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Hepatoprotective Activities of Ethanol Extract of *Desmodium velutinum* (Stem)

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ABSTRACT

The liver is a large organ with two major lobes with the right lobe being six times larger than the left lobe in normal adult. The right lobe is found behind the rib cage with its upper border at about the level of the fifth rib. The circulation system of the liver is characterized by a dual blood supply approximately 80% of this blood supply enters from the portal vein and 20% from the hepatic artery. The scope of herbal medicine is sometimes extended to include fungal and bee product as well as minerals. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such insects, fungi, herbivorous mammals. The stem was air-dried for eighteen (18) days, after which it was homogenized to powder first by cutting it to pieces and grounding,

Keywords: Hepatoprotective, activities, ethanol, extract, stem, minerals.

INTRODUCTION

The liver is a large organ with two major lobes with the right lobe being six times larger than the left lobe in normal adult. The right lobe is found behind the rib cage with its upper border at about the level of the fifth rib [1]. The circulation system of the liver is characterized by a dual blood supply approximately 80% of this blood supply enters from the portal vein and 20% from the hepatic artery [2].

Liver disease is still a worldwide health problem. It seems that conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious effect [3]. There are a number of medicinal plant preparations in global level recommended for the treatment of liver disorder. Many formulations containing herbal extract are sold in the global market for liver disorder but management of liver disorders using herbal preparations is continuously ongoing [4]. Several traditional plants have been extensively used in global traditional disorder. In view, of several undesirable side effect of synthesis drugs, there is growing focus to follow synthesis research methodology and evaluate scientific basis for the traditional herbal medicine that are claimed to poses hepatoprotective activity [5]. It is also gaining great acceptance from the public, medical and biochemical properties of the liver. Although modern drugs are effective in preventing hepatodisorders. Their uses are often limited because of the side effect and the problem posed by the high cost of it [6]. Natural plants have been a valuable source of medicinal agent in proven potentially useful drugs which can often be recognized from their relative importance and use in folk medicine [7].

Extract of *Desmidum velutimum* are traditionally used in some ailment conditions. *Desmidum velutinum* Dc (Fabaceae) commonly known as “Ikeagwuani” in Igbo. It is a perennial or emi-erect shrub of

tropical and sub-tropical regions, up to 3m high, branches are often dark-red (Pal. The plant is used traditionally for treatment of a number of diseases like Jaundice, rheumatism, puerperal fever, paralysis, oedema, filarial and post-natal care to avoid secondary complication. It also provides general support to the body during periods of influenza, cough, cold, neuralgia and headache, it is also used as a dietary supplement [8].

The flavonoid and alkaloid fraction of *Desmodium velutinum* have been known to possess antioxidant and anti-inflammatory activities. Whereas its water decoction extract possesses anti-nociceptive activity, ethanol extract of the plant stem has also been shown to have hypotchelesterolemic and antioxidant effect in iso-oproterenol induced myocardial infarction [9]. Hence *D. velutinum* stem may be a source of a pharmacological active agent useful in the treatment of aches and pains. Identification and characterization of ingredients of herbal drugs by morphological and histological characteristics along with its phytochemical and molecular signature can help in solving the problem.

Aim Of The Study

The aim of this work is the Hepatoprotective activity of ethanol extract on D.V stem in albino wister rat or the protective activity of liver on ethanol extract of D.V stem.

MATERIALS AND METHOD

Identification and Extraction of Plant Material

The stem of a healthy fresh *Desmodium velutinum* (DV) plant was identified and authenticated by a plant taxonomist Prof J.C. Okafor of the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Agbani, Enugu Slate. The sample was obtained at Umueze-Akwunanaw Town in Nkanu West Local Government Area of Enugu State at the early hours of November 12, 2017.

