Bioactive constituents and biochemical assessment of *Argemone mexicana linn* and *Urtica dioca* leaf extracts using male albino rat

Abstract

Urtica dioca (stinging nettle) and *Argemone mexicana linn* are medicinal plants used in most part of Nigeria in treatment of diseases. The present work evaluated bioactive constituents and biochemical effect of *Argemone mexicana linn* and *Urtica dioca* extracts using male albino rat model. A total of eighteen male albino rats were used for the study. Group I served as control, Group II received 300mg/kg of *Urtica dioca* while Group III received 300mg/kg body weight of *Argemone mexicana linn*. Preliminary phytochemical study showed strong presence of flavonoid, tannin, saponin, alkaloid, cyanogenic glycoside and oxalate while the quantitative phytochemical and vitamin results showed high amount of these active compounds. Biochemical results from this study showed very low hepatotoxic and renal toxicity profile while the lipid profile indicates hypocholestrolemia potentials of the plants. This study opined that in as much as very low toxicity was recorded from intake of *Urtica dioca* (stinging nettle) and *Argemone mexicana linn* ethanol leaf extracts at 300mg/kg body weight, proper care should be taken regarding the dose used for medicinal purposes as continuous usage at high quantity may predispose the human health.

Key words: Bioactive, *Urtica dioca* , *Argemone mexicana* ,biochemical

Introduction

In most developing countries of the world, there is an increasing demand for plant based remedies for human health problem. This upsurge in affinity for herbal remedy is probably due to easy accessibility, non-involvement of expert consultation, inexpensive as well as inadequacy of the primary health care (Udoamaka and Jose, 2014). Many medicinal plants are traditionally used in treatment of various human diseases. *Urtica dioica* (stinging nettle), is a perennial plant belonging to the genus *Urtica* and of the family *Urticaceae*. The stem is erect and the leaves are dark-green (Ahmed and Parasuraman, 2014). The plant leaves possess sharp spines with stinging hairs that irritates when in contact with the skin. The plant is central in management of wide array of diseases due to its acclaimed role as a blood sugar reducing plant as well as in diseases related to internal bleeding, anaemia, diabetes and asthma (Bisrat and Fekadu, 2015). Ahmed and Parasuraman (2014) also posited that traditionally, *Urtica dioica* has been effective in management of rheumatism, arthritis, gout,

eczema, anemia, urinary tract infections and kidney stones. *Argemone mexicana* Linn a prickly annual herb belonging to the family *Papaveraceae* and is widely distributed throughout the subtropical and tropical regions of the world. Traditionally, *Argemone mexicana* Linn is used in treatment of malaria, cataracts, skin diseases and jaundice (Sneha and Sanjay, 2011). This work is aimed at profiling the bioactive constituents of these plants as well as their biochemical effect using male albino rats.

Method of analysis

Sample collection

Fresh young leaf of *Urtica dioca* and *Argemone mexicana linn* were harvested from Umuoda Amuzu in Aboh Mbaise L.G.A of Imo State. Clean tap water was used in washing the samples prior to analysis.

Preparation of ethanol leaf extracts of *Urtica dioca* and *Argemone mexicana linn*

Exactly 200g of the powdered plant were measured into a conical flask and 500ml of 70% ethanol were added and left at room temperature for 48 hours. The extract was filtered. The filtrate was evaporated to dryness on a water bath (50^0C) to give the crude extract, which the mass was determined

Experimental design

Plant screening of *Urtica dioca* and *Argemone mexicana linn* leaves involved preliminary qualitative and quantitative phytochemical analysis as well as vitamin composition while biochemical analysis involved eighteen healthy male albino rats of about six weeks old that were utilized for this experiment. The animals adapted to laboratory conditions for one week after grouping them into three groups of six rats. All the animals received food and water throughout the experimental period. Group I served as control, Group II received 300mg/kg body weight of ethanol leaf extract of *Urtica dioca* while group III received 300mg/kg body weight of ethanol leaf extract of *Argemone mexicana linn*. Plant extracts were given via oral guavage on daily basis while animals were sacrificed according to NIH, (1985) regulations

Determination of phytochemical constituents

In determination of preliminary qualitative phytochemical constituents, the method described by Adetugi and Popoola(2001) was adopted in determination of saponin and tannin while the method by Sofaworo, 1993 was employed to determine alkaloid. Trease and Evans, (1989) was used to evaluate flavanoid and oxalate while the method of Stephen (1970) was used to determine cyanogenic glycoside.

Quantitative phytochemical composition

The procedures described by Obadoni and Ochuko(2001) was adopted in evaluation of saponin and oxalate content while that of Boham and Kocipai (1974) was used to determine flavonoid composition. The method of Harbone (1973) was employed in alkaloid and cyanogenic glycoside determination while Van-Burden and Robinson, (1981) was used to determine tannin content

Determination of vitamin composition

The method of AOAC(1975) was employed to determine riboflavin, tocopherol and retinol content while that of Barakat (1973) was used to determine thiamine, niacin and ascorbic acid content.

