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Detection Of Some Active Compounds In Three Herbal Plants Nasturtium Officinale), Hibiscus Sabdarriffa L. And Purtulacaoleracea And Their Effect On Reducing Blood Glucose Level In Diabetic Patients

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Abstract

Hyperlipidemia is a known complication of diabetes mellitus.The use of anti-diabetic drugs continuously may cause side effect or toxicity, for this reason there is a requirement for using an alternative and safe drugs for blood glucose reduction. The aim of the present study was to examine the antidiabetic effects of the water extracts of three herbal plantswatercress (Nasturtium officinale),Gugarate(Hibiscus sabdarriffa L.) andberberine (Purtulacaoleracea).This study was conducted in Baghdad city (Karkh and Rusafa) on 60 diabetic patients to evaluate aqueous extracts of three medicinal herbs;Watercress (Nasturtium officinale), Gujarates (Hibiscus sabdarriffa L.) and Berberine (Purtulacaoleracea) in reducing blood glucose levels. The total duration of the experiment was 6 weeks during the period from 16/5/2021 to 1/7/2021. Results shows highly significant different are accounted amongall probable pair wised. For summarizes of preceding results, it could be conclude thatstudying influences of an assignable factor, "The different Groups of Herbal Plants" has resulted meaningful influences on studied marker.

Keywords: active compounds,herbal plants, diabetic patients

INTRODUCTION

Diabetes mellitus is a metabolic disorder resulted in elevation the blood glucose levels in humans. This may either be triggered by the inability of β-cells of the pancreas to secrete insulin or the failure of the liver/skeletal muscles cells to respond to insulin action (1).The breakdown of insulin producing cells by auto immune response which lead to Type 1 diabetes mellitus whereas Type 2 diabetes mellitus are

caused by insulin resistance and insulin secretory defect due to the failure of β-cells (2).The use of anti-diabetic drugs continuously may cause side effect or toxicity, for this reason there is a requirement for using an alternative and safe drugs for blood glucose reduction (3).Herbal plants have been used by traditional practitioners in management and control of diabetes in some countries with reducing effect of their components as compared to the use of anti-diabetic drugs, pharmacological activities of medical herbs as antioxidant, cytoprotective, anticancer, antiulcer, diuretic, hepatoprotective,anti diabeticand other activities (4). Certain botanical products from plants have been widely used in diabetes care because of their anti-oxidation, anti-inflammation, anti-obesity and anti-hyperglycemia properties (5).

Beberine(Portulacaoleracea)from the family of Oleracea isa herbaceous with thick, green leaves that have medicinal properties.It isfound in most regions as wild or cultivated herb.Berberine has been shown to regulate glucose and lipid metabolism in vitro and in vivo(6) Jun, yin et al., 2008). Botanical products are mixtures of multiple compounds, compared to other products from generally safe plants, berberine is a single purified compound, and has glucose-lowering effect in vitro and in vivo (7).

Watercress(Nasturiumofficinale)is a member of the family Brassicaceae, originated in the Mediterranean region coastand other countries(8, 9)the local Iraqi and Jordanian name is jarjeer (10). The plant consumed as tonic, rubefacient, astringent, digestive,stimulant, to increase sexual desire (aphrodisiac) and diuretic (11, 12).Different phytochemicals have been identified in watercress including flavonoids, phenolics, glucosinolate, Vitamin C, carotenoids, and others (13).

Gujarate tea (HsabdariffaL.) belong to the family Malvaceae, it is used in beverages and in herbal medicines. Hibiscus sabdariffaextracts of different parts have been reported to exhibit antibacterial, anti-oxidant, nephro-and hepato-protective effects (14, 15, 16)..

This study was conducted to evaluate the efficacy of berberine, gujarates and watercressaqueous extracts in diabetic patient with type 2 diabetes. Diabetic patients and poorly controlled diabetic patients were treated with the extracts for 45 days. Blood glucose levels were used to determine the efficacy of the aqueous extracts of these herbs. This study concentrated on the hypoglycemic and hypolipidemic effects of aqueous extracts of diabetic patients was evaluated.

Materials and Methods

This study was conducted in Baghdad city (Karkh and Rusafa) on 60 diabetic patients to evaluate aqueous extracts of three medicinal herbs;Watercress (Nasturtium officinale), Gujarates (Hibiscus

sabdarriffa L.) and Berberine (Purtulacaoleracea) in reducing blood glucose levels. The total duration of the experiment was 6 weeks during the period from 16/5/2021 to 1/7/2021.