The stem were dried under room temperature for eighteeh (18) days, after which it was homogenized to powder first by cutting it to pieces and grounding it with an Electric grinder [10], made in France). A 130g of the grounded sample was soaked in 130ml of Ethanol for 12 hours (Batch extraction), filtered and the filtrate kept, the residue was then subjected to further extraction using in Ethanol by means of Soxhlet apparatus (continuous extraction) and filtrate is collected, together with that obtained in Batch extraction were subjected to heating (at 40°C.) in a distillatory to vaporize the Ethanol leaving only the plant extract. The resulting solid extract weighing 18.3g where divided into two portions of 6.3g and l2g and placed in separate containers (sterile) and stored at 4°C in a refrigerator. The plant extract weighing 6.3g was later used for the experimental animal model while the one weighing 12g was used for phytochemical analysis.

Phytochemical Analysis

The phytochemical test oh the solid extract was carried out based on procedures outlined by [11] and later modified by [12].

Steroid Determination

- Weigh lg of sample, macerate with 20mls of ethanol and filter.
- Pipette 2mls of the filtrate, add 2mls off color reagent, stand for 30 and measure absorbance.

Saponin Determination

- Weigh lg with 10mls of petroleum ether and decant in to a beaker.
- Add another 10mls of petroleum, decant into, a beaker combine-the filtrate andevaporate to dryness.
- Add 6 mls of ethanol, Pipette 2mls into a test tube and add 2mls of color reagent.
- Stand for 30min and measure absorbance at 550Nm.

Flavonoid Determination

- Weigh 1g of the sample,

macerate with 20mls of ethyl acetate, filter and pipette 5mls of the filtrate.
- Add 5mls of dilute ammonia slake, collect the upper layer and measure absorbance at 490Nm.

Reducing Sugar Determination
- Weigh lg of the sample macerate with20mls of distilled water and pipette lml of filtrate.
- Add lml of alkaline copper reagent, boil for 5min and cool.
- Add 1 ml of phosphomolybdic acid reagent, 7mls of distilled water and then measure absorbance at 420nM.

Alkaloid Determination
- Weigh lg of the sample, macerate with 200mls of 20% H_2S0_4 in ethanol (1:1) and filter.
- Pipette Imlof the filtrate, add 5mls of 60% H_2S0_4 and 0.5% foiTnaldehyde in 60% H_2SO_4 and mix.
- Allow to stand for 3hrs, measure absorbance at 565Nm

Terpenoid Determination
- Weigh 1g of the sample macerate with 50mls of ethanol and filter.
- Pipette 2.5mls of the filtrate, add 2.5mls of 5% aqueous phosphomolybdic acid solution and add 2.5mls conc. $H_2St)_4$ gradually mixing it.
- Allow to stand for 30min, make up to 12.5mls with ethanol and measure absorbance at 700Nm.

Glycoside Determination
- Weigh 1 g of the sample, add 2.5mls of 15% lead acetate and filter.
- Add 2.5mls of chloroform, shake vigorously, collect the lower layer and evaporate to dryness.
- Add 3mls of glacial acetic acid, add 0.1ml of 5% ferric chloride and 0.25ml conc. H_2SO_4 and shake.
- Put in the dark for 2hrs and measure absorbance at 530Nm.

Tannin Determination
- Weigh 1g of the sample, macerate with 50mls of ethanol and filter.
- Pipette 5mls of the filtrate, add 0.3ml of 0.1M ferricyanide in 0.1M HC1, add 0.3ml of 0.0005M potassium ferricyanide and measure absorbance at 720nM.

Cyanide Determination
- Weigh 1g of the sample, macerate with 50mls of distilled water, stand for 24hrs and filter.
- Pipette 1ml of the filtrate, add 4mls of alkaline picrate solution, boil for 5min, cool and measure absorbance at 490nM.

Soluble Carbohydrate Determination
- Weigh lg of the sample, macerate with 50mls of distilled water and filter.
- Pipette 1 ml of the filtrate, add 2mls of saturated picric acid and measure absorbance at 530nM.

