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ANTIOXIDANT AND PROTECTIVE EFFECT OF AROMATIC PLANTS BLEND INFUSION AGAINST OXIDATIVE STRESS OF STREPTOZOTOCINE AND CARBON TETRACHLORIDE IN RATS

Khaled F. El-massry*, Ahmed H. El-Ghorab, Manal M. Ramadan and Ahmed M. Gad

Flavour and Aroma Department - National Research Center, Dokki, Cairo, Egypt

ABSTRACT

Aim of the work: The present study aimed to use some aromatic plants as ingredients to prepare a healthy drink with an acceptable flavour and taste and to evaluate its chemoprotective activity against oxidative stress induced by streptozotocin (STZ) and carbon tetrachloride (CCl_4) in rats**. Materials and Methods:** Infusion of guava leaves, linden, ginger, corn silk and chamomile was prepared, its volatiles were isolated and analyzed using GC and GC/MS, sensory evaluation as well as the total phenolic content were determined. The chemoprotective effectiveness of the blend infusion was tested by two separate biological experiments against the oxidative stress of STZ and CCl_4. Serum glucose level was determine as well as liver function, kidney function, lipid profile, plasma hemoglobin, antioxidant enzymes and malonaldehyde (MDA) in some organs were investigated. **Results:** Thirty nine compounds were identified in the volatiles of blend infusion. The main components were 1,8-cineol (35.97%), cumene (7.12%), guryunene (5.25%), β- patchoulene (4.55%), citronellol (2.97%) and α- zingiberene (1.76%) and these compounds are related to the characteristic volatiles of the different aromatic plants that constitute the blend infusion. The blend infusion exhibits high score for all sensory attributes

The results showed that the supplementation with blend infusion to intoxicated rats either with STZ or CCL_4 with blend infusion for four weeks significantly ameliorate most of the toxic effects and protecting pancreas, kidney and liver through improving their antioxidant status. **In conclusion**: The aromatic plants blend infusion was safe and effective in controlling hyperglycemic effects of STZ and improve lipid metabolism as well as its hepatoprotective activity against CCl_4 by amelioration of the associated biochemical parameters.

Key words: Aromatic plants, GC-MS, Chemoprotection, Oxidative stress, Hyperglycemia, liver, kidney, Pancreas.

*To whom correspondence; kfarouk@yahoo.com

INTRODUCTION

Herbs and spices 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 **(Wood *et al*., 2001** 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**).**

Free- and exogenous antioxidant defenses; yet these defense systems are not sufficient in critical situations (oxidative stress, contamin-ation, 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 e.g. 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 antiox-idant 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.
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 reasons for such shift towards the use of herbals include the expensive cost of conventional drugs, adverse drug reactions, and their inefficacy. Approximately 800 plants worldwide have been documented to support antidiabetic effects, however a few comp-rehensive 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, carote-noids, selenium, zinc and phytochemicals **(Atoui *et al*., 2005)**. Many therapeutic properties such as neuroprotective, cardiop-rotective, chem.-oprotective, anticarcinog-enic, hepatoprotc-tive, hypoglycemic and anti- inflammatory have been attributed to herbal preparations **(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).**
The present study aimed to use a miture of aromatic plants namely ginger, guava leaves, linden, corn silk and chamomile for the preparation of an acceptable drink and to evaluate its chemopreventive activity against oxidative stress induced by STZ and CCL_4.

MATERIAL AND METHODS

Chemicals and kits.

Most of the kits used in this study were commercially purchased from Randox Laboratories (San Fransisco, CA, USA) and Biomerieux Laoratory of reagents and products (Mercy Letoile, France). All chemicals used were of analytical grade.

Plant materials and Preparation of blend infusion

Dry guava leaves, corn silk, linden flowers, chamomile and ginger root were purchased from local market and were identified by the department of Botany, Fac. Sci., Cairo university. The plants were separately grounded and blended at variable ratios (45% guava leaves, 35% linden, 10% ginger, 5% corn silk and 5% chamomile). One gram of the grounded 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 the blend infusion was estimated and scored by 15 assessors (Flavour and Aromatic 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 **(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 then extracted with 100 ml of dichloromethane using a liquid- liquid continuous extractor for 6 h. 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 gas chromatographic (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

Total phenolic content was determined in the blend infusion with Folin- Ciocalteu reagent as described by **Kahkonen *et al*. (1999)** using gallic acid as the standard.