Experimental Design

Diabetic patients were grouped in three group (20 patient for each one) and treated with each herb extract by oral administration of 20 ml for 6 weeks. Blood glucose levels were measured in mg/dl every 2 weeks for all patients. The data were arranged in tables to be ready for statistical analysis.

Preparations of the extracts

Dried leaves (50 gm) were crushed in electrical grinder and powdered for every one of the three herbs, then extracted with 250 ml of distilled water. The solution was left overnight then filtered with whatman filter paper and centrifuging at 10000 rpm for 5 minutes at 25 C^0 the solution was ready for oral root administration.

Detection of the active compounds

Some active ingredients were detected using chemical reagents to determine the presence of active compounds without quantification or determination of their chemical structure (due to the text book of Natural product Isolation, by Richard 2003).

Detection of alkaloids

By taking 10 ml of the extractthen boiled with 50 ml of distilled water after adding of 4% of hydrochloric acid, then filtered the solution and cooled. 0,5 ml of the supernatant was tested with mayer solution, appearance of white precipitate indicates the presence of alkaloids (17).

Detection of Saponins

Two methods were used for detection of saponins, The first one; the aqueous extract of the herbal plants powder was shaken vigorously with distilled water in a test tube. Formation of foam standing a little time indicates a positive result. The second method, by adding 5 ml of aqueous extract of the herbal plants to 1-3 drops of 3% ferric chloride solution, white precipitate was appeared which indicates a positive result (17).

Detection of flavonoids

Plant extracts were partitioned with petroleum ether using Buckner funnel. The aqueous layer was mixed with the ammonia solution and the presence of dark color is an indicator for the presence of flavonoids (18).

Detection of polyphenols

Ferric chloride (5%) in water extracts produces brown precipitate appear in presence of polyphenols (17).

Investigation of Blood glucose levels

Supplementation of herbal plant extracts for 6 weeks (one dose 20 ml taken every day fasting at the morning). Measuring glucose level for the diabetic patients every week. The data were arranged in tables to be ready for statistical analysis.

Results and Findings

Chemical detection of active compounds

The aqueous extracts ofWatercress (Nasturtium officinale), Gujarates (Hibiscus sabdarriffa L.) and Berberine (Purtulacaoleracea)were showed the presence of

Table (1): Detection of some active compounds in three aqueous extracts of the herbal plants

		Alkaloids	Saponins	Flavonoids	polyphenols
1	**Watercress**	**+**	**+**	**+**	**+**
2	**Gujarates**	**Trace**	**+**	**+**	**+**
3	**Berberine**	**+++**	**Trace**	**+**	**+**

Effect of plant extractson blood glucose levels

Effect ofWatercress (Nasturtium officinale**), Gujarates (**Hibiscus sabdarriffa L.**) andBeberine (**Purtulacaoleracea**) aqueous extracts (20 ml) on blood glucose level for 60 diabetic patient**

Statistical Analysis[1()]

The following statistical data analysis approaches were used in order to analyze and assess the results of the study under application of the statistical package (SPSS) ver. (22.0) :

(1)All the StatisticalAnalysis and Findings results wereSupervised Emeritus Prof.(Dr.) AbdulkhaleqA Al-Naqeeb, College of Health and Medical Technology, Baghdad –Iraq.

1. **Descriptive data analysis**:
2. Mean value, Standard Deviation, Standard Error, and (95%) Confidence interval for population Mean values and the two extremes values (min. and max.) for assuming that data under lying followed (Normal Distribution Function).

a- Graphical presentation by using :

- Bar Charts. -Stem-Leaf Charts.

2. Inferential data analysis:

These were used to accept or reject the statistical hypotheses, which included the following :

a- The One-Sample Kolmogorov-Smirnov (K-S) test.Test procedure compares the observed cumulative distribution function for a variable with a specified theoretical distribution, which may be normal. The Kolmogorov-Smirnov Z is computed from the largest difference (in absolute value) between the observed and theoretical cumulative distribution functions. This goodness-of-fit test tests

b- whether the observations could reasonably have come from the specified distribution.

c- The Paired-Samples T Test procedure compares the means of two variables for a single grouptwo related groups. It computes the differences between values of the two variables for each case and tests whether the average differs from 0.

d- The One-Way ANCOVA. isa short for Analysis of Covariance. Tests for the difference in mean scores. The repeated measures ANCOVA compares means across one or more variables that are based on repeated observations while controlling for a confounding variable. A repeated measures ANOVA model can also include zero or more independent variables and up to ten covariate factors. Again, a repeated measures ANCOVA has at least one dependent variable and one covariate, with the dependent variable containing more than one observation.

