

Evaluation of some Liver Markers and Lipid Peroxidation of Triton-X100-Fat-Induced Albino Rats Exposed to Water Extract of *Desmodium velutinum* Stem

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ABSTRACT

This study investigated some Liver Markers and Lipid Peroxidation of Triton-X100-Fat-Induced Albino Rats Exposed to Water Extract of *Desmodium velutinum* Stem. A total of thirty (30) male albino rats weighing between 180g and 200g were obtained and randomized into six (6) groups of five (5) rats each. Group A was the negative control group and the rats were fed with grower's mash and water for 3 days, groups B-F were fed with triton-X100 solution only on the first day to make them hyperlipidemic. Then, group B rats were also fed with grower's mash and water for 3 days, but, without treatment. Group C rats were fed with grower's mash and water and were orally administered simvastatin drug for 3 days. Group D rats were fed with grower's mash and water and were orally administered 50mg/kg of aqueous extract of *D. velutinum* for 3 days. Group E rats were fed with grower's mash and water and were orally administered 100mg/kg of aqueous extract of *D. velutinum* for 3 days and Group F rats were fed with grower's mash and water and were orally administered 200mg/kg of aqueous extract of *D. velutinum* for 3 days. At the end of the 3 days feeding period, the blood was collected by cardiac puncture after mild anaesthesia with chloroform, and the biochemical parameters were evaluated using standard methods. Statistical analysis of the results obtained were performed by using ANOVA tests to determine if significant difference exists between the mean of the test and the control group. The limit of significance was set at $p < 0.05$. The results showed that in liver function test, *D. velutinum* did not increase the liver enzymes. Hence, its consumption could not have adverse effect on the liver. This result showed that there was a significant increase ($p < 0.05$) in liver markers in hyperlipidemic untreated groups compared to normal control. Also, there was a significant decrease ($p < 0.05$) of hyperlipidemic treated with 200mg of *D. velutinum* compared to hyperlipidemic untreated.

Keywords: Liver Markers, Lipid Peroxidation, Triton-X100, Albino rats and *Desmodium velutinum* Stem.

INTRODUCTION

A medicinal plant is any plant which in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs [1,2,3,4]. Plant contains a large number of bioactive phytochemicals that are responsible for pharmacological action of plants and used for development of drugs [5, 6, 7, 8]. Man has used plant-based drugs for health care delivery over the centuries [9, 10, 11]. Disease remedies from plant sources for mankind are as old as human history and are still in use to date [12, 13, 14]. It is estimated that about

75% of useful bioactive plant derived pharmaceuticals used globally are discovered by systemic investigation [15, 16, 17]. Traditional medicine is defined by the World Health Organization as "the total combination of methods and practice, whether explicable or inexplicable, used in the diagnosis, prevention or elimination of mental, physical or even social diseases [18,19]. The use of traditional medicine in various therapies by indigenous populations all over the world cannot be over emphasized as 80% of the world's population depends on it [20]. With the

poor economic status of many underdeveloped countries, this form of medical practice is worthy of consideration. This is because it is cheaper, more affordable, more acceptable and easily accessible when compared to orthodox form [20]. However, it has the following challenges in the form of criticisms, shortcomings and inadequacies leveled against traditional medicine such as bad record keeping as well as its vulnerability to medical error in the areas of diagnosis and prescription as stated by the orthodox practitioners [21]. *Desmodium velutinum* is a semi-woody erect shrub of 3m high, of savanna

woodland and old clearings throughout the region from Senegal to Nigeria, and from Cameroun to Zaire and Angola; commonly in the Asian and American tropics. *Desmodium velutinum* has been reported in traditional medicine to have medicinal properties. One of the medicinal uses is in the treatment of fever. *Desmodium velutinum* is also used in the treatment/ management of abdominal pain and general body pain. *Desmodium velutinum* serves many other therapeutic purposes such as antidiarrhea, anti-inflammatory, anti-nephrolithic, and anti-bacteria. Its leaf extract has been reported to possess anti-diabetic properties.

