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Phytochemical screening using GC-FID and sub-chronic assessment of Hydroethanolic leaf extract of *Ageratum conyzoides* Linn on albino rats

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Abstract
Background: *Ageratum conyzoides* Linn finds application in ethnomedicine, hence there is need to evaluate its toxic effect. The present study investigates the phytochemical compositions and the sub-chronic assessment of the hydroethanolic leaf-extract of *A. conyzoides* on albino rats.
Materials and Methods: Gas Chromatography Flame Ionization Detector (GC-FID) was used to determine the phytochemical composition of the plant while sub-chronic assessment was done using Haem Analyzer and Randox kits. Data analysis was done using ANOVA with Turkey's Multiple Comparison Test using MiniTab version 14.0. Values were considered significant when $p<0.05$.
Result: Nine alkaloids were detected with mitraphyline having the highest concentration (9.23mg/100g). Humulene exhibited 27.21mg/100g, highest compared to the 44 terpenes detected. Carotene had 23.4mg/100g out of the 10 carotenoids. A very high salicyclic acid concentration (77.15mg/100g) relative to the 11 phenolic compounds detected. Twenty one (21) flavonoids were detected and kaempterol had the highest concentration (19.11mg/100g). Six known hydroxycinnamic acids were detected and chlorogenic acid showed highest concentration (14.85mg/100g). There was no significant difference ($p<0.05$) between the weights of the control and test rats. The group C showed significant increase ($p<0.05$) in the weight of the liver, compared to the control, group A. increase in the other organs were not significant ($p<0.05$). Liver function indices did not show significant charge except for the total bilirubin (48.30±5.6mg/dl) and (38.36±5.2mg/dl) for groups C and A respectively at $p<0.05$. The renal function indices (creatinine and urea) did not exhibit significant change ($p<0.05$) between test groups compared to the control. Total blood protein increased but not significantly ($p<0.05$) in the test groups compared to the control. Total blood glucose decreased significantly ($p<0.05$) from 76.40±11.2mg/dl to 69.40±12.0mg/dl in groups A and C respectively.
Conclusion: The above results suggest that *Ageratum conyzoides* is toxicologically safe at sub-chronic low doses and as such a good source of nutraceuticals.

Keywords: Phytochemical, *Ageratum conyzoides*, nutraceuticals, concentration, chromatography

Introduction
The importance and uses of plants in the treatment of ailments especially as complimentary alternative medicine have gained more popularity. The accessibility and affordability of herbal remedies by a greater population in the tropical and sub-tropical regions account for the increase in demand. Moreso, international demand in medicinal plants is gradually increasing due to bioprospecting activities as well as search for sources of new drugs.
One of such commonly used medicinal plants found in Africa including nigera is *Ageratum conyzoides. Ageratum* is derived from the Greek word 'ageras', meaning non-aging, referring tgo the longitivity of the whole plant (Burkill, 1985). The plant has about 30 different species and originated in the tropical America (Okunade, 2002). *Ageratum conyzoides* is popularly known as goat-weed because it has odour likened to that of male goats in Australia, hence the common name 'goat-weed' (Igoli *et al.,* 2005).
A. conyzoides is an annual branching herb which grows approximately up to 1m in height. The stem and leaves are covered with fine white hairs. The leaves are ovate and measures up to 2.6cm long. The flowers are purple and white, less than mm across and arranged in close terminal inflorescence. *A. conyzoides* is an invasive weeds, with a wide range of ecosystem in tropica and sub-tropical countries (Batish, 2004a) [7].
A. conyzoides has a long history of traditional medicinal uses especially in Africa and Asia. It has found application in the treatment of various diseases such as colic, ophthalmia,

headache and dyspnea (Al-Magboul *et al.,* 1977) [9]. Furthermore, it has been implicated as purgative in wound dressing, ulcer treatment and treatment of mental and infectious diseases (Adewole and Okunade, 2001).