Biological evaluation

Animals and diets

Forty eight male Swiss albino rats with initial weights ranging from 150 to 170g were provided from the breeding unit of the National Research Center (Cairo). The animals were maintained under laboratory condition for an acclimatization period for one week before performing the experiments. Throughout the experimental period the rats were fed on standard pellets (purchased from Cairo Company of Oil & Soap, Egypt). 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 divided into four groups (six rats/group) including the control group (Gr.I); the group received the prepared blend infusion *ad libitum* for four weeks instead of drinking water (Gr.II); the group i.p. injected with a single dose of STZ (52 mg/kg b.w.)(Gr.III) and the group drink the blend infusion for two weeks followed by STZ injection and continued on the blend infusion drink for another two weeks (Gr.IV).

Hepatoprotective activity

Another 24 rats were divided into four groups (6rats/group) and treated as follows: the control group (Gr.I); Gr.II afforded the blend infusion *ad libitum* for four weeks; Gr.III, i.p. injected with CCL_4 (1.195 ml/Kg b.w.) three times a week for two weeks **(Mac Sween *et al*. (1994)** and Gr.IV, maintained on the blend infusion for four weeks and injected with CCL_4 during the 3^{rd} and 4^{th} weeks (three times/week).

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 test 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

After the collection of blood samples, all animals within different treatment groups were sacrified and samples of liver, 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 analyses

The following biochemical anlyses were carried out: plasma glucose **(Trinder, 1969)**, haemoglobin **(International com-m.ittee for standardization in hema-tology of the European society of hematology, 1965)**, Triglycerides (TG) **Wahlefeld (1974),** total cholesterol (TC) (**Allain *et al*., 1974),** HDL cholesterol (HDL-C) **(Finley *et al*., 1978),** urea **(Tabacco *et al*., 1979)** and creatinine **(Bartel *et al*., 1972)**. The activities of glutathione reductase (GR) (**Goldberg and Spooner, 1983),** glutathione peroxidase (GPx) **(Paglia and Valentine, 1967)**, superoxide dismutase (SOD) (**Nishikimi *et al*., 1972),** glucose-6-phosphate dehydr-ogenase (Glu-6-PDH) (**Lohar and Wall, 1974), and** plasma total antioxidant capacity (TAC) (**Koracevic *et al*., 2001)**. Malondiadehyde (MDA) was determined spectrophotometrically according to **Ohkawa *et al*. (1979).** The activities of transaminases (ALT & AST) **(Bergmeyer *et al*., 1976),** alkaline phosphatase (ALP) (**Rosalki *et al*., 1993)**, γ-glutamyltransferase (γ-GT) (**Szasz, 1976)** and lactate dehydrogenase (LDH), **(Anon, 1972).** Total and direct bilirubin **(Jendrassik and Grof, 1938),** total proteins **(Peters, 1968)** and albumin (**Doumas and Biggs, 1972)**.

Statistical analysis

All experimental data were 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-cineol (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 this concern, **Ramadan *et al*. (2008)** reported the predominance of 1,8-cineol and other volatile components in the essential oil of Egyptian *P. guajava* leaves volatile oil. **Da- Silva *et al*. (2003) and Chen *et al*. (2007)** reported the presence of α- zin giberene as the major constituent in 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 cineol 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).**

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).**

Hypoglycemic study

Glucose level and hemoglobin concentration

Supplementation with blend infusion to rats (Gr. II) did not affect plasma glucose level and blood hemoglobin concentration. In STZ treated rats (Gr. III) a significant ($p < 0.001$) increase in fasting blood glucose level (330%) was found compared to the control rats (Gr.I). On the other hand, diabetic animalsdrink the herbal infusion (Gr.IV), showed a significant decrease in blood glucose compared to the diabetic group (Gr.III)

(Table 3).Blood hemoglobin concentration was significantly decreased in diabetic rats (Gr.III). Obviously, supplementation of blend infusion to diabetic rats significantly improve Hb con-centration, compared to diabetic rats (Table 3).

