

Valley International Journals

Open Access Journal

New Thinking New Innovation

International Journal of Medical Science and Clinical Inventions

Volume 1 issue 6 2014 page no. 345-351 ISSN: 2348-991X Available Online At: http://valleyinternational.net/index.php/our-jou/ijmsci

Phytochemical Compositions, Antihyperlipidemic And Hepatoprotective Effects Of *Brassica Oleracae* Var. *Capitata L.* Leaf Extracts On Triton-Induced Hyperlipidemic Rats.

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Abstract: The antihyperlipidemic and hepatoprotective effect of *Brassica oleracae* var. *capitata L.* were investigated on triton-induced hyperlipidemic rats. Standard procedures were used to determine the phytochemical constituents. Hyperlipidemia was induced in albino rats by administration of a single dose of freshly prepared Triton X-100 (100mg/kg, i.p) after an overnight fast of 18 hours. The result of the phytochemical analysis showed appreciable level of Alkaloids (95.53±1.01mg/100g), Phenols (166.83±2.21mg/100g), Flavonoids (38.02±1.60mg/100g), Saponins (33.80±0.48mg/100g), and low level of Tannins (2.84±0.60mg/100g). The administration of Triton X-100 caused significant (p<0.05) increase in the level of serum total cholesterol (TC), Triglycerides (TG), Low density lipoprotein cholesterol (LDL-c), very low density lipoprotein cholesterol (VDL-cL) respectively and significantly (p<0.05) decrease High density lipoprotein cholesterol (HDL-c) level compared with normal control group. However, treatment of hyperlipidemic rats with Methanolic extract and Petroleum ether extract at 100mg/kg, 300mg/kg and 500mg/kg body weight of animal caused significant (p<0.05) decrease in the levels of serum TC, TG, LDL-c, VLDL-c respectively and significant (p<0.05) increase in HDL-c level at a dose dependent manner compared with the hyperlipidemic control. Methanolic extract and Petroleum ether extract at 500mg/kg body weight were observed to elicit the highest hypolipidemic effect on serum lipid profile by decreasing the levels of TC by 41.3% and 40.9%, TG by 56.7% and 48.7%, LDL-c by 44% and 48.5%, VLDL-c by 56.7% and 48.7% respectively, and increases the level of HDL-c by 115% and 94.5%. The extracts (in a dose dependent manner) also exhibited correction of altered biochemical parameters-SGOT, SGPT and Alkaline phosphatase. Treatment with Methanolic and Petroleum ether extracts at 500mg/kg body weight exhibited quite competitive potential and generally elicited comparable effects when compared with the reference drug Atorvastatin (10mg/kg body weight) which indicates that Brassica oleracae var. Capitata L. extracts could be explored as alternative therapeutic agent in the treatment of hyperlipidemia with favourable hepatoprotective activity.

Keywords: *Brassica oleracae*, Hyperlipidemia, Cholesterol, Triglycerides, HDL-c, LDL-c, VLDL-c, Triton X-100.

I. INTRODUCTION

One of the most significant discoveries in preventive medicine is that elevated levels of lipids in the blood accelerate atherosclerosis. Along with high blood pressure, inactivity, smoking, and diabetes, high lipids had proven to be one of the most important promoters of heart disease, strokes, and peripheral vascular disease [1]. Hyperlipidemia is a metabolic disorder specifically characterized by elevated serum total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and decreased high density lipoprotein levels [2].

Evidence from epidemiological and population studies showed that hyperlipidemia is the etiological factor in the development of coronary heart disease, and potentiating of arteriosclerosis [3]. Recent studies have however reported that increased High density Lipoprotein (HDL) appears to retard or prevent the development of arteriosclerosis while reduced levels are associated with increased risk of coronary artery diseases; also blood lipid levels, particularly total cholesterol, triglyceride and Low density lipoprotein cholesterol (LDL-c) are usually related to promoting atherosclerosis [4], hence interventions that lower these lipid levels can retard or reverse the progression of the processes.