Although, *A. conyzoids* is a delicacy for domestic guinea-pigs, horses and cattle (Dagar and Dagar, 1996), it is not eaten by humans except when taken for medicinal purposes. Therefore, there is need to screen for the phytochemical constituents of the plant and as well as evaluate the toxicity of the plant using some toxicological parameters.

Materials and Methods

Plant

Ageratum conyzoids leaves were harvested in the court yard of Faculty of Biological and Physical Sciences, Abia State University, Uturu, Nigeria. It was identified at the Taxonomy Department of Forestry Research Institute of Nigeria (FRIN) Ibadan to correspond with the Voucher specimen in the herbarium, with number FHI 106480.

The air-dried leaves were ground into powder and 1000g was divided into 2 parts. The first part 750g was used for different solvent extracts in GC-FID. The second part, 250g was soaked in 2L of ethanol-water (90:10) for 72h; with occasional stirring. The solution was filtered and evaporated using a rotary evaporator. The dry extract yield was 1.2%. Approximate concentrations of the extract were made in 2% aqueous Tween 80 and used in the animal experiments.

Methods

Gas Chromatographic determination of phytochemicals

Alkaloids: Alkaloid determination was carried out using the modified method of Ngounou *et al.* (2005) [5].

Terpenoids: Accelerated solvent extraction method of Jitka *et al.* (2007) was carried out to extract the terpenes.

Carotenoids: Carotenoids were extracted using Tagaki, (1985) method.

Phenolic compounds: Phenolic compounds were extracted according to the USEPA official methods such as 3540B (1994).

Flavonoids and hydroxycinnamic acids

Extractions were carried out by the method of AOAC (2009). The different solvent extract were subjected to GS-FID separation and identification protocol.

Animals

Albino rats of either sex weighing 120-180g were purchased from the Department of Animal Science, University of Nigeria, Nsukka. They were housed at the animal house of the Department of Biochemistry, Abia State University, Uturu. The rats received standard rat diets and water a libitum. Accepted guidelines for animal care and handling were followed (OECD, 2008). Ethical approval was obtained from the University Ethical Committee.

Acute Toxicity Test

Determination of LD_{50} as described by Lorke (1983) was carried out using 12 albino rats.

Phase 1

The animals were divided into 3 groups of 3 rats each, and were administered with different doses (10, 100 and 1000mg/kg) of the extract respectively. The animals were observed for 24 hours for morbidity and mortality.

Phase 2

Phase 2 involves the use of three rats, distributed into 3 groups of a rat per group. The animals were given higher doses (1600, 2900 and 5000mg/kg) of extract respectively, and were monitored for 24 hours for morbidity and mortality.

LD_{50} was calculated as

$$LD_{50} = \sqrt{D_o} \times \sqrt{D_{100}}$$

Where D_o = higher dose that gave mortality

D_{100} = lower does that gave mortality.

Sub-Chronic Toxicity Test

Oral toxicity study was carried out by administering repeated doses according to OECD guideline 407 (OECD, 2008) [4]. A total of 42 animals were used for the investigation, 12 rats for the lethal dose (LD_{50}). The remaining 30 rats were divided into 3 groups (A, B, and C) of 10 rats (male and female) per group. Group A received 5mlkg body weight of distilled water. A served as the control. Groups B and C received 400mg/kg and 800mg/kg body weight respectively. The animals received the extracts daily for 28 days and were observed for morbidity and mortality. The animals body weight were measured weekly.

The animals were fasted overnight and anaesthetized with either on the 29th day. Blood samples for haematological analysis were collected in EDTA sample bottles while blood sample for biochemical analysis were without EDTA. Packed cell volume (PCV), haemoglobin, red blood cell count (RBC), mean corpuscular haemoglobin concentration (MCHC), platelets count (PC), neutrophil, basophil, eosinophil and lymphocytes were determined using automatic haematology analyzer (6605477-Coulter AC.T, Beckman Coulter).

Serum obtained after centrifugation without anticoagulant were used for biochemical analysis. Standardized diagnostic kits (Randox Lab Ltd UK) were used. Determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, conjugated bilirubin, unconjugated bilirubin, total protein, total blood glucose, creatinine and urea were measured spectrophotometricall. Weight of the organs were also taken.