Cemek *et al*., (2008), studied the antihyperglycemic and antioxidative activities of the ethanolic extract of aerial part of the *Matricaria chamomilla* L. 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. Moreover, **Akhani *et al*., (2004)** studied the effect of the juice of ginger administration for 6 weeks on STZ-induced diabetic rats. The authers reported that treatment with ginger juice 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. Furthermore, ginger aqueous extract could be of great value in managing the effects of diabetic complications in human subjects **(Al-Amin *et al*., 2006 and Singh *et al*., 2008).** and the extract of corn silk could be used as antidiabetic agent **(Rau *et al*., 2006)**.

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

The present study demonstrated that supple-mentation of hot water infusion of the five blended aromatic plants reduced plasma glucose level and improved hemoglobin level in STZ-induced diabetic rats and this could be explained by the higher antioxidant activity, the higher phenolic content and the hypoglycemic activity of the individual plant.

Lipid profile, kidney function and antioxidant biomarkers

The plasma triglyceride (TG), total cholesterol (TC), LDL- cholesterol (LDL-C) and LDL/HDL ratio were significantly decreased in blend infusion supplemented rats (Gr.II). However, these levels were significantly elevated in the STZ- diabetic rats. Supp-lementation of the blend infusion to diabetic rats significantly reduced lipid profile levels compared to diabetic rats (Table 3). The treatment with blend infusion improved 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. Treatment with the extract lowered serum total cholesterol, triglycerides and increased the HDL-cholesterol levels compared with pathogenic diabetic rats. On the other hand, ginger extract 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 significantly reduced these levels compared to diabetic group (Table 3). In this regards, **Hisaki *et al*. (2005)** proposed that the oxidative stress induced by STZ alters glomeruli function, resulting in the progression of diabetes and induces renal dysfunction. These authors 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. On the other hand, concentration of malonaldehyde (MDA) in liver, spleen and kidney were significantly elevated compared to the non- diabetic groups.

Supplementation of blend infusion to diabetic rats significantly increased the activities of GR and GPx as well as plasma total antioxidant capacity level and reduced the MDA conce-ntrations, compared to group III. (Table 4).

The results of the present study demonstrated an elevation of MDA in STZ-induced diabetic rats organs along with decrease in the antioxidant enzymes activity. Earlier 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 showed that the 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.

Hepatoprotective Study

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 (Gr.II) were comparable to those of control group (Gr.I). In CCl_4- intoxicated rats (group III), all the tested biochemical parameters were markedly disturbed. Supplementation of herbal infusion to intoxicated rats (Gr.IV) significantly improved liver function tests and these alterations appeared to be counteracted by infusion supplementation (Gr.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)**.

Many compounds are known to be beneficial against carbon tetrachloride-mediated liver injury and 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 this 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. The extract decreased the activity of ALT, the levels of total bilirubin, the final malonaldehyde, 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 against the hepatot-oxicity 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 have a hepatoprotective effect against D-galactosamine (D-GalN)/ lipopol-ysaccharide (LPS)-induced liver injury in mice. The authors isolated five flavonol glycol-sides as the hepatoprotective const-ituents of the tilia extract, that strongly inhibited serum GPT and GOT elevations in D-GalN/LPS-treated mice. **Manuele *et al*. (2008)** reported that *Tilia cordata* flowers extract are rich in α-pinene and β-pinene, that may thus constitute a potential source of monoterpenes with immunomodulatory acti-vity. Moreover, high performance liquid chromatography analysis indicated that the ethanol extract of tilia was constituted prin-cipally of tiliroside, quercetin, quercitrin, kaempherol, and their glycosides and these results supported the use of Tilia species intraditional medicine **(Herrera- Ruiz *et al*., 2008).** Plant polyphenols are reported to exhibit antioxidant and antiinflammatory effects. Flavonoids of Germ-an 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 hepatoprot-ective effects of the blend infusion reported in the current study might be due to the 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 diabete