Experimental Animal Model

Healthy male Albino wistar rats 12 in number with mean weights of 1.50±0.60kg where obtained from a Veterinary medicine practitioner at the University of Nsukka. (UNN), Enugu State. The rats were randomly distributed into four (4) groups of three (3) rats in each group. They were housed separately in a well-ventilated cage and fed with water and grower's mash (Guinea feed Nigeria) for three (3) while they acclimatized to their new environment (Applied Biochemistry Laboratory, Enugu Stale University of Science and Technology).

Group 1 (Control; recipients normal feed and water only): Starting from the fourth day the rats in Gp (1) continued to receive the normal feed for 10 days thereby serving as the control.

Group 2 (Recipients of high lipid food - Cow's brain): The rats in

Gp (2) inaddition to their normal fed received 6ml of a high lipid food containing 3.6g of the cow's brain in 500ml of distilled water forming a semi-solid mixture. The rats received the cow's brain twice daily (morning and evening) for 7 days.

Group 3 (recipients of cow's brain plus treatment with Atorvastatin): The rats in this group received the normal feed and the cow's brain like the Gp (2) rats for 7 days, after which they were now fed with normal feed and received treatment with 2ml of dissolved Atorvastatin drug for the next 3 days.

Group 4 (Recipients of cow's brain plus treatment with Ethanol extract of DV stem): Like the Gp 2 rats they received normal feed and cow's brain for 7days, before they were now feed with normal feed plus treatment with 0.5ml of the liquid Ethanol extract of DV stem for the following 3 days.

NB- The high lipid food, dissolved drug doses and the DV extract doses where oral'.} Administered through a syringe (needle part: removed).

Collection of Blood Sample

During blood sample collection all rats in every group had to be sacrificed and this was on the eleventh (11th) day except for rats in Gp (2) which were sacrificed on their eight (8) day of the experiment. Before sacrificing the rats they were first suffocated by subjecting to a mild anesthesia (with chloroform) and then dissected through the aid of a dissection set and the blood samples collected into an EDTA tube. About 5-9mls of blood samples was collected from each group.

RESULTS

The following tables gives the result of the phytochemical analysis of *Desmodium velutinum* (DV) and the liver function test done on the Albino wistar rats. Table (1) gives the qualitative phytochemical analysis done on the leaf and stem of DV using (N-hexane or ethanol or water as the medium of extraction for the leaf) and (ethanol or N-hexane as medium of extraction for the stem). Table (2) gives the result of the quantitative analysis for the phytochemicals, while Table (3) is the liver function test for the rats in Group 1 (group that were feed normal diet only), Group 2(group feed on normal diet + cow's brain only), Group 3 (group feed on normal diet + cow's brain and treatment with Atorvastatin) and Group 4 (group feed on normal feed + cow's brain and treatment with ethanol extract of DV stem.

Table 1: Qualitative Phytochemistry Result *of Desmodium velutinum*

Phytochemicals/Sample Code	A	B	C	D	E	F	G	H
Tannin	+++	++	+++	+++	+++	+++	+++	+++
Alkaloid	+++	++	+++	+++	+++	+++	+++	+++
Carbohydrate	+	+	++	++	+	+	+	+
Saponin	+	+	+	+	+	+	+	+
Steroid	+	+	+	+	+	+	+	+
Hydrogen Cyanide	+	+	+	+	+	+	+	+
Flavonoid	++	++	++	++	++	++	++	++
Reducing sugar	++	++	++	++	++	++	++	++
Terpenoid	+	+	+	+	+	+	+	+

Date =>+ - Mild concentration, ++ Moderate concentration, +++ = High concentration.