Statistical Analysis

The results were expressed as mean ± standard deviation. Statistical analysis was performed by one-way analysis of variance (ANOVA) with Tukey's multiple comparison test using minitab (version 14.0). Values were considered significant when $p<0.05$.

Results

Tables 1- represent the results of the GC-FID of the different phytochemical screened. Mitraphyline showed the highest concentration (9.11mg/100g) of the alkaloids detected in Table 1. While in Table 2, showing the terpenes, humulene was detected to have 27.21mg/100g. Carotene was found to be the highest in concentration (7.46mg/100g) of carotenoids. The phenolic compound with the highest concentration in the plant sample was salicyclic acid (77.15mg/100g). Table 5, showed the flavonoids and kaemferol has the highest concentration (19.11mg/100g). chlorogenic acid, one of the hydrocinnamic acids detected in the *Ageratum conyzoides* has the highest concentration of 14.85mg/100g as shown in Table 6.

From Table 7, the mean body weight increased in the animals administered 400mg/kg body weight (Group B), when compared with the control. The animals that received 800mg/kg (Group C) body weight had less weight gain than

the Group B. the increase in Group B and the decrease in Group C compared with B are not significantly different at $p<0.05$. But no significant difference was observed in the organ weights of heart, spleen, kidney, kidney and testis except in the liver at $p<0.05$.

The haematological parameters in Table 9 showed marginal increase in Groups B and C animals when compared with the control, Group A. These increases were not significantly different at $p<0.05$.

Table 10, showed the biochemical parameters for both liver and kidney biomarkers. The total blood glucose level was significantly ($p<0.05$) decreased in both Groups B and C. While the other parameters on the table did not show significant increase or decrease when compared with the control.

Table 1: Alkaloids identified in the hexane leaf extract of Ageratum *conyzoides* extract by gas chromatography method, showing their retention time (RT) and amount (mg/100g).

Name	Retention time (min)	Amount (mg/100g)
9-Octadecanamide	11.673	5.62869
Dihydro-oxo-demethoxyhaemanthamine	12.562	2.5946
Augustamine	13.974	7.61346
Oxoassoanine	15.037	1.96294
Criname-3-alpha-ol	16.251	2.35000
Buphanidrine	17.097	7.97504
Powelline	18.596	1.47533
Udulatine	19.525	1.12162
Abelline	20.541	5.54940
6-hydroxybuphanidrine	21.105	5.89277
6-hydroxypowelline	21.673	1.52700
Crinamidine	22.364	5.62689
6-hydroxyundulatine	24.613	2.58739
Expoxy-3,7-dimethocriname-11-one	25.483	1.77170
1-Beta,2Beta-Expoxyambelline	24.613	2.66208
Akuammidine	26.503	2.87460
Mitraphylline	27.063	9.11125
Voacangine	27.431	3.21442

Table 2: Terpenes identified in the chloroform leaf extract of *Ageratum conyzoides* extract by gas chromatography method, showing their retention time (RT) and amount (mg/100g)

