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Effect of Leaf Essential Oil of *Citrus sinensis* at Different Harvest Time on Some Liver and Kidney Function Indices of Diabetic Rats

O. Soji-Omoniwa, N. O. Muhammad, L. A. Usman, B. P. Omoniwa

***Abstract*—**This study was conducted to investigate the effect of the leaf essential oil of *C. sinensis* harvested at 7.00a.m and 4.00p.m on some Liver and Kidney function indices of diabetic rats as well as investigate the effect of time of harvest on the observed effect. Experimental animals were divided into 4 groups (A, B, C and D). Diabetes mellitus was induced in all animals, except the normal control group (Group A), by injecting 150mg/kg body weight of alloxan monohydrate intraperitoneally. Group A received distilled water while group B (diabetic control group) was not treated. Group C and D were treated with leaf essential oil of *C. sinensis* harvested at 7.00 a.m and 4.00p.m respectively at a dose of 110 mg/kg body weight every other day for 15 days. Alkaline phosphatase (ALP), Alanine Transaminase (ALT) and Aspartate Transaminase (AST) activity was evaluated in the serum, Liver and Kidney of studied animals. Total and Direct Bilirubin level, Total Protein and Globulin, Creatinine and Urea level were also evaluated. Result showed that creatinine and urea, serum ALP, AST and ALT levels was significantly reduced ($p < 0.05$), while the levels of total Protein and Globulin increased significantly ($p < 0.05$) for the treated animals compared to the diabetic control group. In conclusion, the leaf essential oil of *Citrus sinensis* ameliorated the impaired renal and liver function; however, the time of harvest of the leaf does not significantly affect its ameliorative effect.

Keywords*—C.sinensis*, Function indices, Harvest time, Leaf essential oil.

I. Introduction

RESEARCH into the medicinal uses of essential oil obtained from many plant sources have gained popularity recently and many of the essential oils have been shown to possess medicinal properties [1]-[3]. Correlation has been established in many cases between the medicinal potentials of these essential oils and their phenolic compounds. Essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. They are also known as volatile oils, ethereal oils or aetherolea, or simply as the "oil of" the plant from which they were extracted, such as *oil of clove*. Oil is "essential" in the sense that it carries a distinctive scent, or essence of the plant.

Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer, and often are based solely on historical accounts of use of essential oils for these purposes. Studies have shown that the yield of plant material, the essential oil content and quantitative composition of plants can be influenced by harvest time, ecological and climate conditions [4]. Essential oil is also affected by season as reported by [4]. They reported that the amount of essential oils and the concentrations of the phenolic constituents were the lowest in spring and the highest in June/July, the full blooming period. They determined that essential oil content were between 4.8-10.8% and major components were carvacrol (481.9-489.9 mg/mL), γ- terpinene (40.7-65.2 mg/mL), p-cymene (40.2-54.0 mg/mL) from June to July. Previous report showed that the highest essential oil content (2.33%) was at the full-flowering stages. This is due to high yields of fresh and dry biomass and content of oil at this stage [5].

Variation in the effect of harvesting hour on the essential oil content of Lemon balm grown in the Eastern Mediterranean was studied. The highest essential oil content (0.081%) was obtained at 6.00a.m when the plants were covered in dew. The study concluded that morning (6.00a.m) and evening (7p.m) harvests had yield advantage over noon (12p.m) harvest [6]. A similar pattern was also reported in a previous work [7]. They attributed the differences in the essential oil content to available moisture as well [7]. Another report however stated that there was no significant effect in harvest hour on composition and content of essential oil of *Ocimum basilicum* major components [8].