Key: A = N-hexane extract of leaf.
B = Ethanol extract of leaf.
C = Water extract of leaf.
D = Ethanol extract of stem.
E = N-hexane extract of stern.
F = Water extract of kola pod
G = N-haxane extract of kola pod
H = water extract of DV stem

Table 2: Quantitative phytochemistry result of *Desmodium velutinum*

Phytochemicals Sample Code	A	B	C	D	E	F	G	H
Tannin	2.64±0.015	2.56±0.003	2.82±0.004	2.14±0.003	3.31±0.0070	3.20±0.004	.54±0.004	2.62±0.004
Alkaloid	2.14±0.003	3.16±0.003	3.28±0.089	3.45±0.006	3.45±0.006	3.25±0.003	.62±0.003	3.76±0.004
Carbohydrate	1.43±0.004	1.91±0.004	1.43±0.003	1.92±0.003	1.49±0.003	1.66±0.003	.59±0.003	1.89±0.003
Saponin	1.45±0.002	1.22±0.004	1.05±0.003	1.34±0.004	1.43±0.004	1.10±0.003	.16±0.004	1.44±0.004
Steroid	0.69±0.004	0.24±0.005	0.63±0.004	0.64±0.004	0.55±0.007	0.62±0.004	.68±0.059	0.65±0.002
Hydrogen Cyanide	0.64±0.003	0.56±0.003	0.63±0.003	0.52±0.003	0.58±0.020	0.48±0.006	.49±0.003	0.67±0.004
Flavonoid	8.25±0.003	3.92±0.003	3.82±0.003	2.94±0.003	3.35±0.004	3.62±0.004	.68±0.004	3.67±0.004
Reducing Sugar	326.090±0.005	360.806±0.004	321.743±0.003	334.743±0.003	373.682±0.006	360.864±0.005	62.485±0.00	304.357±0.005
Terpenoid	0.737±0.002	0.45±0.004	0.28±0.005	0.37±0.002	0.39±0.003	0.38±0.003	.31±0.004	0.31±0.002

Data are means of triplicate determinations +- standard deviation (SD)
Data in the same Column bearing different superscript differed significantly ($P<0.05$)

Key: A = N-hexane extract of leaf.
B = Ethanol extract of leaf.
C = Water extract of leaf.
D = Ethanol extract of stem.
E = N-hexane extract of stern.
F = Water extract of kola pod
G = N-haxane extract of kola pod
H = water extract of DV stem

Table 3: Result of liver function test of Albino wistar rat feed with various samples

Parameters	Normal feed (GP-1)	Cow's brain (GP-2)	Lipitor (GP-3)	Ethanol extract of stem (GP-4)
Total bilirubin	0.71±0.01	0.90±-0.01	0.40±0.00	0.81±0.01
Directbilirubin	0.10±0.00	0.18±0.00	0.20±0.00	0.10±0.00
Indirect bilirubin(mg/dl)	0.60±0.00	0.70±0.00	0.50±0.21	0.7±0.00
Total serum/plasma protein(g/dl)	4.85±0.07	3.35±0.07	1.55±0.07	5.05±0.07
Albumin (mg/dl)	2.15±0.01	3.71±0.01	0.70±1.14	4.00±0.00
Globulin (g/dl)	2.40±-0.00	1.85±0.07	0.81±0.01	1.00±0.00
Serum plasma alkaline phosphate(ALP) (KA units/100ml)	3.50±0.00	5.01±0.01	1.51±0.01	3.00±0.00
Serum plasma phosphate acid phosphate(units/ml)	0.51±0.01	1.20±0.00	1.20±0.00	0.31±0.01
Creatinine phosphate kinase (units/ml)	30.00±0.00	35.00±0.00	0.41±0.01	10.45±0.07
Aspartate amino transferase (AST) (u/1)	11.00±0.00	13.00±0.00	8.75±0.07	8.01±0.00
Alanine amino transferase(ALT) (u/T)	13.91±0.01	15.00±-0.00	11.00±0.00	10.00±0.00
y-Glutamyltransferase (GGT) (u/1)	6.91±0.01	8.01±0.01	3.71±0.01	5.01±0.01
Lactate dehydrogenase (LDH) (u/ml)	2.50±0.00	3.34±0.01	1.41±0.01	1.06±0.01

