu-6-PDH), plasma total antioxidant capacity (TAC) were measured using the methods of Goldberg and Spooner (1983), Paglia and Valentine (1967), Nishikimi, et al. (1972), Lohar and Wall (1974) and Koracevic, et al. (2001), respectively. Malondiadehyde (MDA) was determined spectrophotometrically according to Ohkawa, et al. (1979). Transaminases (ALT & AST), alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GT), and lactate dehydrogenase (LDH), activities were determined according to the methods described by Bergmeyer, et al. (1976), Rosalki, et al. (1993), Szasz (1976), and Anon (1972), respectively. Total and direct bilirubin, total proteins and albumin levels were determined in plasma samples according to the colorimetric methods described by Jendrassik and Grof (1938), Peters (1968) and Doumas and Biggs (1972), respectively.

Statistical analysis

All experimental data are expressed as mean ± S.E. Significant differences among the groups were determined by one-way analysis of variance (ANOVA) using the SPSS statistical analysis program. Statistical significance was considered at $p \leq 0.05$. All the statistical analysis was carried out according to Baily (1994).

RESULTS AND DISCUSION

Volatile Constituents

The chemical composition of the blend infusion volatiles was shown in Table1. The constituents were listed in order of their elution from the DB5 column. Thirty nine compounds were identified. The main constituents identified in the volatiles of blend herbal infusion were 1,8-cineole (35.97%), cumene (7.12%), guryunene (5.25%), β- patchoulene (4.55%), citronellol (2.97%) and α- zingiberene (1.76%) The reported components are related to different chemical classes namely, monoterpenes (M) (18.38%), light oxygenated compounds (LOC) (54.62%), sesquiterpenes (S) (24.97%) and heavy oxygenated compounds (HOC) (2.03%). It is obvious that these compounds are related to the characteristic volatiles of the different aromatic plants that constitute the blend infusion.

In literature, Ramadan, et al. (2008) reported the predominance of 1,8-cineole and other volatile components in the essential oil of Egyptian *P. guajava* leaves volatile oil. Chen, et al. (2007) and Da- Silva, et al. (2003) were reported the presence of α- zingiberene as the major constituent in the ginger oil. El-Ghorab, et al. (2007) found that the volatile extract from Egyptian corn silk contained α-terpineol, citronellol and α-terpineol and other compounds.

Phenolic content and Sensory evaluation

The content of phenolic compounds was calculated as milligram gallic acid equivalent per liter of herbal infusion. The total phenolic content of blend infusion was relatively high (552± 31 mg GAE/L). Also, the herbal infusion was subjected to a detailed sensory analysis concerning aroma, taste, colour and appearance and the total quality scores (TQS) of infusion was calculated. The blend infusion exhibits high scores for all sensory attributes (Table 2).

The high aroma quality of blend infusion is mainly ascribed to its aroma attributes and this is mainly due to the characteristic volatile constituents of blend. The presence of 1,8 cineole at high concentration (35.97%) confirms the presence of fresh and minty note (Boelens and Boelens, 1997). Linalool (0.65%) and α-terpeneol (1.26%) which are responsible for the floral note (Kumazawa and Masuda, 2002). Citronellol (2.97%), possesses a fresh rosy odour and sabinene

(1.10%), which is one of the chemical compounds that contributes to the spiciness of black pepper (Arctander, 1969).

Table 1. The Chemical composition of the volatile compounds of the aromatic plants blend infusion

