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# The Effects of Ethanol Exract of *Desmodium velutinum* Stem on Liver Markers of Albino Wistar Rats Fed with High Fat Diet

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**Abstract:** The effect of ethanol stem extract of *Desmodium velutinum* on some liver enzymes of albino Wistar rats fed with high fat diet was analyzed. Twenty four healthy albinoWistar rats were divided into four groups of six rats each. Group I rats were fed with normal feed, group II rats were fed with 10mg/ml of high fat diet, group III rats received 10mg/ml of high fat diet + 5mg/kg of atorvastatin while group IV rats were fed with 10mg/ml of high fat diet + 5mg/kg of ethanol stem extract of *Desmodium velutinum*. The rats were sacrificed at the end of the experiments which lasted for two weeks. The results showed that ethanol stem extract of *Desmodium velutinum* possesse shepato protective activity on the fatty liver of albino Wistar rat fed with high fat diet. *Desmodium velutinum* showed variable amount of phytochemicals including flavonoids, alkaloids, saponins, tannins and terpenoids which were determined quantitatively and qualitatively using standard methods. The significant effect of *Desmodium velutinum*e than olstem extract was compared with that of standard drug atorvastatin and the result suggest that the ethanol stem extract of *Desmodium velutinum* could be used in the treatment of liver diseases.

**Key words:** *Desmodium velutinum* · High fat fed rats · Atorvastatin and Hepatoprotective activity

## INTRODUCTION

Liver is the largest and most complex internal organs of the body. It plays an important role in the maintenance of internal environment through its multiple and diverse functions [1]. Liver is involved in several vital functions such as metabolism, secretion and storage [2]. Hepatitis or inflammatory disorder involves inflammation and damage to the hepatocytes [2-4]. Hepatitis is one of the most prevalent diseases in the world. Every year 18,000 peoplesuffer liver cirrhosis caused by viral hepatitis [5- 8]. Ectopic fat storage occurs in obesity, particularly in the liver leading to a condition termed non alcoholic fatty liver disease (NAFLD) characterized by varying degree of liver injury that progresses from steatosis to tratohepatitis, fibrosis and necrosis [9]. Due to its prominent association with insulin resistance/obesity, NAFLD is regarded as the hepatic manifestation of metabolic syndrome. Liver has great capacity to detoxicate toxic substances and at the same time synthesize useful principles [10]. Therefore damage to liver inflicted by hepatotoxic agents is of grave consequences [11-13]. Experimental studies have reported that animal fed with high fat diet (HFD) for more than two months develops weight, hyperlipidemic, hyperglycemia, oxidative stress and insulin resistance (IR). Besides, consumption of a calorie-rich diet results in lipid accumulation, excess production of inflammatory cytokines and macrophage infiltration that favours the progression of liver disease. Many medicinal plant/indigenous plant have been mentioned and well established as hepatoprotective agents [14- 17].

*Desmodium velutinum* is a medicinal plant that is used in the treatment of many diseases. The extract of *Desmodium velutinum* showed significant anti-pyretic activity on experimental rats. *Desmodium velutinum* is very rich in alkaloids and related amino compounds. It is a source of flavonoids, saponins and pharmacological

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active agent used in the treatment of aches and pains. *Desmodium velutinum* being one of the important medicinal plants could have ameliorative effect on the liver disorders [18- 20].

## MATERIAL AND METHODS

**Identification and Extraction of *Desmodium velutinum* Plant (STEM):** Fresh stem of *Desmodium velutinum* were obtained from Umueze Awkunanaw at Nkanu-West Local Government of Enugu State. The plant was authenticated by Prof. J.C. Okafor, a plant taxonomist with Biotechnology Department, Enugu State University of Science and Technology Enugu in the month of February 2013. The stem was dried at room temperature for eighteen (18) days. The dried stem was ground into fine powder with the aid of a clean dry electric grinder. 130g of the ground stem was soaked in 130ml of ethanol for 24h, filtered and then extracted with ethanol by hot-continuous percolation method in a Soxhlet apparatus. The ethanol extract was concentrated with the aid of a rotary evaporator. The concentrated solid extract was weighed and placed in a sterile container labeled and stored at 4°C in a refrigerator. The phytochemical analysis on the solid extract was carried out based on procedures outlined by Wang*et al.* [21].