DISCUSSION

From the results of the qualitative and quantitative phytochemistry of *Desmodiwn velutinum* in chapter four it is evident that DV contained in high or moderate concentration with steroid being an exception those phytochemicals known for their reputable benefits as having medicinal values (flavonoid, alkaloid, tannin, reducing sugars) and nutritive values (carbohydrate and reducing sugars) but in mild concentration those that are associated toxicity but may still be useful in medicine (hydrogen cyanide, terpenoid and saponin). In essence medicinal values of DV is just being under estimated by insufficiency in research pertaining to it. The following account of the importance of the various phytochemicals will substantiate the above claim:

Tannin (+++): This is known to be important in the breakdown of sugar aiding digestion. The high concentration of DV means that it could be important in management of digestive problems.

Alkaloid (+++): These have been known for their efficacy in combating malaria(qunine). Also effective in the treatment of high blood pressure (reserpine) etc. So DV may have any similar benefit.

Carbohydrate (++): It is metabolized for energy generation or other bodies need, so DV can be regarded as being nutritive.

Saponin (+): These are known to be associated with toxicity though it has other medicinal use, therefore DV may either have mild toxicity effect or not.

Steroid (+): This is known to be a blood cell builder, DV may not

have a blood boasting property due to the mild concentration of steroid.

Hydrogen cyanide (+): It is known to be a highly toxic substance found in cassava and can cause partial paralysis and goitre. Its mild concentration in DV means that DV could not lead to such diseases.

Flavonoids (++): these are well known for their functional role as antioxidants which remove toxic substances(free radicals) from the body, therefore the moderate concentration of flavonoid in DV could have antioxidant activity and probably the property exploited by DV extract in improving the oxidative stress that result in abnormal liver lipid metabolism induced by high lipid diet. Thus improving the altered level of liver enzymes in the blood.

Reducing sugars (++): These are sugars with aldehyde functional group which allows them to act as reducing agents. It therefore may play a complementary role as antioxidants with flavonoids in reversing the effect of oxidative stress in the hepatocytes induced by high lipid diet.

Terpenoids (+): This biological compound has been reported to be toxic as well as havingcertain medicinal values such as anti-bacterial activities, but their mild concentration in DV may not account for these properties. This finding is in agreement with the research work of [13], [14].

Now in the case of how the extract of DV stem affects the level of liver enzyme parameters as against Atorvastatin we consider the liver function test result in table 3. from the result shown in table (3) it can be observed that the group of rats treated with ethanol extract of stem had better results in the improvement of the alterations in the level of parameters of liver injury induced by cow's brain in the blood than did the group treated with *Atorvastatin* in almost all the result, the antioxidant property of DV shown in its phytochemistry may be responsible for this result. Ethanol extract of DV stem gave a better result over *Atorvastatin* by tending to bring the parameters of liver injury as close as possible to normal. The group fed with cow's brain (the initial cause of liver injury) serves as reference point, while the group that were fed only on normal feed (growers mash) served as the control. The following assessment of the liver function test result gives credit to the above claim.

NB: Group l (Gp 1) is the control which received the normal feed only, Group 2(Gp 2) the group that received cow's brain without any treatment, Group 3(Gp 3) received the cow's brain and treatment with Lipitor (*Atorvastatin*) and Group 4(Gp 4) the group that received cow's brain but treatment with Ethanol extract of DV stem, this finding is in line with the research work of [15].