Compound Name	Area %	KI	Identification Methods
Monoterpenes (M)			
Santolina-*triene*	1.05	908	KI &MS
Cumene	7.12	926	KI &MS
□-Pinene	0.34	936	KI &MS&St
Verbenene	1.09	976	KI &MS
β-Pinene	2.33	980	KI &MS
Sabinene	1.10	984	KI &MS
Mesityllene	2.78	994	KI &MS
P-Cymene	0.76	1026	KI &MS
β-Ocimene(z)	1.43	1040	KI &MS
□-Terpinene	0.38	1062	KI &MS
Light Oxygenated Compounds (LOC)			
Isovaleric acid	0.12	831	KI &MS
Hexenal (E-2-)	0.21	854	KI &MS&St
Heptanone (3-methly-4-)	1.93	929	KI &MS
Heptanone(5-methly-3-)	3.05	943	KI &MS
Isopropyl Tiglate	0.7	973	KI &MS
Hexenol Acetate(-E-3-)	3.76	1004	KI &MS
Cineole (1,8)	35.97	1033	KI &MS&St
Linalool Oxide (cis)	1.05	1074	KI &MS
Iso-Terpinolene	1.23	1086	KI &MS
Linalool	0.65	1098	KI &MS
Terpin-4-ol	1.24	1156	KI &MS&St
Phenyl-tert-butanol	0.48	1156	KI &MS
□-Terpineol	1.26	1198	KI &MS&St
Citronellol	2.97	1234	KI &MS
Sesquiterpenes (S)			
Copaene(α-)	0.77	1376	KI &MS
β-patchoulene	4.55	1380	KI &MS
Cyperene	0.84	1398	KI &MS
Aromadendrene	0.98	1436	KI &MS
Thuyopsadiene	3.48	1462	KI &MS
Guryunene(ע)	5.25	1473	KI &MS
Curcumene(ע)	1.61	1480	KI &MS
β-Selinene	1.22	1489	KI &MS
□- Zingiberene	1.76	1490	KI &MS
β-Guaiene(Trans)	0.94	1500	KI &MS
α-Bisabolene(z-)	0.98	1504	KI &MS
β-Bisabolene	1.14	1509	KI &MS
□-Cadinene	1.45	1524	KI &MS
Heavey Oxygenated Compounds (HOC)			
Elemol	1.58	1549	KI &MS
Cubenol	0.45	1644	KI &MS
M (monotepene)	18.38		
LOC (light oxygenated compound)	54.62		
S (sesquterpene)	24.97		
HOC(Heavy oxygenated copmpounds)	02.03		

In the present study aromatic plants which are expected to possess promising antioxidant activities were selected and mixed at variable ratios in blend. Plant phenolic compounds have been considered to have multiple biological effects including antioxidant activity (Ito, et al., 2005). The most important volatile constituents identified in the blend infusion (Table 1) were 1,8 cineol, cumene, guryunene, β-patchoulene, linalool, α terpineol, terpin-4-ol, α-pinene and sabinen, most of them have antioxidant activity (Perry, et al., 2003).

The replacement of drinking water with blend infusion to rats of (group II) did not affect food and drink consumption, body weight of rats (data not shown) and all the studied biochemical parameters.

Glucose and hemoglobin level

For antihyperglycemic properties study, the STZ-induced diabetic rats is one of animal models of human diabetes mellitus (DM). DM is a serious endocrine disorder that is characterized by the disruption of intermediary metabolism due to insufficient insulin activity, insulin secretion, or both (Amos, et al., 1997).

Supplementation with blend infusion to rats of (group II) did not affect plasma glucose level and blood hemoglobin concentration. Their levels in these rats were on par with that of the control rats (group I). In STZ- induced diabetic rats (group III) there was a significant ($p < 0.001$) increase in fasting blood glucose (330%) compared to the control rats (group I). On the other hand, there was a significant decrease in fasting blood glucose in diabetic rats treated with herbal infusion (50%) (Group IV) compared to diabetic rats (group III). Blood hemoglobin level was significantly decreased in diabetic rats (group III). Obviously, supplementation of blend infusion to diabetic rats significantly improved that level compared to diabetic rats (Table 3).

Table 2. The sensory quality scores of the aromatic plants blend infusion*

Quality	Maximum Score	Score
Aroma	35	32.1±3.5
Taste	35	30.3±2.4
Colour	15	12.4±1.7
Appearance	15	13.6±1.2
Total quality score	100	88.4±7.9

**The total phenolic content was 552±31 mg GAE/L*

Cemek, et al., (2008), studied the antihyperglycemic and antioxidative activities of the aerial part of the *Matricaria chamomilla* L. ethanolic extract (MCE) in streptozotocin (STZ) induced diabetic rats and found that the extract significantly reduced postprandial hyperglycemia and oxidative stress as well as augmented the antioxidant system. This ascribed to protective effect on beta-cells in STZ-diabetic rats so diminished the hyperglycemia-related oxidative stress.

Akhani, et al., (2004) studied the effect of the juice of ginger for 6 weeks on STZ- induced diabetic rats. The auther reported that treatment with ginger produced a significant increase in insulin levels and a decrease in fasting glucose levels in diabetic rats as well as decrease in serum cholesterol, serum triglyceride and blood pressure in diabetic rats. Ginger aqueous extract could be of great value in managing the effects of diabetic complications in human subjects (Al-Amin, et al., 2006). Rau, et al., (2006) reported that extract of corn silk could be used as antidiabetic agent.