AIM AND OBJECTIVES OF THE STUDY

The aim of this research was to investigate the effects some Liver Markers and Lipid Peroxidation of Triton-X100-Fat-Induced

Albino rats exposed to water extract of *Desmodium velutinum* stem.

SPECIFIC OBJECTIVES OF THE STUDY

- To determine the effect of the water extract of *Desmodium velutinum* stem on some liver marker enzymes (ALP, AST, ALT).

- To determine the effect of the water extract of *Desmodium velutinum* stem on lipid peroxidation (MDA).

SIGNIFICANCE OF THE STUDY

The result from this research work may help to widen the people's knowledge on

traditional medicine and their possible efficacies over synthetic drugs.



Figure 1: Image of *D. velutinum* plant (locally called “Ikeagwuana”)

MATERIALS AND METHODS

PLANT MATERIAL-COLLECTION AND IDENTIFICATION

The plant sample was collected by the herbalist who uses it for pain remedy. The plant was thereafter prepared according to standard procedures of herbarium specimen preparation and preservation as follows; clean cut of the stem was made

using a matchet, unnecessary twiggy shoots was cut away, the sample was cut into pieces and dried quickly under room temperature and the powdered sample was obtained.

METHODOLOGY

One gram (1g) of sample was weighed and transferred in a test tube and 15ml ethanol and 10ml of 50%*m/v* potassium hydroxide were added. The test tube was allowed to react in a water bath at 60°C for 60mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot

water and 3ml of hexane, which was all transferred to the funnel. This extracts were combined and washed three times with 10ml of 10%*v/v* ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200ul was transferred to a vial for analysis.

EXTRACTION OF *D. VELUTINUM* USING WATER

Seventy (70) grams of powdered *D. velutinum* was soaked in 500ml of distilled

water and allowed to stand for 48 hours. It was stirred at 2hours interval to achieve

complete extraction. After 48 hours, it was sieved using muslin cloth and afterwards filtered using whatmann no1 filter paper.

Biological yield of the extract= $\frac{\text{weight of extract}}{\text{Weight of extraction}}$

$$\frac{5.73}{70} \times \frac{100}{1} = 8.2\%$$

The filtrate was concentrated using waterbath set at 70°C. After concentration, the weight of the extract was 5.73g.

EXPERIMENTAL ANIMAL MODEL

A total of 30 healthy albino Wister rats with mean weight of 1.93kg were obtained from Chris's experimental animal farm and research laboratory, Awka, Anambra state. They were randomized into six (6) groups of five (5) rats each and were housed separately. They were fasted for 18hours after which they were fed with grower's mash and water. A solution of triton-X100(Polyethylene Glycol Octyl Phenyl Ether which is a non-ionic surfactant, or detergent which dissolve lipid to increase the permeability of cell membrane to antibody) was made by mixing 1ml of 100% triton-X100 and 9ml of distilled water. A known antilipidemic drug: simvastatin (brand name- Zocor, 10mg) was prepared by dissolving 5mg (half of one tablet) in 2ml of distilled water. Also, Desmodium velutinum stem water extract weighing 5.73g was dissolved in 15ml of distilled water forming a liquid drug extract.

Group I rats were fed orally with only grower's mash and water for three (3) days. Group II rats were fed orally with Triton-X100 solution on the first day only. Then, with growers mash and water, morning and night for the three (3) days. Group III rats were fed orally with Triton-X100 solution

on the first day only. Then, with growers mash and water, morning and night for three (3) days, and were also, orally administered the simvastatin drug solution, once a day for the same three (3) days.