Name	Retention time (min)	Amount (mg/100g)
Acetic acid	4.725	1.3067
Butanol	5.071	4.11114
Butanoic acid	6.388	1.20116
2-methyl butenoic acid	7.641	3.04827
2-methyl butenoic acid	8.046	1.14142
2-methylbutenoic acid ethyl ester	8.883	8.94140
2-methylbutenoic acid ethyl ester	9.125	5.08059
Azulene	9.362	19.04213
Alpha pinene	9.860	7.30451
Cis ocimene	10.924	6.2473
Myrcene	12.240	7.8926
Allo ocimene	12.993	9.31155
Pinene-2-ol	13.194	3.67806
Apha thujene	13.806	8.73124
Gama terpinene	14.162	4.14474
Citral	14.930	1.38581
Camphor	15.070	18.36817
Neral	15.328	1.96752
1,8-cineole	16.546	5.05398
Borneol	17.528	26.58686
Linalool	17.657	7.42601
Citranellal	18.208	7.42601
Nerol	18.464	7.42601
Alpha terpineol	18.743	1.02757
Terpinen-ol	19.000	5.91286
Citronellol	19.490	1.36038
Apha terpinenyl acetate	21.138	2.60900
Ethyl cinnamate	21.395	2.20688
Bornoel acetate	21.769	1.77418
Neryl acetate	21.769	11.74175
Geranyl acetate	21.991	3.25177
Germacrene acetate	22.414	3.24844
Beta caryophyllene	22.852	1.24450
Cyperene	23.389	5.43150
(6)-shogaol	24.076	2.40003
Alpha copane	24.737	7.97427
Humulene	25.869	27.21342
Valencene	27.326	1.17291
Beta selinene	28.180	9.38152
Aromadendrene	29.043	1.23826
Gama muurolene	30.003	1.18499

Table 3: Carotenoid identified in the acetone leaf extract of *Ageratum conyzoides* extract by gas chromatography method, showing their retention time (RT) and amount (mg/100g)

Name	Retention time (min)	Amount (mg/100g)
Malvidin	19.399	1.0620
Beta-cryptoxanthin	20.535	1.0898
Lycopene	21.501	3.0411
Carotene	22.689	23.5450
Lutein	23.230	11.4730
Xanthophyll	24.033	4.0032
Anthrera-xanthin	24.882	3.9008
Asta-xanthin	25.615	8.3133
Viola xanthin	26.355	3.3420
Neo-xanthin	27.121	6.7579

Table 4: Phenolic acid methanolic identified in the acetone leaf extract of *Ageratum conyzoides* extract by gas chromatography method, showing their retention time (RT) and amount (mg/100g)

Name	Retention time (min)	Amount (mg/100g)
Phenylacetic Acid	7.090	7.93417
Salicylic Acid	8.000	77.15173
Cinamic Acid	8.522	2.39022
Protocatechnic Acid	10.380	9.34124
Vanillic Acid	11.453	4.35492
P-hydroxybenzoic Acid	12.828	7.15674
Gallic Acid	13.263	15.92019
Ferulic Acid	14.925	22.10566
Syringic Acid	15.316	2.85351
Piperic Acid	15.504	5.99905
Sinapinic Acid	16.250	4.10327

Table 5: Flavonoids identified in the methanolic leaf extract of *Ageratum conyzoides* extract by gas chromatography method, showing their retention time (RT) and amount(mg/100g)

Name	Retention time (min)	Amount (mg/100g)
Catechin	13.538	3.576
Raveratrol	15.032	1.796
Apigenin	16.039	3.406
Diaidzein	16.247	2.113
Butein	16.673	3.360
Naringenin	16.784	9.998
Biochain	17.094	6.243
Luteolin	17.363	2.213
Kaemferol	18.055	19.113
Epicatechim	19.523	9.69364
Epicatechim-3-gallate	21.824	1.07264
Quercetin	22.605	5.13208
Isorhamnein	23.968	4.40460
Gallocatechin	22.605	2.3986
Myricetin	24.791	1.32905
Naringin	27.061	1.20460
Kaempferol-3-arabinoside	27.289	9.23094
Quercitrin	27.643	4.56696
Isoquercetin	27.899	4.88972
Orientin	28.196	7.23090
Isoorientin	28.839	4.32175

Table 6: Hydrocinnamic Acid identified in the acetone leaf extract of *Ageratum conyzoides* extract by gas chromatography method, showing their retention time(RT) and amount(mg/100g)

Name	Retention time (min)	Amount (mg/100g)
P-coumarin	7.535	1.20192
P-coumaric acid	10.920	1.58228
Caffeine acid	13.842	1.55272
Scopoletin	6.659	3.64532
Chlorogenic acid	18.417	14.85610
Chicoric acid	19.619	3.783363

Table 7: Mean body weight (g) of rats administered leaf extract of *Ageratum conyzoides*