Some antidiabetic plants may exert their action by stimulating the function or number of β– cells and thus increasing insulin release. In some other plants, the effect is due to decreased blood glucose synthesis due to the decrease of the activity of enzymes like glucose-6-phosphatase, fructose 1,6-bisphosphatase, etc. in still other plants, the activity is due to slow absorption of carbohydrate and inhibition of glucose transport (Shalev, 1999; Eddokus, 2003; Villasenor, 2006; Tomohiro, et al., 2007).

The present study demonstrated that supplementation of hot water infusion of five blended aromatic plants reduced plasma glucose level and improved hemoglobin level in STZ-induced diabetic rats and this could be explained since the tested blend infusion possess higher antioxidant activity and phenolic content and also due to the hypoglycemic activity of their individual plant components.

Lipid profile, kidney function and antioxidant biomarkers

The plasma triglyceride (TG), total cholesterol (TC), LDL- cholesterol and LDL/HDL ratio were significantly decreased in blend infusion supplemented rats (group II) and their levels significantly elevated in the STZ- diabetic rats. Supplementation of the blend infusion to STZ- diabetic rats (protected group) significantly reduced their levels compared to diabetic rats (group III) (Table 3). The treatment of blend infusion showed to improve lipid profile by reducing the level of total cholesterol, triglycerides, and LDL-cholesterol and in the same time increased the level of HDL-cholesterol.

The lipid lowering and antioxidant potential of ethanolic extract of ginger was evaluated in STZ-induced diabetes rats. The extract treatment lowered serum total cholesterol, triglycerides and increased the HDL-cholesterol levels when compared with pathogenic diabetic rats. *Zingiber officinale* extract treatment lowered the liver and pancreas thiobarbituric acid reactive substances (TBARS) values as compared to pathogenic diabetic rats (Bhandari, et al., 2005). The improvement of lipid profile produced by the treatment with blend fusion could be attributed to the plant phenolics that are found in blended plants.

Plasma urea and creatinine concentration were significantly higher in the diabetic rats than control rats. Supplementation of herbal infusion to diabetic rats (protected rats; group IV) significantly reduced these levels compared to diabetic group (Table 3).

Hisaki (2005) proposed that the oxidative stress induced by STZ alters glomeruli function, resulting in the progression of diabetes and induces renal dysfunction and reported that polyphenol antioxidant treatment attenuated the renal dysfunction, suggesting the beneficial effect of antioxidant treatment in diabetes.

Activities of various antioxidant enzymes (GR, GPx, SOD, Glu.6ph.DH) and the total antioxidant capacity (TAC) were significantly decreased in STZ-diabetic rats (Gr. III). On the other hand, concentration of malonaldehyde (MDA) in liver, spleen and kidney were significantly elevated compared to the non- diabetic groups (groups I & II). Supplementation of blend infusion to rats (group IV) significantly increased the activities of GR and GPx as well as plasma total antioxidant capacity level and reduced the MDA concentrations, compared to group III. (Table 4).

Table 3. Hypoglycemic, hypolipidemic and renal protective activity of the aromatic plants blend infusion

	TG (mg/dl)	TC (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	LDL/HDL	Urea (mg/dl)	Creatinine (mg/dl)	Hemoglobin (mg/dl)	Glucose (mg/dl)
Normal control (Group I)	201±11[a]	192±8.9[a]	101±8.5[a]	86±5.9[a]	1.17 ±0.06[a]	51.2±4.1[a]	0.51 ±0.017[a]	13.61±0.61[a]	92.5±5.3[a]
Blend supplemented (Group II)	178±9 [b]	170±6.2[b]	85 ±4.5 [b]	83±6.5[a]	1.02± 0.03[b]	50.0±2.1[a]	0.49±0.19[a]	13.11±0.72[ab]	90±4.8[a]
STZ- diabetic (Group III)	297±13[c]	251±14[c]	147±11.9[c]	100±9.2[b]	1.47±0.04[c]	143±9.9[b]	3.82 ±0.09[b]	11..64±0.51[c]	410±31[b]
Protected group (Group IV)	211± 12[a]	203±11[a]	99 ±4.9[a]	101±8.2[b]	0.98±0.01[b]	88..3±6.4[c]	1.98±0.07[c]	12.97±0.55[b]	218±14[c]

TG:Triglyceride; TC: Total cholesterol ; a,b and c: same scripts in the same column indicate no significant differences (P≤0.05).