**Experimental Animal Model:** Twenty four apparently healthy albino Wistar rats with mean weight of 1.50 ± 0.60kg were obtained locally from Nsukka, Enugu State, Nigeria. The rats were housed separately and fed with water and growers mash (Guinea feed Nigeria) and allowed for 3days acclimatization. Group I rats were fed with normal feed, group II rats were fed with 10mg/ml of high fat diet, group III rats received 10mg/ml of high fat diet + 5mg/kg of atorvastatin while group IV rats were fed with 10mg/ml of high fat diet + 5mg/kg of ethanol stem extract of *Desmodium velutinum*.

**Collection of Blood Samples:** The collection of blood samples from the rats was simply done by dissection of the rats, followed by cardiac puncture after a mild anesthesia with chloroform. About 5-9mls of blood sample were collected in the EDTA tube from each group using a medical syringe. Serum was separated from the blood after clotting by centrifugation and then used for analysis.

Blood samples were collected from the rats in groups I and II on 7$^{th}$ day of oral feeding of the rats with high fat from cow's brain and normal feed (Grower's mash and water) respectively. Blood sample were collected from the rats in groups III and IV on day 3 of oral administration of a known drug (atorvastatin; Lipitor) and the liquid extract (Ethanol extract of *Desmodium velutinum* stem mixture) respectively.

**Determination of Liver Markers:** Liver markers of total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, aspartate amino transferase and alanine amino transferase were assayed according to the authors Jendrassik and Grof (1938); Reitman and Frankel (1957) and Klein *et al.* (1960) as outlined in the Radox kit.

## RESULTS AND DISCUSSION

Liver is the key organ in the metabolism, detoxification and secretory functions of the body, its disorders are numerous with no effective remedies however, the search for new medicines isstill on [22]. Many folk remedies from plant origin have been long used for treatment of liver diseases. Management of liver disease is still a challenge to the modern medicine.

Table 1: Quantitative analyses of the ethanol stem extract of *Desmodium velutinum* showing the phytochemical composition of sample (mg/100g)

| Phytochemical samples | Quantitative composition (mg/100g) |
|---|---|
| Soluble carbohydrate | 1.90±0.003 |
| Cyanide | 0.52±0.003 |
| Reducing sugar | 3.34±0.003 |
| Saponins | 1.34±0.004 |
| Tannins | 2.14±0.003 |
| Flavonoids | 2.94±0.003 |
| Alkaloids | 3.45±0.006 |
| Steroids | 0.64±0.004 |
| Terpenoids | 0.37±0.002 |

Table 2: Qualitative phytochemical analyses of ethanol stem extract of *Desmodium velutinum*

| Phytochemical samples | Qualitative analysis of the samples |
|---|---|
| Tannins | ++ |
| Alkaloids | +++ |
| Carbohydrates | ++ |
| Saponins | + |
| Steroids | + |
| Hydrogen cyanide | + |
| Flavonoids | ++ |
| Reducing sugar | +++ |
| Terpenoids | + |

Keys

+++ = Relative High Abundance of Compound

++ = Moderate Abundance of Compound

+ = Relative low Presence of Compound

- = Not Detected.