Week	Control (A)	Extract	
		400mg/kg (B)	800mg/kg (B)
0	128±5.2	131±4.2	125±4.3
1	135±4.6	136±3.9	129±4.7
2	138±4.8	140±4.1	135±4.1
3	144±5.5	147±4.5	139±5.6
4	148±5.1	152±5.2	145±7.2

Table 8: Mean organ weight (g) of rats administered leaf extract of *Ageratum conyzoides*

Organs	Control (A)	Extract	
		400mg/kg (B)	800mg/kg (B)
Heart	0.31±0.02(10)	0.31±0.03(10)	0.30±0.05(10)
Liver	2.10±0.4(10)	2.40±0.9(10)	2.60±0.17(10)
Spleen	0.10±0.02(10)	0.14±0.16(10)	1.90±0.08(10)
Kidney	0.51±0.06(10)	0.50±0.07(10)	0.58±0.05(10)
Testis	0.90±0.05(4)	0.70±0.30(4)	0.69±0.34(4)

No. of rats per group indicated in parenthesis; *p<0.05 control vs. extract.

Table 9: Haematological parameters of rats administered leaf extract of *Ageratum conyzoides*

Organs	Control (A)	Extract	
		400mg/kg (B)	800mg/kg (B)
WBC (10^3 µ/L)	9.10±0.70(10)	9.60±0.60(10)	8.40±0.90(10)
RBC (10^6 µ/L)	7.70±0.50(10)	7.80±0.30(10)	8.30±0.20(10)
Haemoglobin (g/dl)	14.40±0.90(10)	14.60±2.10(10)	14.90±0.80(10)
PCV (%)	46.02±2.30(10)	48.00±3.40(10)	48.00±4.20(10)
MCHC (%)	35.00±0.60(10)	39.00±3.80(10)	42.00±1.20(10)
Platelets (10^3 µ/L)	942.00±48.00(10)	826.00±70.00(10)	982.00±42.00(10)
Neutrophils (%)	68.00±2.00(10)	73.00±11.35(10)	74.30±2.80(10)
Leucocytes (%)	38.00±.27(10)	44.00±7.21(10)	44.70±3.05(10)
Monocytes (%)	2.00±0.60(10)	3.20±2.80(10)	3.60±1.20(10)
Eosinophils (%)	0.60±0.25(10)	1.20±0.36(10)	1.40±0.60(10)

No. of rats per group indicated in parenthesis; *p<0.05 control vs. extract.

Table 10: Biochemical parameters of rats administered leaf extract of *Ageratum conyzoides*

Organs	Control (A)	Extract	
		400mg/kg (B)	800mg/kg (B)
AST (U/L)	239.00±28.00(10)	204.00±26.00(10)	227.00±1.80(10)
ALT (U/L)	71.00±13.00(10)	68.00±6.00(10)	62.00±7.00(10)
ALP (U/L)	268.00±58.00(10)	210.00±45.00(10)	220.00±54.00(10)
Conjugate bilirubin (mg/dL)	0.72±5.00(10)	0.64±6.2(10)	0.60±7.4(10)
Unconjugated bilirubin (mg/dL)	0.81±4.00(10)	0.68±5.20(10)	0.63±5.00(10)
Total blood glucose (mg/dL)	76.40±11.20(10)	71.20±9.50(10)	69.40±12.00(10)
Creatinine (mg/dL)	6.90±1.20(10)	6.20±0.90(10)	5.50±0.7(10)
Urea (mg/dL)	48.00±6.00(10)	36.00±8.00(10)	38.00±5.00(10)

No. of rats per group indicated in parenthesis; *p<0.05 control vs. extract.

Discussion

The phytochemical composition of *Ageratum conyzoides* was investigated. Mitraphyline is among the pentacylic oxindle alkaloids found in plants (Keplinger *et al.,* 1999). Reports have shown that pentacyclic alkaloids exhibit antiproliferative effects through stimulation of apoptosis (DeMartino *et al.,* 2006; Gurrola-Diaz *et al.,* 2011; Pilarski *et al.,* 2010). Insight into mechanism of anticancer activity of pentacyclic oxindole alkaloids of *Uncaria tomentosa* by means of a computational reverse virtual screening and molecular docking approach

(Pawel Kozielewicz *et al.,* 2014).