Group IV rats were fed orally with Triton-X100 solution on the first day only. Then, with growers mash and water, morning and night for three(3) days, and were also, orally administered 50mg/kg aqueous extract of *D. velutinum*, once a day for the same three(3) days. Group V rats were fed orally with Triton-X100 solution on the first day only. Then, with grower's mash and water, morning and night for three (3) days, and were also, orally administered 100mg/kg aqueous extract of *D. velutinum*, once a day for the same three (3) days. Group VI rats were fed orally with Triton-X100 solution on the first day only. Then, with grower's mash and water, morning and night for three (3) days, and were also, orally administered 200mg/kg aqueous extract of *D. velutinum*, once a day for the same three (3) days. All the administrations were done orally with the use of an oral intubation tube.

Nutritional value of the grower's mash (Guinea feed Nigeria)

Metabolizable Energy (ME).....	2800kcal/kg (min)
Crude protein (CP).....	18.00% (min)
Crude fat.....	5.00% (max)
Crude fiber (CF).....	8.00% (max)
Calcium.....	1.00% (min)
Phosphorous.....	0.40% (min)
Lysine.....	1.00% (min)
Methionine.....	0.45% (min)
Methionine + Cysteine.....	0.80% (min)
Threonine.....	0.60% (min)
Tryptophan.....	0.20% (min)

COLLECTION OF BLOOD SAMPLES

The collection of blood samples from the rats in each group was done on the third day of the experiment, at the early hours of the day, immediately after feeding the

animals. This was done by cardiac punctures after mild anesthesia with chloroform. About 4-6mls of blood of different rats were collected using insulin

syringes, and deposited differently in labeled EDTA and other tubes. The collected blood samples were centrifuged

at 2000rpm for 30minutes to get the serum and plasma.

BIOCHEMICAL ANALYSIS

LIVER FUNCTION TEST

Serum biochemical indices routinely estimated for liver functions were analysed. They include: alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline

phosphatase (ALP), direct and total bilirubin. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacture's instruction.

LIPID PEROXIDATION

Lipid peroxidation was determined by the thiobarbuturic acid-reacting substances (TBARS) assay method. The reaction depends on the formation of complex between melondialdehyde (MDA) and thiobarbituric acid (TBA). 0.4ml of the serum was collected into the test tubes, 1.6ml of 0.25N HCl was added together with 0.5ml of 15% trichloroacetic acid 0.5ml of 0.375% of thiobarbituric acid and then mixed thoroughly.

The reaction mixture was then placed in 100^oc boiling water for 15 minutes, allowed to cool and centrifuged at 3000rpm for 10 minutes. The supernatant was collected and the optical density recorded at 532nm against reagent blank containing distilled water.

The lipid peroxide activity was calculated using the formular:

$$\frac{\text{Optical density}}{\text{Time}} \times \frac{\text{extinction coefficient}}{\text{amount of sample}}$$

Where the extinction coefficient value is $1.56 \times 10^{-5} \text{M}^{-1} \text{CM}^{-1}$

Optical density= 532nm, time= 15mins, amount of sample= 15mg.

The unit is expressed as $\mu\text{mol}/\text{MDA}/\text{mg}$ of serum.