For the abbreviations of the comparison significant (C.S.), we used the followings:

- NS : Non significant at $P>0.05$
- S : Significant at $P<0.05$
- HS : Highly significant at $P<0.01$

This paragraph presents the findings of the data analysis systematically in tables and these correspond with the objectives of this study and as follows:

Normal Distribution Function (Goodness of Fit test)

Table (1) represented one-sample "Kolmogorov-Smirnov" test procedure comparing the observed cumulative distribution function for studied readings with a specified theoretical distribution, which proposed normal shape (i.e. The Bell Shape).

Table (1): Normal distribution function test due to different groups in relative to different of studied Marker (Blood Glucose) test

Groups (Herbal Plants)	One-Sample Kolmogorov-Smirnov Test			Test Dist.
	Periods	Test Statistic	Statistics (*)	
Nasturtium Officinale	First week	No.	20	Normal
		Kolmogorov-Smirnov Z	0.891	
		Asymp. Sig. (2-tailed)&C.S. (*)	0.405 (NS)	
	Sixth week	No.	20	Normal
		Kolmogorov-Smirnov Z	0.563	
		Asymp. Sig. (2-tailed)&C.S. (*)	0.909 (NS)	
Hibiscus Sabdariffalinn	First week	No.	20	Normal
		Kolmogorov-Smirnov Z	0.547	
		Asymp. Sig. (2-tailed)&C.S. (*)	0..926 (NS)	
	Sixth week	No.	20	Normal
		Kolmogorov-Smirnov Z	0.838	
		Asymp. Sig. (2-tailed)&C.S. (*)	0.483 (NS)	
PortulacaOleracea	First week	No.	20	Normal
		Kolmogorov-Smirnov Z	0.526	
		Asymp. Sig. (2-tailed)&C.S. (*)	0.945 (NS)	
	Sixth week	No.	20	Normal
		Kolmogorov-Smirnov Z	0.961	
		Asymp. Sig. (2-tailed)&C.S. (*)	0.314 (NS)	

(*) NS: Non Sig. at P>0.05

Results shows that test's distribution are normal for the studied readings concerning different groups in relative to different of the studied Marker (i.e. Blood Glucose) either for measuring at the first week, or at the sixth week, since all observed significant levels are accounted (P>0.05), and that could be enables of applying the conventional statistical methods, either for descriptive methods for estimations by points and intervals, such as (mean, standard deviation, standard error, 95% confidence interval for the population mean values, ….etc), or applying an inferential statistics of parametrical methods, such as (One-WayANCOVA), which supposed that underlying data are follows (Normal Distribution Function).

Table (2) represents a summary statistics base line outcomes of studied readings concerning (Blood Glucose) test for studied different groups of (Herbal Plants), such as: [mean values, standard deviation, standard error of mean values, 95% confidence interval for mean value, and the two extremes values of the studied readings (i.e. minimum and maximum)].

Table (2): Descriptive Statistics of Base Line for "Blood Glucose" test at the studied of different groups

Herbal Plants	Periods	No.	Mean	SD	SE	95% C.I.		Min.	Max.
						L.b.	U.b.		
Nasturtium Officinale	First week	20	288.3	90.5	20.3	245.9	330.6	115	450
	Sixth week	20	294.6	101.7	22.8	247.0	342.2	114	455
Hibiscus Sabdarriffa L	First week	20	102.9	13.2	3.0	96.7	109.0	85	133
	Sixth week	20	97.7	12.2	2.7	92.0	103.4	82	125
PurtulacaOleracea	First week	20	165.3	46.8	10.5	143.3	187.2	86	250
	Sixth week	20	138.9	38.1	8.5	121.0	156.7	88	216

By reviewing the results of the statistics concerning studied marker for the two periods (1st week, and 6th week), it is clear that the results hasa little bit regressed in a way that is consistent in general along (Hibiscus Sabdarriffa L, and PurtulacaOleracea)of (Herbal Plants) groups, while a reversed response are accounted with (Nasturtium Officinale) of of (Herbal Plants) group, since mean value since of the slight rise that accompanies after passing period of 6 week.