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

Compound Name	Area %	KI[a]	Identification Methods[b]
Monoterpenes (M)			
Santolina-*triene*	1.05	908	KI &MS
Cumene	7.12	926	KI &MS
α-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
γ-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	1943	KI &MS
Isopropyl Tiglate	0.7	973	KI &MS
Hexenol Acetate(-E-3-)	3.76	1004	KI &MS
Cineole (1,8)	35.97	033	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
α-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
α- 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
β-Cadinene	1.45	1524	KI &MS
Heavy Oxygenated Compounds (HOC)			
Elemol	1.58	1549	KI &MS
Cubenol	0.45	1644	KI &MS
M		18.38	
LOC		54.62	
S		24.97	
HOC		02.03	

KI: Kovat index; MS: Mass spectra, ST: available standards.

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

Table 3. Hypoglycemic, hypolipidemic and renal protective activity of the aromatic plants blend infusion in STZ- treated rats

	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: In each column means with different letters are significantly different ($P \leq 0.05$).

Table 4. The antioxidant activity of the aromatic plants blend infusion in STZ-treated rats

	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]
(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: In each column means with different letters are significantly different ($P \leq 0.05$).

Table 5. The hepatoprotective activity of the aromatic plants blend infusion in CCL4-treated rats

	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.

In each column means with different letters are significantly different ($P \leq 0.05$).

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التأثير الوقائي ومضاد للأكسدة لمنقوع خليط من النباتات العطرية ضد الأجهاد التأكسدى النتاتج من الإستربتوزوتوسين ورابع كلوريد الكربون فى الفئران

خالد فاروق المصري ، أحمد حسن الغراب ، منال محمد رمضان، أحمد محمد جاد

قسم كيمياء مكسبات الطعم والرائحة – المركز القومي للبحوث

تهدف هذه الدراسة إلى تحضير مشروب صحي(شاى) ذو طعم ورائحة مقبولة من المستخلص المائى لبعض النباتات العطرية ودراسة كفاءته للوقاية من أثارالشقوق الحرة الناتجة من المعاملة بمادة الستربتوزوتوسين (STZ)ورابع كلوريد الكربون (CCl_4)فى فئران التجارب.

تم تحضير المستخلص المائى من مخلوط من خمسة نباتات عطرية (اوراق الجوافة و الزنجبيل وشواشى الزرة والكاموميل والتيليو) التي لها نكهة وطعم مقبول بنسب مختلفة وتقييمها حسيا لأختيار افضلها من حيث النكهة. كما تم تقدير المحتوى الكلي للفينولات وتم فصل والتعرف على المركبات الطيارة فى منقوع هذه النباتات وتحليلها بجهاز التحليل الغازي الكروماتوجرافي- طيف الكتلة كما ونوعا كما تم دراسة التأثير الوقائى للمستخلص المائى من هذه النباتات (infusion) فى الفئران المعاملة بمادتى الستربتوزوتوسين أو رابع كلوريد الكربون.

وقد أظهرت التحاليل وجود مركبات عطرية ذات اهمية كبيرة كمضادات أكسدة ومسئولة عن النكهة مثل 1,8-cineol , cumene, guryunene, β- patchoulene, citronellol and α-zingiberene

كما أظهرت النتائج أن المستخلص المائى للنباتات العطريه تحت الدراسه قد أظهرت إنخفاضا معنويا في مستوى سكر الدم (خافض لسكر الدم) ومستوى الدهون بالدم (خافض للدهون) وتحسين للؤشرات الحيوية الخاصة بالكلى وإرتفاع معنوى لمستوى الإنزيمات الحامية (مضادات الأكسدة الطبيعية) وتقليل معنوى لسمية رابع الكربون على الكبد (مواد حاميه للكبد). مما يؤكد أمكانية أستخدام المستخلص المائى للنباتات العطرية تحت الدراسة كمشروب صحى خافض لمستوى السكر بالدم وحامى للكبد والاعضاء الجسم المختلفة من الأثار الضارة الناتجة من الأجهاد التأكسدى.