RESULTS

RESULT OF LIVER FUNCTION TEST

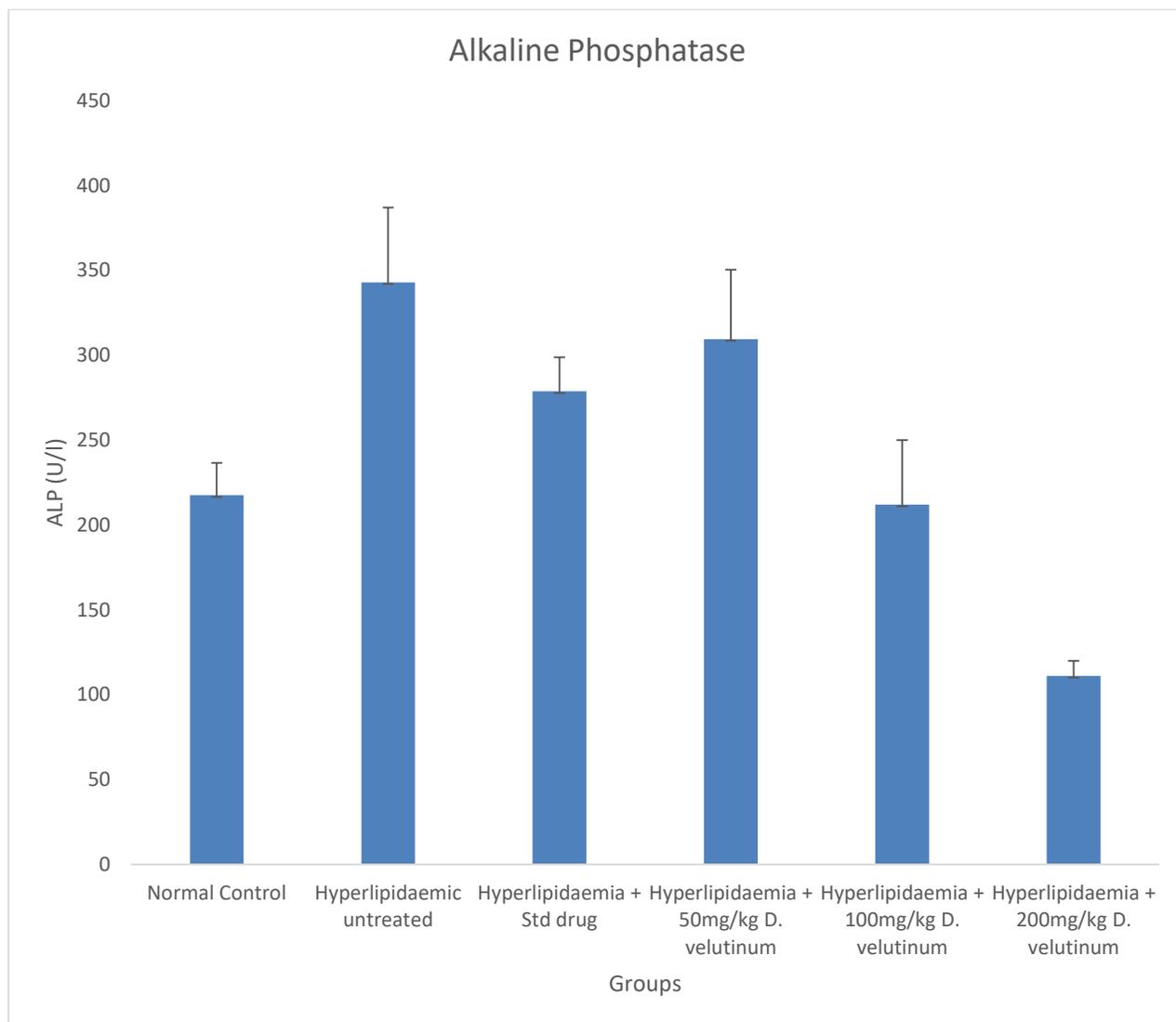


Figure 2: Effect of aqueous extract of *D. velutinum* on the alkaline phosphatase of triton x100-induced hyperlipidaemic rats.

This result showed that there was a significant increase ($p < 0.05$) of alkaline phosphatase in hyperlipidemic untreated animals compared to normal control. Also,

there is a significant decrease ($p < 0.05$) of hyperlipidemic treated with 200mg of *D. velutinum* compared to hyperlipidemic untreated.