Figure (1) represent graphically plotting of cluster bar chartsand Stem-Leaf plots concerning "Blood Glucose" teston the subject of different groups at the two different periods.

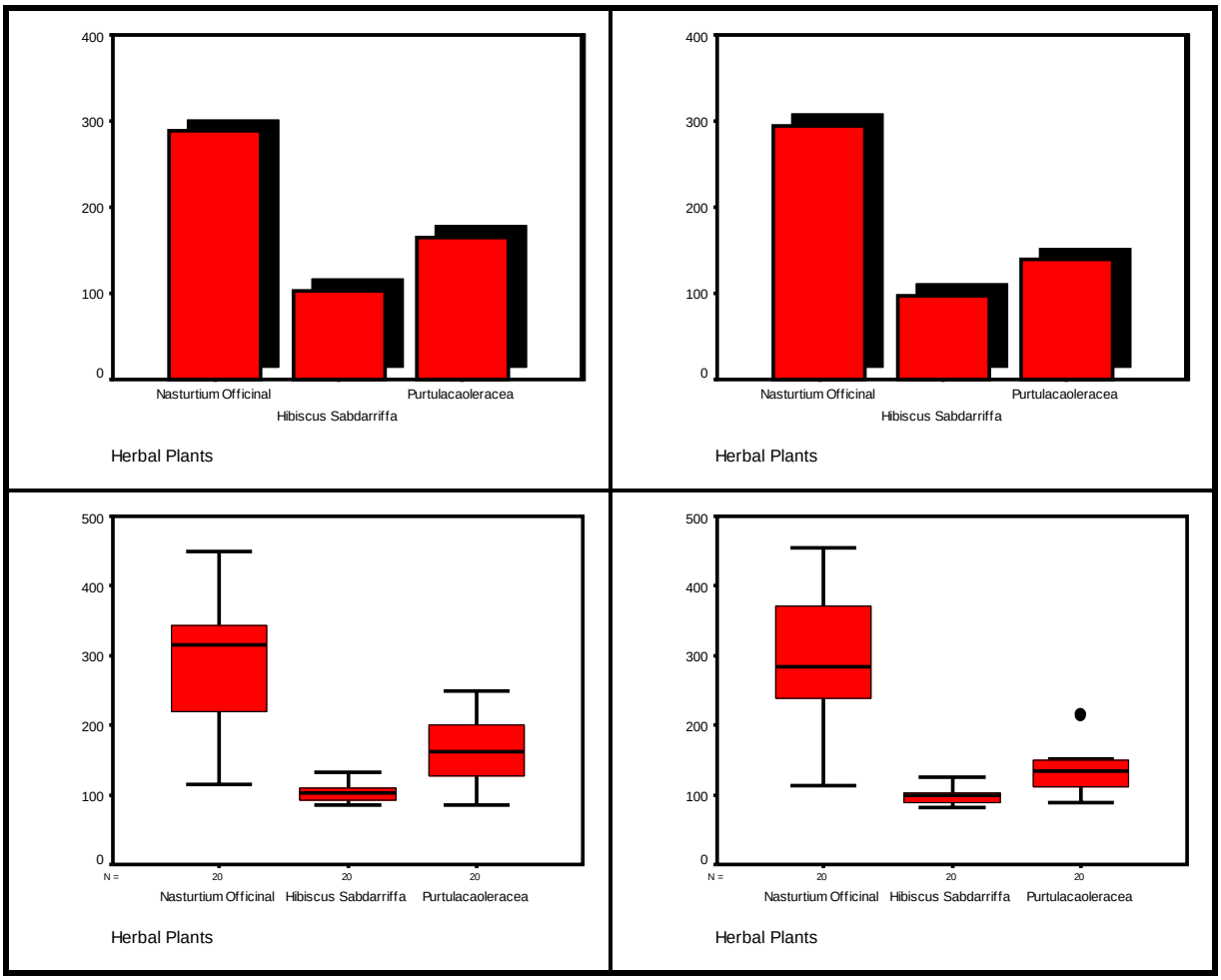

Figure (1): Stem - Leaf Plots for (Total Blood Glucose test) distributed by the studied Herbal Plants groups atthe two different periods

Table (3)shows testing matched paired t-test of studied marker (Blood Glucose) to indicate the extent to which the marker 's decline level has been tested after six weeks in each "Herbal Plants" groups. The results of the matched pairs t- test were shown no significant different are accounted for "Nasturtium Officinale" group at P>0.05, while significant differences were reported for "Hibiscus Sabdarriffa L" group at P<0.05, as well as highly significant differences were reported for "PurtulacaOleracea" group at P<0.01.