Leaf extracts of *C. sinensis* have been used in Nigerian local folk medicine to treat neurological disorders and to facilitate the digestion of food. It has also been used as an antidiabetic, antibacterial, antifungal, hypotensive, antioxidant, insect repellent, larvicidal, antiviral, uricosuric, anti-yeast, antihepatotoxic and antimutagenic agent [9]-[13]. In Odummara Obi-orodo, of Imo State, Nigeria, herbalists use concoction made from *Citrus sinensis* leaf, *Magnifera indica*, *Carica papaya*, *Psidum guajava* and other plants' leaf to treat malaria [14]. The oils are generally in use in many foods, confectionery, drug, cosmetic, and flavoring products [9] and there have been claims on its use in managing several ailments in folk medicine and in some instances substantiated with scientific data. It is believed among some Nigerian Herbalists

O. Soji-omoniwa is with the Department of Biochemistry, Faculty of Natural and Applied Science, Plateau State University, Bokkos, Plateau State, Nigeria.(phone: +234 7034205898; e-mail: adejillian234@yahoo.com).

N.O. Muhammad is with the department of Biochemistry and Molecular Biology, Federal University, Dutsin-Ma, Nigeria. (phone: +234 8033931900 e-mail: drmno02@gmail.com).

L. A. Usman is with the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. (phone: +234 8035032378)

B.P. Omoniwa is with the Department of Science Laboratory Technology, University of Jos, Jos, Nigeria. (phone:+2348037362620; e-mail: sojiomoniwa@gmail.com).

that leaf "sleeps" at certain time of the day, hence affecting the therapeutic benefit of such plant. This study was conducted to investigate the ameliorative effect of the leaf essential oil of *C. sinensis* harvested at 7.00a.m and 4.00p.m on some liver and kidney function indices of diabetic rats.

II. Materials and Methods

A. Materials

Alloxan monohydrate and dimethylsulfoxide (Sigma Chemical Company, St. Louis, Mo, USA), Accu-chek active glucometer and strips (Roche Diagnostic, Mannheim, Germany) and OHAUS analytical balance (Ohaus Corporation, NJ, USA), were used. Fresh leaves of *Citrus sinensis* were obtained from the Junior Staff quarters of the University of Ilorin, Nigeria where a voucher specimen of the plant was deposited. Identification of the leaf was carried out at the Plant Biology Department of the University of Ilorin. Albino rats (*Rattus norvegicus*) were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria.

Assay kits for alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total and conjugated bilirubin were products of Randox Laboratories, Co- Antrim, United Kingdom. All other reagents used were of analytical grade and were prepared in all glass distilled water.

B. Methods

1. Essential Oil Extraction

Pulverished leaves of *Citrus sinenis* (800g) were hydrodistilled for 3 hours in a Clevenger type apparatus. Five (5) percent v/v of the resulting oil was prepared, using saline solution of dimethylsulphoxide (DMSO) [15].

2. Gas Chromatography/Mass Spectrometry (GC-MS) Analyses

A Hewlett-packward ITP5890A GC, interfaced with a VG analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2ml/min. The MS operating conditions were: ionization voltage 70eV, ion source 230ºC. The GC was fitted with a 25m × 0.25mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15m. The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the oils were computed in each case from GC peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the retention time of series of n-alkanes) and mass spectra with those of authentic samples and with data from literature.

3. Experimental Animals

Forty (40) male albino rats of *norvegicus* strain (150–200g) were housed in standard cages and allowed to acclimatize to animal house for 14 days. All rats were maintained under standard laboratory conditions (12-h light/dark cycle, 25±2ºC). They were fed with standard rat chow and tap water *ad libitum.* Animals were then randomly selected into 4 groups (i.e. A, B, C and D) of 10 rats each representing (respectively) the Normal Control, Diabetic Control, Diabetic treated with 110 mg/kg b.wt. leaf essential oil of *Citrus sinensis* harvested at 7.00a.m and the last group treated with essential oil from leaf harvested at 4.00p.m.

4. Induction of Experimental Diabetes

After fasting for 8 hours, animals in the diabetic groups were subjected to a single intraperitoneal injection of freshly prepared 150 mg/kg body weight alloxan monohydrate dissolved in sterile distilled water. 48h after alloxan injection, fasting blood glucose (FBG) was determined using AccuChek active glucometer and compatible strips. Rats showing glucose concentration above 110 mg/dl were considered diabetic.