Table 4. The antioxidant activity of the aromatic plants blend infusion

	Antioxidant activities					MDA (mg/100g tissue)				
	GR (U/L)	**SOD (U/L)**	**GPx (U/L)**	**TAC (U/ml)**	**G-6-pH.DH (U/g Hb)**	**Liver**	**Spleen**	**Kidney**	**Heart**	**Lung**
Normal control (Group I)	1135±98[a]	211±19[ab]	1661±71[a]	2.11±0.13[a]	12.89±1.5[a]	2.94±0.12[a]	2.86±0.10[a]	5.88±0.18[a]	1.71±0.09[a]	0.75±0.10[a]
Blend supplemented (Group II)	1398±197[b]	221±21[a]	1837±79[b]	3.89±0.14[b]	13.21±1.8[a]	2.01±0.10[b]	2.0±0.09[b]	4.48±0.11[b]	1.68±0.10[a]	0.79±0.11[a]
STZ- diabetic Group (Group III)	688±49[c]	142±17[c]	1045±56[c]	0.82±0.12[c]	7.76±1.1[b]	3.87±0.21[c]	3.98±0.11[c]	7.51±0.17[c]	2.69±0.12[b]	0.80±0.14[a]
Protected group (Group IV)	1006±58[a]	189±16 [b]	1597±68[a]	1.97±0.11[a]	11.93±1.2[a]	3.06±0.12[a]	3.0±0.08[a]	6.10±0.15[a]	1.95±0.11[c]	0.76±0.12[a]

GR: Glutathione reductase; SOD: Superoxid Dismutase; GPx: Glutathion peroxidase (U/L); TAC Total antioxidant capacity (U/ml) and G-6-Ph.DH: Glucose-6- ph.dehydrogenase (U/g Hb); MDA; Malondialdehyde . a,b and c: same scrips in the same coloumn indicate no significant differences (P ≤0.05).

The results of the present study demonstrated elevated MDA in STZ-induced diabetic rats organs along with decrease in the antioxidant enzymes activity. Earlier there have been many reports documenting elevated lipid peroxide levels and diminished antioxidant status in diabetic subjects (Sato, et al., 1979). As diabetes and its complications are associated with free radical mediated cellular injury (Oberley, 1988) herbal hypoglycemic agents were administered to diabetic rats to assess their anti-oxidant potential. The monoterpenoids 1,8-cineole, linalool, and α-pinene present in the volatiles of blend fusion have been reported to be antioxidant, further to this any potential synergistic interactions could change the antioxidant profile of a whole plant extract (Perry, et al., 2003).

Our results show that blend infusion not only have hypoglycemic activity but they also significantly reduce the MDA levels in diabetic rats. Moreover, following treatment the activity of the antioxidant enzymes were also increased. The herbal hypoglycemic agents may also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds (Gupta, et al., 2002), or by increasing the synthesis of anti-oxidant molecules.

The results of hepatoprotective effects of blend infusion on CCL_4- intoxicated rats are shown in Table 5. The activities of liver enzymes; ALT, AST, ALP, γGT, LDH and total proteins, albumin, globulin and A/G ratio as well as total, direct and indirect bilirubin levels in infusion supplemented rats (group II) were comparable to those of control group (group I). In CCl_4- intoxicated rats (group III), all the tested biochemical parameters were markedly disturbed. Supplementation of herbal infusion to intoxicated rats (protected group IV) significantly improved liver function tests and these alterations appeared to be counteracted by infusion supplementation (group IV). The present study showed for the first time that blend infusion of five aromatic plants possess hepatoprotective activity as evidenced by the significant inhibition in the elevated levels of serum enzyme activities as well as other biochemical parameters (Table 5).

It is well established that CCl_4 hepatotoxicity by metabolic activation, therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($^{*}CCl_3$). This free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethylperoxyl free radical leads to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis, and finally, results in cell death (Britton and Bacon 1994).