Table 3: Liver function tests of rats feed with various samples

| Parameters | Group 1Rats fed with normal feed | Group2 Rats fed with 10mg/ml of high fat diet | Group 3 Rats fed with 10mg/ml of high fat diet +5mg/kg of atorvastatin | Group 4 Rats fed with 10mg/ml of high fat diet+5mg/kg of ethanol stem extract of *Desmodium velutinum* |
|---|---|---|---|---|
| Total bilirubin (mg/dl) | 0.71±0.0l | 0.91±0.10 | 0.40±0.00 | 0.61±0.01 |
| Direct bilirubin (mg/dl) | 0.08±0.00 | 0.11±0.00 | 0.09±0.00 | 0.08±0.00 |
| Indirect bilirubin (mg/dl) | 0.60±0.00 | 0.70±0.00 | 0.50±0.21 | 0.40±0.00 |
| Serum plasma Alkaline phosphate (ALP) (units/100ml) | 3.50±0.00 | 5.01±0.01 | 1.51±0.01 | 3.00±0.00 |
| Aspartate amino Transferase (ALT) (u/l) | 11.00±0.00 | 13.00±0.00 | 8.75±0.07 | 8.01±0.00 |
| Alanine amino Transferase (ALT) (u/l) | 13.91±0.00 | 15.00±0.00 | 10.71±0.00 | 11.00±0.01 |

Results as mean ± standard deviation;$p<0.05$ as significant and$p>0.05$ as non-significant and n=6.

In Ayurveda, various herbal and herbomineral preparations are extensively used for the treatment of various liver disorders [23]. Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP which are enzymes originally present in higher concentration in cytoplasm. An elevation in the levels of serum maker enzymes is generally regarded as the most sensitive index of the hepatic damage. The elevation of ALP in liver tissue of high-fat fed rat indicates the disturbed excretory function of liver. As we know, the enzymes ALT and AST are present in the hepatic and biliary cells [24, 25]. Observed elevated level of these enzymes in serum and liver tissue of high fat from cow's brain fed to the albinoWistar rats indicate that these elevations could be due to hepatocytes usually released into circulation thereby increasing their serum levels under hepatocellular injury or inflammation of the biliary tract cells. In table three liver markers of AST, ALP and ALT of albino wistar rats fed with high fat diet in group 2 increased significantly ($p>0.05$) when compared with that of groups 3 and 4 rats that were treated with standard drug atorvastatin and ethanol stem extract of *Desmodium velutinum respectively*.

Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary diseases and severe disturbance of hepatocellular function [26, 27]. In the present investigation, the rats fed with high fat diet significantly increased levels of bilirubin as compared to control rats (Group 1). This trend of result collaborated with the statement that induced hepatitis is characterized by increased levels of bilirubin in serum [28]. In table 2, which is the qualitative analysis of the phytochemistry, tannins, alkaloids, flavonoids, saponins and terpenoids were found to be present. Some of these phytochemicals are antioxidants which fight against unstable molecules (Free radicals) in the body system. Saponinsreduce blood cholesterol by preventing its re-absorption and also flavonoids which has protective power against liver diseases reduces cholesterol in the body system [5, 8,10]. Findings on the rats fed with atorvastatin and ethanol stem extract of *Desmodium velutinum* in separately mixing with high fat diet showed the appreciable normal level of enzyme profile of (ALT,AST and ALP) and total protein in serum and liver tissue as well as bilirubin in serum of albino wistar rats.

The result on atorvastatin group indicated that atorvastatin was effective in the treatment of liver fibrosis induced in rats by either high fat diet administration or bile duct ligation indicated that atorvastatin inhibit both hepatic inflammation, collagen synthesis in the liver. In this study the atorvastatin treated group (In table 3) with great prevention on the alteration of biochemical changes which indicates that atorvastatin act as a hepato-protector from high-fat toxicity in rat, research recently points to an unfavourable effect of atorvastatin to cause insulin resistances ambient glycemia in hypercholesterolemic patients. Further, the atorvastatin therapy is associated with a decrease in cardiac outcomes, including recurrent heart attack [22, 23]. The present investigation showed that *Desmodium velutinum* ethanol

extract of the stem act as an important mediator of enzyme resistance in high fat diet induced obesity, through its ability to decrease the elevated activity of ALT,ALPand AST at the cellular level.

## CONCLUSION

In conclusion, *Desmodium velutinum* ethanol stem extract has been shown to be a potential hepatoprotective drug as indicated by its ameliorative effects on the liver damages due to high fat fed diets.

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