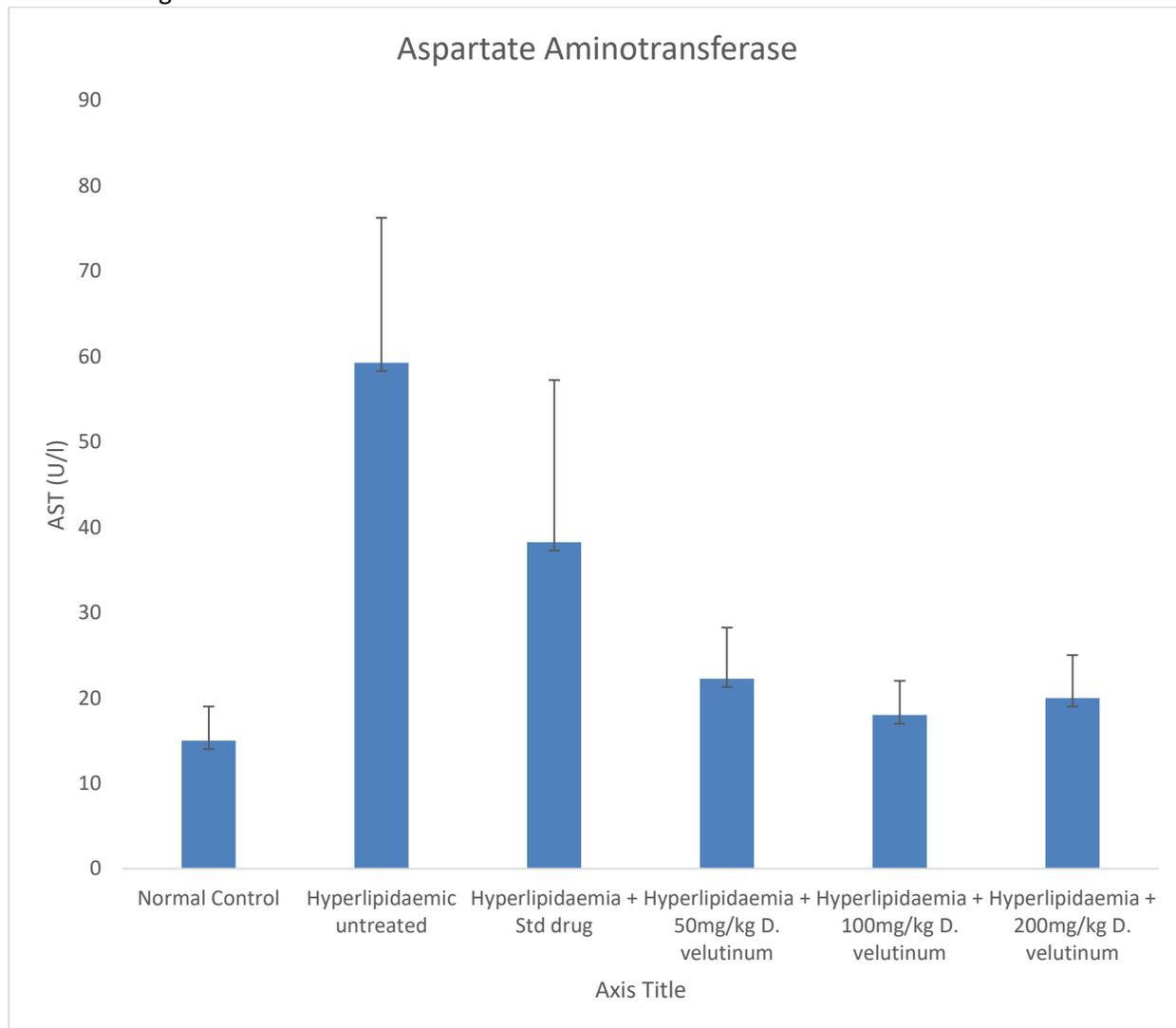


Figure 3: Effect of aqueous extract of *D. velutinum* on the aspartate aminotransferase of triton x100-induced hyperlipidaemic rats.

