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Bioactive Constituents and Biochemical Assessment of *Argemone mexicanna linn* and *Utrica dioca* Leaf Extracts in Albino Rat Model

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Utrica dioca (stinging nettle) and *Argemone* m*exicanna 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 mexicanna linn* and *Utrica 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 *Utrica dioca* while Group III received 300mg/kg body weight of *Argemone mexicanna linn*. Preliminary phytochemical study showed the strong presence of flavoniod, 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 suggest that even though there was low observed toxic response from intake of *Utrica dioca* (stinging nettle) and *Argemone mexicanna linn*, proper care should be taken regarding the dose used for medicinal purposes as continuous usage at high quantity may predispose the human health.

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1. 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 [1]. 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). *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. This work is aimed at profiling the bioactive constituents of these plants as well as their biochemical effect using male albino rats.

2. METHODS OF ANALYSIS

2.1 Sample Collection

Fresh leaf of *Utrica dioca* and *Argemone mexicanna linn* were harvested from Umuoda Amuzu in Aboh Mbaise L.G.A of Imo State. The samples were carefully washed with clean tap water before analysis.

2.2 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.

2.3 Experimental Design

Plant screening of *Urtica dioca* and *Argemone mexicana linn* leaves involved preliminary qualitative Eighteen healthy male albino rats of about six weeks old were used for the study. The animals were randomly divided into three groups of six rats each. They were housed in standard cages and allowed to acclimatize to laboratory conditions for one week prior to commencement of experiment. The animals were allowed free access to commercial rat feed and water *ad libitum.* Group I served as control, Group II received 300mg/kg body weight of ethanol leaf extract of *Utrica dioca*_while group III received 300mg/kg body weight of ethanol leaf *Argemone mexicanna linn*. At the end of the experiment which lasted for twenty eight days, the animals were sacrificed in accordance with the National Institute of Health guidelines for the care and use of laboratory animals [2]. Blood samples were collected by cardiac puncture into anticoagulant free tubes with cork for biochemical analysis.

2.4 Determination of Phytochemical Constituents

In determination of preliminary qualitative phytochemical constituents, Adetugi and Popoola [3] was adopted in determination of saponin and tannin while Sofaworo, 1993 was employed to determine alkaloid. Trease and Evans, [4] was used to evaluate flavanoid and oxalate while the method of Stephen [5] was used to determine cyanogenic glycoside.

2.5 Quantitative Phytochemical Composition

Obadoni and Ochuko [6] was adopted in evaluation of saponin and oxalate content while Boham and Kocipai [7] was used to determine flavonoid composition. The method of Harbone [8] was employed in alkaloid and cyanogenic glycoside determination while Van-Burden and Robinson, [9] was used to determine tannin content.

2.6 Determination of Vitamin Composition

The method of AOAC[10] was employed to determine riboflavin, tocopherol and retinol content while Barakat [11] was used to determine thiamine, niacin and ascorbic acid content.

2.7 Biochemical Analysis

Serum alanine aminotrnsferase (ALT) and serum aspartate aminotransferase activities were determined by the method of Reitman and Frankel [12] while Alkaline phosphatise activity was determined by Bassey et al., [13] using commercial diagnostic kit. Serum total protein

was determined by the method of Henry et al., [14] while lipid profile- Total cholesterol, high density lipoprotein, Low density lipoprotein and serum triglyceride was determined using Randox reagent kit by the method of Fossati and Prencipe, [15]. The assay of total bilirubin was done using the method of Jendrasik and Groff, [16]. Urea was done using Urease Berthlor method while creatinine was determined by the method of Heinegard and Triderstorm [17].

2.8 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 soft ware. Means were separated using One way analysis of variance.

3. RESULTS AND DISCUSSION

Phytochemicals or plant chemical are bioactive non-nutrient plant compound that provide a range of activities against oxidative damage [18]. Findings from this study showed both qualitative and quantitative phytochemical constituents of Utrica dioca and *Argemone* mexicanna 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 [19]. Vitamins are antioxidants that play important role in cellular defense strategy against oxidative stress (Fikriye and Omer, 2005). Findings from the study showed appreciable amount of vitamin in the two plants studied. This shows that extracts of these plants can serve as free radical scavenger. The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism and analysis of liver enzymes such as AST, ALT and ALP have been reported as a rough proportionality of the extent of liver damage [20]. Friday [21] noted that cell-derived enzymes have high activity in cells and spill in plasma when the cell is damaged or the enzymes produced in excess.

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

Saponin	+	+
Flavoniod	++	++
Alkaloid	++	+
Tannin	++	++
Cyanogenic glycoside	+	+
Oxalate	+	++

Table 2. Quantitative phytochemical constituents of *Urtica dioca* and *Argemone mexicana linn*(%)

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*

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±0.11	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*

Result of the 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 Utrica dioca and *Argemone* mexicanna 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 [20]. 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, [20] 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 Utrica dioca and *Argemone* mexicanna 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 reported to signify liver injury [22]. 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. Total bilirubin level was not significantly increased. This however suggests that intake of the extract may not be associated with diseases like jaundice. The Kidney is highly susceptible to toxicants for two reasons. A high volume of blood flows through it and it filters large amounts of toxins which can concentrate in the kidney tubules causing: decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones. 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). It has been reported that high blood urea is associated with increased tissue protein catabolism, excess breakdown of blood protein and diminished excretion of urea [21]. 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. Lipid profile results presented in Table (6) shows significant increase in HDL, decrease in total cholesterol, LDL and TG. Increased lipids in the blood are considered to increase the risk of myocardial infarction [23]. Cholesterol is an essential substance involved in many cellular functions, including the maintenance of membrane fluidity, production of vitamin D on the surface of the skin, production of hormones and possibly helping cell connections in the brain [24]. Increased level of circulating cholesterol and its accumulation in heart tissues are well known for their role in cardiovascular damage [25]. The decrease in cholesterol level is 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 [26].The result show a decrease in triglyceride in a dose dependent ratio. High Density Lipoprotein (HDL) level is a very well known measure of cardiac health due to their strong inverse relationship with coronary artery disease. The protective role

of HDL-C against CVD has been suggested to occur in several ways such as serving as transport particles for excess cholesterol to the liver, where it is converted into bile acids and excreted [24]. There was a significant increase in the HDL levels of groups II and III relative to control ($P<0.05$). This could be as a result of some bioactive compounds in Utrica dioca and *Argemone* mexicanna linn 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 Utrica dioca and *Argemone* mexicanna linn extracts may have cholesterol reducing properties which is necessary in prevention of arteriosclerosis [27-29].

5. CONCLUSION

The result of this study has shown that Utrica dioca and *Argemone* mexicanna linn ethanol extract at 300mg per kg body does not show evidence of cumulative toxicity and may as well be useful in reduction of hypercholesterolemia. This however may suggest that increased and prolonged intake of these plant extracts may have adverse health effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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