Analysis of selected biochemical parammeters

Liver integrity enzyme assessment such as serum alanine aminotrnsferase and serum aspartate aminotransferase were assayed using Reitman and Frankel (1957) method while Bassey *et al.,* (1946) was employed in assessment of alkaline phosphatase activities. In serum total protein determination, Henry *et al.,* (1974) was adopted while Fossati and Prencipe, (1982) was employed in lipid profile evaluation. In total bilirubin assay, Jendrasik and Groff,(1938) was used while Heinegard and Triderstorm (1973) was adopted for creatinine assay.

Expression of results and statistical analysis

Results are presented as mean of triplicate determinations ± standard deviation. Statistical anaylsis was done using students package for social sciences version 20 computer software. Means were separated using One way analysis of variance.

Results

Table 1.Preliminary qualitative constituents of *Urtica dioca* and *Argemone* mexicana linn

Components	*Urtica dioca*	*Argemone mexicana*
Saponin	+	+
Flavoniod	++	++
Alkaloid	++	+
Tannin	++	++
Cyanogenic glycoside	+	+
Oxalate	+	++

Legend: + = present, ++ = strongly present

Table2.Quantitative phytochemical constituents of *Urtica dioca* and *Argemone mexicana linn*(%)

Components	*Urtica dioca*(%)	*Argemone mexicana* (%)
Saponin	8.84±0.32	2.01±0.01
Flavoniod	23.68± 0.33	14.22±0.02
Alkaloid	7.26±0.14	4.36±0.09
Tannin	14.31±0.31	12.82±0.08
Cyanogenic glycoside	1.79±0.01	3.32±0.20
Oxalate	8.53±0.01	7.53±0.21

Values are mean of triplicate determination ± standard deviation

Table 3. Vitamin composition (mg/g) of *Urtica dioca* and *Argemone mexicana linn*

Components	*Urtica dioca* (mg/g)	*Argemone mexicana* (mg/g)

Riboflavin	2.68±0.02	1.80±0.03
Thiamine	4.41±0.01	3.90±0.21
Niacin	19.00±0.04	7.12±0.00
Ascorbic acid	1.90±0.00	1.50±0.05
Tocopherol	13.45±o.11	11.8±0.02
Retinol`	4.40±0.03	3.80±0.01

Values are mean of triplicate determination ± standard deviation

Table 4: Liver integrity of rats administered *Urtica dioca* and *Argemone mexicana linn* extract.

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Protein (mg/dl)	Albumin (mg/dl)	Total bilirubin
Group I	61.51±0.46	33.94±0.12	83.21±0.07	9.13±0.56	7.01±0.01	0.19±0.10
Group II	62.62±0.91*	34.50±0.14*	84.07±0.09*	9.31±0.27*	7.41±0.05*	0.20±0.01*
Group III	63.33±0.04*	34.63±1.09*	83.31±0.04*	9.27±0.32*	7..35±0.15*	0.21±0.04*

Values are mean of triplicate determination ± standard deviation.
* indicate no significant difference from the control.

Legend:
Group I = Control
Group II = 300mg/kg body weight of ethanol leaf extract of *Urtica dioca*
Group III= 300mg/kg body weight of ethanol leaf extract of *Argemone mexicana linn*

Table 5: Serum urea (mg/dl) amd serum creatinin (mg/dl) of rats administered *Urtica dioca* and *Argemone mexicana linn* leaf extract

Groups	Urea (mg/dl)	Creatinin (mg/dl)
Group I	32.12±0.09	0.45±0.21
Group II	34.03±0.16*	0.47±0.23*
Group III	34.16±0.14*	0.49±0.25*

Values are mean of triplicate determination ± standard deviation.
* indicate no significant difference from the control.

Legend:
Group I = Control
Group II = 300mg/kg body weight of ethanol leaf extract of *Urtica dioca*
Group III= 300mg/kg body weight of ethanol leaf extract of *Argemone mexicana linn*

Table 6. Lipid profile of rats administered *Urtica dioca* and *Argemone mexicana linn* extract

Groups	TC(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	TG(mg/dl)

Group I	91.02±0.85	30.29±1.41	21.31±0.04	77.13±0.6
Group II	80.12±1.71*	33.07±1.02*	16.16±0.15*	72.38±1.01*
Group III	79.05±1.98*	35.21±0.767*	14.01±1.21*	79.55±0.55*

Values are mean of triplicate determination ± standard deviation.
* indicate significant difference from the control