This result showed that there was a significant increase ($p < 0.05$) of aspartate aminotransferase in hyperlipidemic untreated animals compared to normal control. Also, there was a significant decrease ($p < 0.05$) of hyperlipidemic

treated with 50mg of *D. velutinum*, hyperlipidemic treated with 100mg of *D. velutinum* and hyperlipidemic treated with 200mg of *D. velutinum* compared to hyperlipidemic untreated.

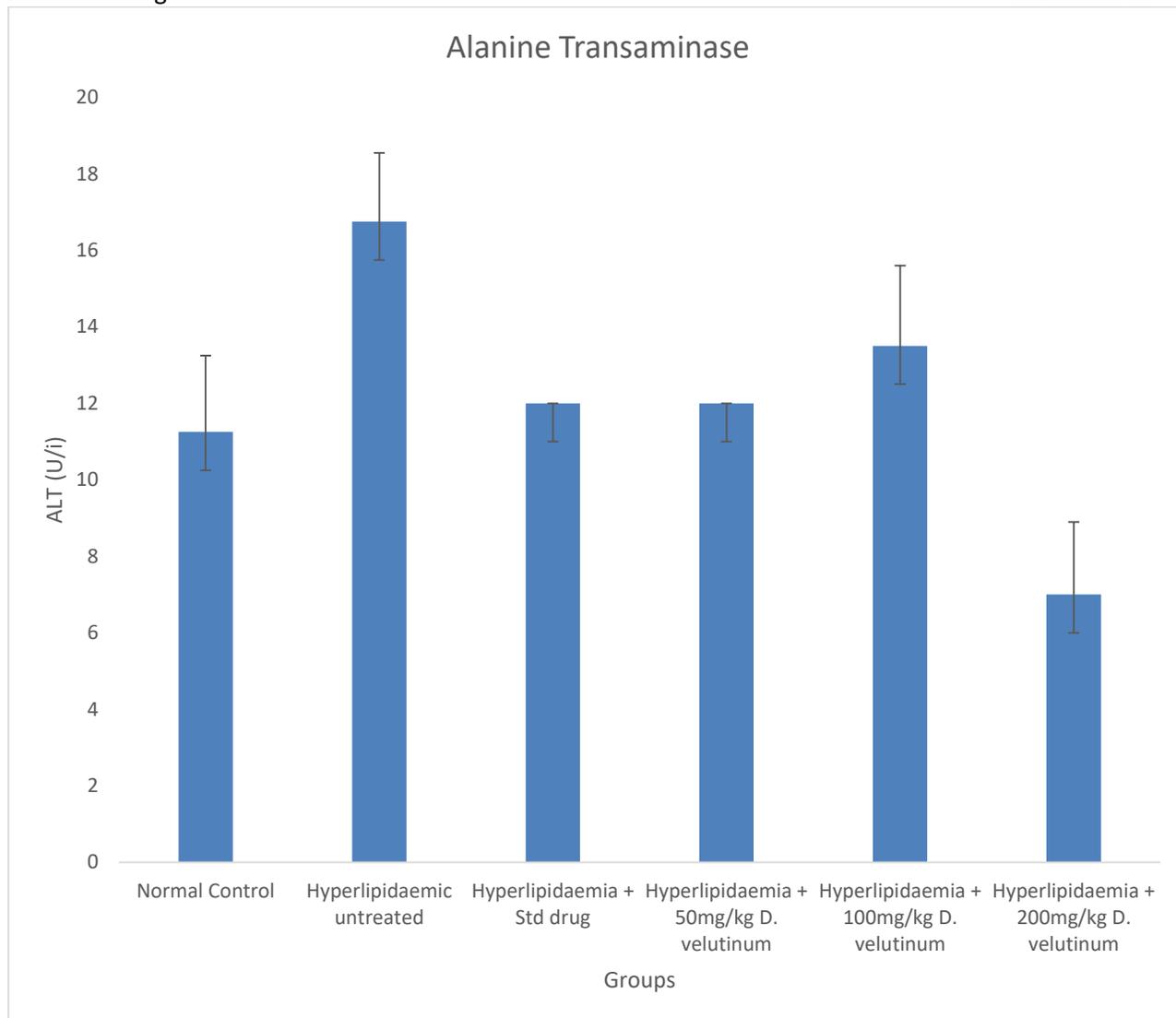


Figure 4: Effect of aqueous extract of *D. velutinum* on alanine transaminase of triton x100-induced hyperlipidaemic rats.