Humulene is an essential oil of the monocyclic sequiterpenes, first extracted from hops (*Humulus lupulus*) from where the name was derived. Anti-inflammatory effects of humulene was reported by Passosa *et al.* (2007). Inhibitory effects of humulene on tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL1-β) has been reported by Fernandes *et al.* (2007).

Chlorogenic acid (CGA) is a group of phenolic secondary metabolites produced by a certain plant species like coffee (Clifford, 1999) [29]. Accumulating evidence has demonstrated that CGA exhibits many biological properties, including antibacterial, antioxidant and anti-carcinogenic activities (Kassai *et al.,* 2000; dos Santos *et al.,* 2006; Feng *et al.,* 2005) [30-32]. Recent report claims that chlorogenic acid modulates glucose and lipid metabolism *in vivo* in both genetically metabolic disordered conditions (Nicasio *et al.,* 2005) [33].

References

1. Yang L, Wang M, Hu E, Liu J, Wu L. Determination of 21 phenolic compounds in soil by ultrasound extratction-gas-chromatography. Se. Pu., 2013; 31(11):1081-1086.
2. Brojendro Singh *et al*. Ethnobotany, phytochemistry and pharmacology of *Ageratum conyzoidees* Linn (Astaeraceae). 2012.
3. Shigeaki T. Determination of green leaf carotenoid by HPLC. Agric. Bio. Chem., 1985; 49:1211-1213.
4. Organization for economic Cooperation and Development Repeated dose oral toxicity test method. In: OECD guideline for testing of chemicals N407, Paris, France. 2008.
5. Ngounou FN, Manfouo RN, Tapondjou LA, Lontsi D, Kuete V, Penlap V *et al*. Antimicrobial ditterpenoids alkaloids from *Erythropheum sauaveolens* (Guill. and Perr.). Brenan. Bull. Chem. Soc. Ethiop. 2005; 19(2):221-225.
6. Ditka F, Lee L, Vlastimil K. GC-MS of terpenes in walnut-tree leaves after accelerated solvent extraction. J. Sep. Sci., 2007; 31(1):162-168.
7. Batish DR, Singh HP, Kahli RK, Johar V, Yadev S. Management of invasive exotic weeds require community participation. Weed Tech., 2004a; 18:1445-1448.
8. Adewole LO. *Ageratum conyzoides* L. (Asteraceae), Fitoterapia, 2002; 73:1-16.
9. Almagboul AZI, Bashir AK, Khalid SA, Farouk A. Antimicrobial activity of venolepin and vernodali. Fitoterapia, 1977; 68:83-84.
10. Organization for economic Cooperation and Development Guidelines of chemical/ section 4. Health effect of test No. 423: Acute oral toxicity. Acute toxic class method. OECD, Paris, France. 2002.
11. Association of Official Analytical Chemis Official method of analysis. Washington DC, USA. 2009.
12. Aiyegoro OAT. Phytochemical screening and polyphenol antioxidant activities of aqueous leaf extract of Helichrysum pedunculatum. Int. J. Mol. Sci., 2009; 10:4990-5001.
13. Andrea L, Judith Katahi VS, Lajos B. The role of antioxidant phytonutrients in the prevention of diseases. 2003; 47(1-4):119-125.
14. Ayoola GA, Folawewo AD, Adiesegun SA, Abioro OO, Adepoju-Bello AA, Coker HAD. Phytochemical and antioxidant screening of some plants of aoocynaceae from South West Nigeria. Afr. J Plant Sci., 2008; 2(9):124-128.
15. Garcia-Sales P, Morales-Soto A, Segura-Carretero A, Fernandez GA. Phenolic compound extraction system for fruits and vegetable samples. Molecules, 2010; 15:8813-8826.