5. Isolation and Homogenization of Tissues

The rats were dissected in order to isolate tissues of interest (liver and kidney). The isolated tissues were cleansed with cotton wool to remove blood stains, weighed and immediately stored in ice cold 0.25M sucrose solution. Homogenization was done using mortar and pestle in ice-cold 0.25M sucrose solution ($1:5^{w}/_{v}$). The homogenates were appropriately diluted with 0.25M sucrose solution before they were used for assay of enzyme activities and protein concentration measurements.

6. Liver Function Test

Serum albumin concentration was quantified by the method described [16]. Total bilirubin concentration in the serum was measured by the method described [17]. Alkaline Phosphatase Activity was determined using the method of [18], AST and ALT using the method of [19].Protein concentration in tissues was determined using the biuret method [20].

7. Kidney Function Parameters

Urea and creatinine levels in the serum were determined using the method of [21].

8. Statistical Analysis

All data were expressed as the mean of ten replicates $\pm$ standard error of mean (S.E.M). Statistical evaluation of data was performed by SPSS version 16.0 using one way analysis of variance (ANOVA), followed by Duncan's multiple range test for multiple comparison. Values were considered statistically significant at $P < 0.05$ (confidence level = 95%).

III. Results

The percentage yield for each time of the day was 0.125 % w/v for 7.00 am leaves and 0.11 %w/v for 4.00 pm leaves.

TABLE I
CHEMICAL COMPOSITION OF LEAF ESSENTIAL OIL OF *CITRUS SINENSIS* HARVESTED AT 7.00A.M AND 4.00P.M

s/n	Name of compound	Retention index	TIME OF THE DAY AREA % 7.00a.m	4.00p.m
1	1R-alpha Pinene	937	1.07	0.81
2	beta-Phellandrene	1053	22.85	18.49
3	beta-Pinene	990	1.39	1.06
5	beta-mycrene	994	2.68	2.76
6	3-carene	1011	12.45	11.63
7	1,3-cyclohexadiene,1-methyl-4-(1-methylethyl)	1018	1.36	-
8	6-octenal,3,7-dimethyl	1159	1.28	1.17
9	3-cyclohexen-1-ol,4-methyl-1-(methylethyl)	1179	3.53	-
10	1,3,8-p-Menthatriene	1111	0.96	
11	D-Limonene	1047	4.96	4.83
12	1,3,6-ocatriene,3,7-dimethyl	1040	8.03	8.02
13	1,4-Cyclohexadiene,1-methyl-4-(1-methylethyl)	1062	1.72	1.84
14	Cyclohexene,1-methyl-4-(1-methylethylidene)	1096	3.1	4.55
15	1,6-Octadiene-3-ol,3,7-dimethyl	1082	11.91	-
16	2,6-octadienal,3,7-dimethyl(E)	1320	2.46	2.6
17	Cyclohexane,1-ethenyl-1-methyl-2-,4-bis(1-methylethenyl)-,(1S-(1.alpha.,2.beta.)	1375	1.79	1.44
18	CaryoPhyllene	1467	2.15	-
19	2,6,9,11-Dodecatetraenal,2,6,10-trimethyl	1706	1.12	0.98
20	6-Octen-1-ol,3,7-dimethyl	1233	-	5.91
21	Benzene,1-methyl-4-(1-methylethyl)	1033	-	1.09
22	Linalyl isobutyrate	1374		12.3
23	alpha-phellandrene	1032		0.57
24	2,6-octadienal,3,7-dimethyl(Z)	1294		2.6
25	Apiol	1680		0.76
26	Naphthalene,1,2,3,4,4a,5,6,8a-octahyro-7-methyl-4-methyl-(1-methylethyl)-,(1.alpha.,4a.beta.,8a.alpha)	1512		1.1
27	1H-Benzocycloheptene,2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl	1499		0.83
28	Santolina triene	908		0.53
	TOTAL	**84.81**		**85.87**

TABLE II
EFFECT OF LEAF ESSENTIAL OIL OF *CITRUS SINENSIS* ON TOTAL AND DIRECT BILIRUBIN OF ALLOXAN-INDUCED DIABETIC RATS