This result showed that there was a significant increase ($p < 0.05$) of alanine transaminase in hyperlipidemic untreated animals compared to normal control. Also,

there was a significant decrease ($p < 0.05$) of hyperlipidemic animals treated with 200mg of *D. velutinum* compared to hyperlipidemic untreated.

RESULT OF LIPID PEROXIDATION

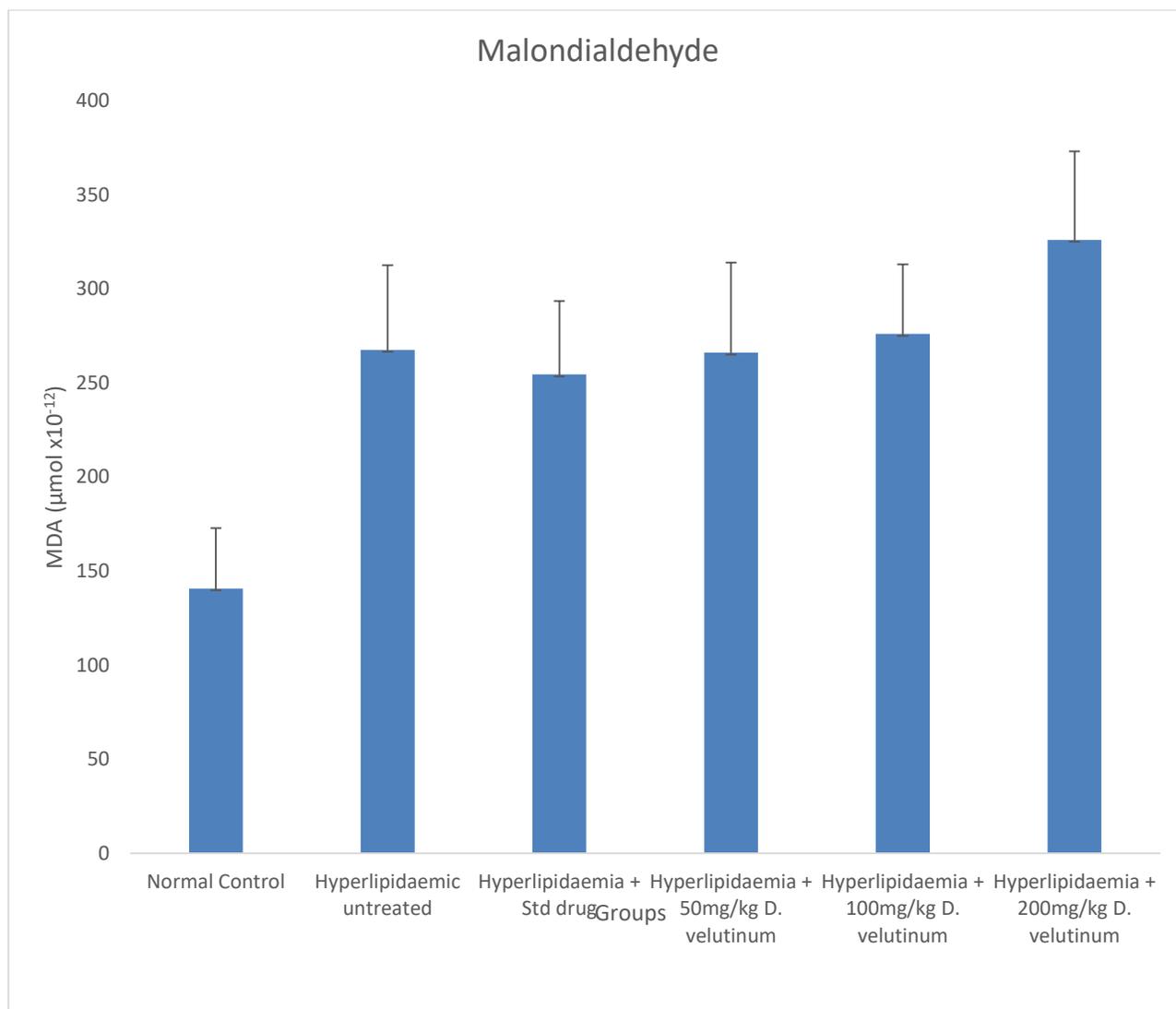


Figure 5: Effect of aqueous extract of *D. velutinum* on malondialdehyde of triton x100-induced hyperlipidaemic rats.

This result showed that there was a significant increase ($p < 0.05$) of malondialdehyde in hyperlipidemic untreated animals, hyperlipidemic treated

with 50mg of *D. velutinum*, hyperlipidemic treated with 100mg of *D. velutinum* and hyperlipidemic treated with 200mg of *D. velutinum* compared to normal control.

DISCUSSION

This research was carried out to evaluate the effects some Liver Markers and Lipid Peroxidation of Triton-X100-Fat-Induced Albino rats exposed to water extract of *Desmodium velutinum* stem. In the normal control group, there was no increase in the concentration of alkaline phosphatase

(ALP), aspartate transferase (AST) and alanine transaminase (ALT) in the blood even after the induction of *D. velutinum*. In hyperlipidemic untreated group, there was a significant increase ($p < 0.05$) of ALP, AST and ALT in the blood with respect to normal control. There was no increase or

decrease of ALP, AST and ALT in hyperlipidemic animals treated with standard drug. In hyperlipidemic treated with 50mg of *D. velutinum*, there was a significant decrease ($p < 0.05$) of AST with respect to hyperlipidemic untreated. In hyperlipidemic treated with 100mg of *D. velutinum*, there was a significant decrease ($p < 0.05$) of ALP and AST with respect to hyperlipidemic untreated. In hyperlipidemic treated with 200mg of *D. velutinum*, there was a significant decrease