Table (3): Matched Paired t- test for testing effectiveness of time period improvement on studied Marker's readings in each of different groups

Herbal Plants	Pairs	Mean Paired Diff.	Matched Paired t-test	d.f.	Sig. (2-tailed)	C.S.
Nasturtium Officinale	First week - Sixth week	-6.35	-0.75	19	0.462	NS
Hibiscus Sabdarriffa L	First week - Sixth week	5.15	2.756	19	0.013	S
PurtulacaOleracea	First week - Sixth week	26.4	5.059	19	0.000	HS

(*) HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05.

Table (4)shows the most common statistical method of testing and analyzing studied marker (Total Blood Glucose test) associated with different source of variation (S.O.V.), such as different groups of "Herbal Plants" along different periods, as well as testing effectiveness of the other source of variations that not included in the studied model (i.e. the Intercept), and finally to interpreted the variations in the marker's readings due to influences by different of studied marker.

Table (4): One –Way ANCOVAof Fixed Model for testingeffectiveness of time period improvement's Marker's readings along different groups

Dependent Variable: Total Blood Glucose test						
Source of Variation (SOV)	Type III Sum of Squares	d.f.	Mean Square	F	Sig.	C.S. (*)
Corrected Model	431473.3	2.0	215736.7	54.2	0.000	HS
Intercept	1880802.2	1.0	1880802.2	472.3	0.000	HS
Groups	431473.3	2.0	215736.7	54.2	0.000	HS
Error	226999.6	57	3982.4			
Corrected Total	2539275.0	60				
R - Squared = 0.655; Adjusted R-Squared = 0.643						

(*) HS: Highly Sig. at P<0.01.

Results shows highly significant influences had been obtained at P<0.01due to different (S.O.V.), as well as intercept (The other sources of variation not included in the studied model) which is recorded a

highly significant effectiveness at P<0.01, and that should informative with the interpret variations among the studied marker's readings.

Finally, determination coefficient (i.e. the R-Square) was recorded (65.5%), which representing the percent value of studied main influences resulted by a different groups that explained amount of variation among the marker's readings along the studied period of time.With respect to that results of (LSD) test, which was applied to know the actual significant levels among all probable pair wised of different groups, as illustrated in table (5).

Table (5): Pair's wisedcomparisons by (LSD) test among studied groups concerning "Blood Glucose"test

Dependent Variable: Blood Glucose				
(I) Group	**(J) Group**	**Mean Diff. (I-J)**	**Sig.**	**C.S. (*)**
Nasturtium Officinale	**Hibiscus Sabdarriffa L**	**196.6**	**0.001**	**HS**
	PurtulacaOleracea	**155.75**	**0.008**	**HS**
Hibiscus Sabdarriffa L	**PurtulacaOleracea**	**-41.15**	**0.040**	**S**

(*) HS: Highly Sig. at P<0.01; **S: Sig. at P<0.01**; **Testing based on LSD test.**

Results shows highly significant different are accounted amongall probable pair wised, as well as the negative sign of mean difference (I-J) refer that (Group–I) has recorded lowoutcomes of marker'sreadings less than (Group–J), while the positive sign of mean difference (I-J) refer that (Group – I) has recorded increasesin the marker'sreadingsmore than (Group–J). For summarizes of preceding results, it could be conclude thatstudying influences of an assignable factor, "The different Groups of Herbal Plants" has resulted meaningful influences on studied marker.

In addition to preceding results, determination coefficient indicating that rather than studied influences of assignable factor, the intercept (i.e. other sources of variation that not included in the studied model or known non assignable factors) should be informative as a one meaningful influences for interpretation studied marker, and finally the percent value of studied main influences result by a different groups that interpretation extremely all the amount of variation among the marker's readings along the studied period of time.

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