Treatment	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)
Normal control	13.8 ± 1.03^{a}	1.92 ± 0.17^{a}
Diabetic Control	19.30 ± 0.25^{b}	2.41 ± 0.45^{a}
Diabetic + 7.00a.m EO	11.28 ± 0.84^{c}	1.30 ± 0.23^{c}
Diabetic + 4.00p.m EO	10.20 ± 0.24^{c}	1.51 ± 0.17^{c}

Values are expressed as mean of ten replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$).
EO – Essential oil

TABLE III
EFFECT OF LEAF ESSENTIAL OIL OF *CITRUS SINENSIS* ON SERUM PROTEINS CONCENTRATION OF ALLOXAN-INDUCED DIABETIC RATS

Treatment	Total protein(g/dl)	Albumin (g/dl)	Globulin (g/dl)
Normal control	4.68 ± 0.31^{a}	3.96 ± 0.11^{a}	2.01 ± 0.63^{a}
Diabetic control	4.48 ± 0.04^{a}	2.67 ± 0.10^{b}	1.06 ± 0.00^{b}
Diabetic + 7.00a.m EO	5.36 ± 0.24^{b}	2.48 ± 0.60^{b}	2.89 ± 0.44^{a}
Diabetic + 4.00p.m EO	5.24 ± 0.14^{b}	2.51 ± 0.17^{b}	2.73 ± 0.29^{a}

Values are expressed as mean of ten replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$).
EO – Essential oil

TABLE IV
EFFECT OF ADMINISTRATION OF LEAF ESSENTIAL OIL OF *CITRUS SINENSIS* ON KIDNEY FUNCTION PARAMETERS OF ALLOXAN-INDUCED DIABETIC RATS

Treatment	Creatinine (mg/dl)	Urea (mg/dl)
Normal control	1.83 ± 1.06^{a}	11.19 ± 0.86^{a}
Diabetic control	8.25 ± 0.85^{b}	35.05 ± 2.15^{b}
Diabetic + 7.00a.m EO	6.76 ± 0.64^{b}	4.52 ± 0.74^{c}
Diabetic + 4.00p.m EO	5.53 ± 0.67^{c}	9.25 ± 0.86^{d}

Values are expressed as mean of ten replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$).
EO – Essential oil

TABLE V
EFFECT OF LEAF ESSENTIAL OIL OF *CITRUS SINENSIS* ON ALKALINE PHOSPHATASE (ALP) ACTIVITY (NM/MIN/MG PROTEIN) IN TISSUES OF ALLOXAN-INDUCED DIABETIC RATS

Treatment	Serum	Liver	Kidney
Normal control	1.18 ± 0.14^{a}	5.42 ± 0.40^{a}	6.34 ± 0.12^{a}
Diabetic control	2.37 ± 1.14^{b}	1.42 ± 0.63^{b}	0.72 ± 0.27^{b}
Diabetic + standard drug	1.08 ± 0.33^{a}	4.46 ± 0.62^{a}	6.22 ± 0.01^{a}
Diabetic + 7.00a.m EO	1.10 ± 0.40^{a}	4.50 ± 0.19^{a}	6.57 ± 0.71^{a}
Diabetic + 4.00p.m EO	1.12 ± 0.30^{a}	4.32 ± 0.37^{a}	5.68 ± 0.95^{a}

Values are expressed as mean of ten replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$).
EO – Essential oil

TABLE VI
EFFECT OF LEAF ESSENTIAL OIL OF *CITRUS SINENSIS* ON ALANINE TANSAMINASE (ALT) ACTIVITY (U/L) IN TISSUES OF ALLOXAN-INDUCED DIABETIC RATS

Treatment	Serum	Liver
Normal control	1.10 ± 0.48^{a}	7.78 ± 0.79^{a}
Diabetic control	2.68 ± 0.28^{b}	0.50 ± 0.00^{b}
Diabetic + 7.00a.m EO	0.83 ± 0.15^{a}	7.32 ± 0.09^{a}
Diabetic + 4.00p.m EO	1.01 ± 0.20^{a}	7.03 ± 4.60^{ac}