of AST and ALT with respect to hyperlipidemic untreated. There was a significant increase ($p < 0.05$) of malondialdehyde in hyperlipidemic untreated group, hyperlipidemic treated with 50mg of *D. velutinum*, hyperlipidemic treated with 100mg of *D. velutinum* and hyperlipidemic treated with 200mg of *D. velutinum*. This showed that increase in lipids increased the level of MDA in the blood and *D. velutinum* did not exert any positive effect in controlling it.

CONCLUSION

D. velutinum is a potential medicinal plant that can be used for therapeutic purposes, or as a precursor for the synthesis of useful drugs. In the liver function test, the result showed no increase in the level of the liver enzymes after induction of *D. velutinum*. This shows that *D. velutinum* did not attack the kidneys and liver, and could be useful in the treatment or regulation of any disease associated to these organs. In lipid peroxidation,

induction of hyperlipidemia increased the level of malondialdehyde (MDA) in the blood. The increase of free radicals causes over production of MDA, which is a marker for oxidative stress and the antioxidant status in cancerous patients. *D. velutinum* showed no significant effect in altering the production of free radicals, and hence could not be used in regulating the production of MDA.

RECOMMENDATIONS

Desmodium velutinum is recommended to herbalists who use medicinal plants for remedies of various diseases. Researchers

should carry out more investigations to find out other health benefits of *Desmodium velutinum*.

CONTRIBUTION TO KNOWLEDGE

D. velutinum did not increase the liver enzymes. Hence, its consumption may

have no adverse effect on the liver and kidney.

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