Legend:
Group I = Control
Group II = 300mg/kg body weight of ethanol leaf extract of *Urtica dioca*
Group III= 300mg/kg body weight of ethanol leaf extract of *Argemone mexicana linn*

Fig 1. *Argemone* mexicana linn

Fig 2 *Urtica dioca*

Discussion

Phytochemicals or plant chemical are bioactive non-nutrient plant compound that provide a range of activities against oxidative damage (Liu,2013). The result of this study showed both qualitative and quantitative phytochemical constituents of *Urtica dioca* and *Argemone* mexicana linn. The increased presence of flavoniods shows that extracts of these plants may have anti- inflammatory potentials. These phytochemicals have been reported to play important role in detoxification of reactive oxygen species (Chandra *et al.*, 2009). Vitamins are antioxidants that play important role in cellular defense strategy against oxidative stress (Fikriye and Omer, 2005). Result showed appreciable amount of vitamin in the two plants studied. This shows that extracts of these plants can serve as free radical scavenger. Metabolism of foreign substances is very central to liver function and hence, its proximity to toxic substance induced injury. Analysis of liver enzymes such as AST, ALT and ALP has been reported as a rough proportionality of the extent of liver damage (Iweala and Osundiya, 2010). Friday (2004) noted that when cells are damaged, cell-derived enzymes exhibit increased activity in cells and spill into the serum. Result of liver enzymes (AST, ALT and ALP) shows no significant increase in their activities relative to control ($P<0.05$). The observed normalcy in enzyme activity may have resulted from phytochemicals substances present in *Urtica dioca* and *Argemone* mexicana linn. This shows there was little or no cellular impairment that may lead to loss of functional integrity of the liver. Total proteins are rough measures of protein status but reflect major functional changes in liver integrity (Iweala and Osundiya, 2010). The measurement of the level of total protein and total bilirubin

is used to assay for the synthetic and excretory function of the liver. Total protein obtained from the present study showed no significant difference ($P<0.05$) compared to control. This may as well be an indication of none impairment of hepatocyte since total protein is synthesized by the liver cells . Iweala and Osundiya, (2010) opined that alteration in the synthesis of total protein could have consequences in over all physiological function of the animals. Albumin result obtained from this study could further portray the medicinal efficacy of *Urtica dioca* and *Argemone* mexicana linn as results showed none significant levels of albumin. This shows that the extracts did not induce much hepatocellular injury in animals as decrease in albumin level is posited to signify optimal functioning of the liver (Saba *et al.*, 2010). An increase in total bilirubin may indicate that the excretory function of the liver has been affected by damage caused to the liver or it could also indicate that a toxic substance might have competed and displaced the binding of bilirubin on albumin or the uptake of bilirubin is inhibited. Findings from this study show that total bilirubin level was not significantly increased. The renal function result obtained from this study (Table 5) shows that there were no significant increase in urea and creatinine levels compared to control ($P<0.05$). Friday (2004), noted that increase in blood urea is in association with tissue protein catabolism, excess breakdown of blood protein and diminished excretion of urea. Hence justifying the medicinal usage of these plants. Non significant increase in creatinine level relative to control may be an indication of none haepatocellular damage by the extract. Increase in high density lipoprotein is considered to inhibit myocardial infarction while accumulation and increase in circulating total cholesterol, low density lipoprotein and triglyceride in the blood and heart tissues are considered to accelerate the risk of myocardial injury (Anuradha *et al.*, 2001).Many cellular functions such as synthesis of vitamin D on skin surface, hormone production as well as membrane fluidity maintenance are associated with cholesterol (Grover *et al.*, 2001; Ugwu *et al*., 2011). Decreased level of cholesterol obtained from the results may be an indication that extract from the plants could be have hypocholesterolemic effect. Triglyceride accumulation in serum has been reported to play a major role in coronary heart diseases (Mahale *et al.*, 2008).The result show a decrease in triglyceride in a dose dependent ratio. High Density Lipoprotein (HDL) is central to transport of excess cholesterol to the liver, where it is converted into bile acids and excretion thereby performing a protective role in myocardial related diseases (Ugwu *et al.*, 2011). Significant increase in HDL levels of groups II and III animals relative to control group ($P<0.05$) as observed from the results could be attributed to some bioactive compounds in *Urtica dioca* and *Argemone* mexicana linn leaves which may have increased the HDL level which is

responsible for transport of cholesterol out of the cell. The results of lipid profile suggest that ethanol leaf extracts of *Urtica dioca* and *Argemone mexicana linn* may be effective in management of myocardial related diseases.

Conclusion

It can be concluded that ethanol leaf extract of *Urtica dioca* and *Argemone* mexicana linn at 300mg per kg body did not show evidence of cumulative toxicity. This however may suggest that increased and prolonged intake of these plant extracts may have adverse health effects.

Ethical Approval

Animal Ethic committee approval has been taken to carry out this study.

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