Table 5. The hepatoprotective activity of the aromatic plants blend infusion

	Plasma liver enzymes activities (U/L)					**Plasma proteins levels (mg/dl)**				**Bilirubin (mg/dl)**		
	γ-GT	**LDH**	**ALT**	**AST**	**ALP**	**T.P**	**ALB**	**GLB**	**A/G**	**Total**	**Direct**	**Indirect**
Normal control (Group I)	26.8±1.2[a]	686±30[a]	48.2±3.8[a]	120±10[a]	360±18[a]	9.3±0.77[a]	5.2±0.61[a]	4.1±o.31[a]	1.26±0.11[a]	0.42±0.021[a]	0.19±0.009[a]	o.23±0.008[a]
Blend supplemented (Group II)	26.13±1.3[a]	671±48[a]	49.1±2.6[a]	123±7.8[a]	371±21[ab]	9.1±0.61[a]	5.0±0.47[a]	4.1±0.28[a]	1.21±0.09[a]	0.4±0.016[a]	0.19±0.019[a]	0.21±0.020[a]
CCl_4- intoxicated (Group III)	31.14±1.5[b]	1572±82[b]	206±11[b]	254±18[b]	817±61[c]	5.7±0.31[b]	3.1±0.22[b]	2.6±0.16[b]	1.19±0.11[b]	1.11±0.08[b]	0.36±0.027[b]	0.75±0.054[b]
Protected group (Group IV1)	27.11±1.4[a]	713±54[a]	52±.3.8[a]	129±6.8[a]	393±29[b]	8.9±0.65[a]	5.0±o.41[a]	3.9±0.24[a]	1.28±0.13[a]	0.42±0.03[a]	0.2±0.017[a]	0.23±0.016[a]

γ-GT: γ-glutamyltransferase ; ALT and AST: Transaminases; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; T.P: Total protein; ALB: Albumin; GLB: Globulin.

Many compounds known to be beneficial against carbon tetrachloride-mediated liver injury exert their protective action by toxin-mediated lipid peroxidation either via a decreased production of CCl_4 derived free radicals or through the antioxidant activity of the protective agents themselves (Gupta and Misra 2006). The mechanism by which tested blend infusion exert its protective action against CCl_4 induced alternations in the liver may be attributed to the antioxidant effect of the blend infusion; but the suggestion needs to be more exploited.

El-Ghorab, et al., (2007) reported that corn silk could be used to produce novel natural antioxidants as well as a flavouring agent in various food products. The hepatoprotective activity of corn silk extracts was studied on an acute hepatitis model induced by CCl_4. The extract showed decrease in the activity of ALT and in the levels of total bilirubin and the final malonaldehyde and diene conjugates as lipid peroxidation products, and absence of decline in the activity of glutathione-dependent enzymes. The extracts exhibited antioxidant effects, which were proved by the reduction of the final and intermediate products of lipoperoxidization (Katikova, et al., 2001).

Ajith, et al. (2007) studied the hepatoprotective effect of aqueous ethanol extract of ginger against acetaminophen-induced acute toxicity and reported that aqueous ginger extract significantly protected the hepatotoxicity as evident from improvement in the activities of serum transaminases, alkaline phosphatase, liver SOD, CAT, glutathione peroxidase and glutathione-S-transferase (GST), and reduced glutathione (GSH) levels.

Matsuda, et al. (2002) reported that ethanolic extract from the flowers of linden was found to show a hepatoprotective effect against D-galactosamine (D-GalN)/ lipopolysaccharide (LPS)-induced liver injury in mice. The author isolated five flavonol glycosides as the hepatoprotective constituents of the tilia extract, that strongly inhibited serum GPT and GOT elevations in D-GalN/LPS-treated mice.

Manuele, et al. (2008) who reports that *Tilia cordata* flowers extract rich in α-pinene and β-pinene, that may thus constitute a potential source of monoterpenes with immunomodulatory activity.High performance liquid chromatography analysis indicated that tilia ethanol extract was constituted principally of tiliroside, quercetin, quercitrin, kaempherol, and their glycosides and these results supported the use of Tilia species in traditional medicine (Herrera, et al., 2008).

Plant polyphenols are reported to exhibit antioxidant and anti-inflammatory effects. Flavonoids of German chamomile are reported to exhibit the hepatoprotective effect (Chamomil represented 35% of blend ingredients). Flavonoids normalized activities of key enzymes of sphingolipid turnover and ceramide contents in the damaged liver and liver cells, and stabilized the hepatocyte membranes (Babenko and Shakhova, 2006 and 2008).

In conclusion, the significant antioxidant activity of blend infusion as well as the potential hypoglycemic and hepatoprotective effects, might be due to scavenging of free radicals metabolites released from the toxicants such as CCl_4 and STZ and could be attributed to the presence of phytochemicals mainly volatile compounds, considering that the guava leaves representing 45% of blend ingredients which are used for several ailments including diabetes (Wyk, et al., 2007).

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