16. Abena AA, Kintasangouloula-Mbaya GS, Diantama J, Bioka D. Analgesic effect of a raw extract of *Ageratum conyzoides* in rats. PMID 8275920.
17. Fisner T. Chemical prospecting: A call for action. In: Borma F.H. and Keller, S.R, (eds). Ecology, Economics and Ethics.
18. Fatema N. Antioxidant and cytotoxic activities of *Ageratum conyzoides* stems. 2012.
19. Harbone JB. Phytochemical method. London Chapman and Hall Ltd., 1973, 49-188.
20. Moura AC, Silva E, Fraga MCW, Afiatpour P, Maja MP. Anti-inflammatory and chronic toxicity study of the leaves of *Ageratum conyzoides* in rats. Phytomedicine, 2005; 12(1-2):138-142.
21. Ashande MC, Mpiana PT, Ngbohia KN. Ethnobotany and pharmacognosy of *Ageratum conyzoides* L. 2015.
22. Bharat P, Sagar R, Sulav R, Pradeep S, Rubin TM. Effects of crude extract of *Ageratum conyzoides* on serum lipid profile in albino mice and its heamostatic effects. J. Plant Bio. Res., 2015; 3(4):1047.
23. Agbafor KN, Engwa AG, Ude CM, Obiudu IK, Festus BO. The effect of aqueous extract of *Ageratum conyzoides* on glucose, creatinine and calcium ions levels in albino rats. Pharm. Chem. Biol. Sci., 2015; 3(3):408-415.
24. Cryer PE. Hypoglycemia. Handbook of physiology section 7, the Endocrine System 11. The endocrine pancreas and regulation of metabolism. Jefferson C.A. and Goodman H. (eds). New York, Oxford University Press, 2001, 1059-1092.
25. Kowalski J, Samojedry A, Paul M, Pietsz G, Wilcozok T. Effect of apgenin, kaempferol and resperatrol on the expression of interleukin-1β and tumor necrosis factor-α gene in J.774.2 macrophases. Pharmacol. Resp., 2005; 57:390-394.
26. Li RI, Mei JZ, Liu GJ. Kaempferol induced apoptosis of human esophageal squamous carcinoma. Nanfang Yike Daxue Xuebao, 2011; 31:1440-1442.
27. Xie FR, Su M, Qiu W, Zhang M, Guo Z, Su B *et al*. Kaempferol promotes apoptosis in human bladder cancer cells by inducing the tumor expressor, PTEN. Int. J. Mo. Sci., 2013; 14:21215-21226.
28. Kao YC, Zhou C, Sherman M, Laughton CA, Chen S. Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoesterogens: a site directed mutagenesis study. Envion. Health Prospect. 1998; 106:85-92.
29. Clifford MN. Chlorogenic acid and other cinnates: nature, occurrence and dietary burden. J. Sci. Food Agric. 1999; 79(3):362-372.
30. Kassai H, Fukada S, Yamaizuma Z, Sugie S, Mori H. Action of chlorogenic acid in vegetables and fruits as an inhibition of 8-hydroxydeoxyguanodine formation *in vitro* and in a rat carcinogenesis model. Food Chem. Toxicol., 2000; 38(5):467-471.
31. Dos Santos MD, Almedia MC, Lopes NP, desouza GEP. Evaluation of the anti-inflammatory, analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. Biol. Pharmaceut. Bulletin, 2006; 29(11):2236-2240.

32. Fang R, Lu Y, Bowman LL, Qian Y, Castranova V, Ding M. Inhibition of activators protein-1, NF-αβ, and MAPKS and induction of phase 2 detoxifying enzyme activity by chlorogenic acid. J. Biol. Chem. 2005; 280(30):27888-27895.
33. Nicasio P, Aguilar-Santamaria I, Aranda E, Ortiz S, Gonzalex M. Hypoglycemic effect of chlorogenic acid content in two Cecropia species. Phytother. Res., 2005; 19(8):661-664.