Values are expressed as mean of ten replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$).
EO – Essential oil

TABLE VII
EFFECT OF LEAF ESSENTIAL OIL OF *CITRUS SINENSIS* ON ASPARTATE TANSAMINASE (AST) ACTIVITY (U/L) IN TISSUES OF ALLOXAN-INDUCED DIABETIC RATS

Treatment	Serum	Liver
Normal control	5.88 ± 0.54^{a}	12.69 ± 1.15^{a}
Diabetic control	7.84 ± 0.52^{b}	4.43 ± 0.93^{b}
Diabetic + 7.00a.m EO	5.20 ± 0.43^{a}	12.05 ± 0.03^{a}
Diabetic + 4.00p.m EO	5.02 ± 0.68^{a}	12.86 ± 1.61^{a}

Values are expressed as mean of ten replicates ± S.E.M and those with different superscripts along a column are statistically different ($p < 0.05$).
EO – Essential oil

IV. Discussion

The concentration of the major chemical constituents of leaf essential oil of *C. sinensis*, beta-Phellandrene, 3-carene, D-Limonene and 1,3,6-ocatriene,3,7-dimethyl decreased in the 4.00pm leaves when compared with the 7.00 am leaves (Table I).

The result of the present study showed that the leaf essential oil of *C. sinensis* improved the liver and kidney function indices and there was no significant difference in the effect of the oil extracted from the leaf harvested at 7.00a.m and 4.00p.m on the liver and kidney function. Enzyme pattern in the tissues may be used to assess liver dysfunction and serum enzyme may be used to corroborate the physiological state of the organs. Typically, during tissue damage, some enzymes find their ways into the serum probably by leakage through disrupted cell membranes. Thus, serum enzymes measurement provides a marker in toxicity studies as well as in clinical diagnosis. In this study, there was a significant increase ($p < 0.05$) in serum ALP, ALT and AST activity of the diabetic untreated animals with a corresponding decrease in the Liver and Kidney. This result suggests possible leakage of these enzymes from the liver and kidney into the serum. Such pattern indicates hepatotoxic and nephrotoxic effect of alloxan. However, administration of leaf essential oil of *Citrus sinensis* to the diabetic animals caused a reversal of the enzyme pattern observed in the serum and tissues. This may be attributed to the presence of monoterpenes, a major component of the plant essential oil which has been reported to have hepatoprotective property and may also possess nephroprotective ability.

Total protein and albumin are parameters used to ascertain the state of the liver's synthetic function. Albumin is a major protein of the plasma and represents about 25% of total hepatic protein synthesis and half its secreted protein. Its synthesis is depressed in a variety of diseases, particularly those of the liver [22]. Bilirubin (total and conjugated) on the other hand could be used to assess the excretory function of the liver [22], [23]. Severe hemolysis causes the release of more bilirubin into the blood which manifests as elevated levels of unconjugated and total bilirubin [24]. Unconjugated and total bilirubin can also increase in the event of low bilirubin conjugation [24]. In this study, the levels of albumin, globulin, total protein and bilirubin were elevated in the group treated with the oils. This may be due to the ability of the extract to cause an alteration in the liver's synthetic function. (Tables II and III).

Serum Urea and Creatinine are indicators of kidney functions. They are usually required to assess the normal functioning of different parts of the nephrons. High blood urea levels in renal disease are a consequence, not a cause, of impaired renal function [24]. Blood creatinine level is raised in cases of renal failure. Result of this study showed a significant increase ($p < 0.05$) in serum creatitine and urea concentrations of the diabetic control group when compared to the normal control group. Treatment with essential oil reduced serum creatinine and serum urea significantly ($p < 0.05$). This result suggests that the essential oil of this plant may be capable of improving renal excretory function.

Overall, the time of harvest however did not affect the effect of the extracted oils on the biochemical parameters studied in the experimental animals significantly.

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