CONCLUSION

The ethanol extract of *Desmodium velutinum* stem contains phytochemical with important medicinal value in high or moderate concentration. The anti-oxidant activity of some of these phytochemical (flavonoid and reducing sugars) could be what is mechanism by which ethanol extract of DV stem in improving the alteration in level of parameters that indicate liver injury in the blood of Albino wistar rats fed with high fat food(cow's brain). Therefore, at the end of this study, a strong conclusion can be deduced that, the ethanolic extract of stems of *Desmodium velutinum* possess hepatoprotective activities better than Atorvastatin when high fat diet is being consumed by Abino wistar rats.

REFERENCES

1. Anderson, K.J., Teuber, S.S., Gobeille, A., Waterhouse, L. and Steinberg, H.M. (2.011). Walnut potyphenolics inhibit *in vitro* human plasrrla and LDL oxidation. *J. Nutr.,* 131: 2837-2842.
2. Anowi, C.F., Ilodigwe, E. E., Ajaghaku, D.L. and Ezenna, C.C. (2.013). Evaluation of antidiarrhoea activity of methanolic and methylene dichloride (50:50) leaf extracts of *Desniodiium velutinum* (Fabaceae). *Herb Form,* 2: 8-12.
3. Anowi, C.F., Onyegbule, A.F., Onyekaba, E. and Azode, C. (2012.). Antipyretic and phytochemical evaluation of the ethanol extract of the leaves of *Desmodium vclutinwn. Asian J. Pharm. Life Sci.,* 2: 135-143.
4. Carithers, L.L., Lien, M., Wang, R. and Wang, J. (2012). Phytochemical analysis of medicinal plants with Liver protective activities. *Chin. J. Integr. Med.,* 18: 790-800.
5. Liu, R.H. (2014). Potential synergy of phytochemicals in cancer prevention: Mechanism of action. J. *Nutr.,* 134: 3479S-3485S.
6. Fakurazi, S., Shari fiidin, S. A. and Arulselvan, P. (2012). *Moringa otcifera* hydroethanolifc extracts effectively alleviate acetaminophen-indticed hepatotoxicity in experimental rats through their antioxidant nature. *Molecules,* 17: 8334-8350.
7. Geetha, T. S. and Geetha, N. (2014). Phytochemical screening, quantitative analysis of primary and secondary metabolites *of Desmodium spp* leaves from Kqdaikanal hills, Tamilnadu. *Int. J. Pharm. Tech. Res.,* 6: 521 - 529.
8. Gyton, E., Araya, H., Horie, T., Hayashi, M. and Awazu, S. (2008). An alteration in the liver microsomal membrane of the rat following paracetamol overdose. *J. Pharm. Pharmacol.,* 39(2): 1047-1049.
9. Habig, W.H., Pabst, M.J. and Jakoby, W.B. (2014). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.,* 24(2): 130 - 139.
10. Harbome, J. B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 1st Edn., Chapman and Hall Ltd., London, UK., pp: 185 - 186.
11. Hayes, R. E. (2009). Modern Nutrition in Health and Disease. 5th Edn., Lea & Febiger, Philadelphia, PA., USA. Pp 1153.
12. Isah, A.O., Agunu, A. and Ddnmalam, U.H. (2014). Analgesic activity of methanol leaf extract of *Desmodium velutinum* (P..Beauv.) DC (PapiliOnaceae). *Niger. J. Scient. Res.,* 13: 22-25.
13. Jayasri, M.A., Lazar, M. and Radha, A. (2009). A report on the antioxidant activity of leaves and rhizomes of *Costus pietus* D. Don. *Int. J. Integr. Bio!.,* 5: 20 - 26.
14. Kamble, G. (2.010). Antibacterial potentials of stern bark extracts of *Leptadenia lancifolia* against some pathogenic bacteria. *Pharmacologyonline*, 3: 1 72 -- 180.
15. Manolio, G., Kromhout, D., Hcrtog, P., Hollman,.C-.H., Hertog, M.G.L.-and Katan, M.B. (201 I). Dietary antioxidant flavonoids and risk of coronary liver disease: The Zutphen elderly study. *Lancet„* 32: 1007-1011.