Numerous traditional medicinal plants are being tested presently for their therapeutic efficacy. Brassica oleracae var. capitata L. has been used in ancient times both as food and as medicine [5]. Recent studies on the extract and juice showed its biological activities such as anti-inflammatory, antioxidant, anti-cancer, cytotoxic and antitumor [6,], anti-HPV, wound healing, cardioprotective, antimicrobial, antifungal and antitrypanonsomal [7, 8]. These properties have been attributed to the substantial amounts of various bioactive compounds in the vegetable. antihyperlipidemic and hepatoprotective effects of Brassica oleracae var. capitata L. leaf extracts on hyperlipidemic rats were investigated in order to source for alternative ethnolipid-associated for disorders medicine hepatoprotective capability and also serve as a useful tool for health and pharmaceutical industries for consideration in drug formulation.

II. MATERIALS AND METHODS

- A. Sample preparation: Fresh samples of Brassica oleracae var. capitata L. were purchased from main market Minna, Niger State and verified at the department of Biological Sciences, Federal University of Technology, Minna, Nigeria. The samples were washed with clean water to remove dirt and other contaminants, sliced into pieces, dried at room temperature for four weeks, ground into powder and stored in an air tight container for further analysis.
- B. Experimental animals: Young adult Swiss albino rats weighing between 180g -200g were used for this study. The rats were obtained from the animal house of Ibrahim Badamasi Babangida University, Lapai, Niger State and transported to the research site. The animals were grouped and housed in cages and maintained under standard laboratory conditions (temperature: $25\pm2^{\circ}\text{C}$) with light and dark cycle (12/12 hours). They were allowed free access to standard dry pellet diet and water ad libitum. The rats were acclimatized to laboratory condition for 2 weeks before commencement of experiment.
- C. Drugs and chemicals used: Total cholesterol, triglyceride, HDL-c, SGOT, SGPT and Alkaline phosphatase wet reagent kits were the products of AGAPPE Diagnostics, Switzerland; Triton X-100 from Sigma chemical Co, London. Atorvastatin was purchased from Pan Lac pharmacy, Minna, Nigeria; Carboxyl methyl cellulose was purchase from Dona Hills Chemical, Minna Nigeria. Other chemicals used were of BDH analytical grade products.
- D. Preparation of extracts: 250g of powdered plant sample was extracted with 750ml methanol and 750ml petroleum ether in separate flat bottom flask using the simple maceration procedure [9]. The containers were sealed with cotton plug and aluminium foil, and kept at room temperature with occasional agitation. The solvents were carefully decanted every 48 hours and fresh solvents introduced for a period of 10 days until soluble matter has been dissolved. After extraction, the samples were filtered with doubled layer cotton cloth and thereafter with Whatman 1 filter paper, and the whole filtrate evaporated to dryness under reduced temperature (40°C) by rotary

evaporator to obtain the Methanolic extract (ME) and petroleum ether extracts (PE) of the plant.

- E. Experimental design and induction of hyperlipidemia: Hyperlipidemia was induced in Wister albino rats by a single intra-peritoneal (i.p) injection of freshly prepared solution of Triton X-100 (100mg/kg body weight) in physiological saline solution after an overnight fasting for 18 hours [10]. The animals were randomly divided into nine (9) groups comprising of five (5) animals each. The first group (group I) was given standard pellet diet, water and orally administered with 5% Carboxyl methyl cellulose (CMC) and serves as the normal control "N-C". The II, III, IV, V, VI, VII, VIII, and IX group animals were injected intra-peritoneally (i.p) with 10% aqueous solution of Triton X-100. After 72 hours of triton injection, the second group received a daily dose of 5% Carboxyl methyl cellulose (CMC) per orally (p.o) for 7 days, and served as the hyperlipidemic control "H-C". The third, fourth and fifth group were administered a daily dose of ME 500mg/kg bw, 300mg/kg bw and 100 mg/kg bw suspended in 5% CMC, p.o., for 7 days, after 72 hours of inducing hyperlipidemia. The sixth, seventh and eighth group were administered a daily dose of PE 500mg/kg bw, 300mg/kg bw and 100 mg/kg bw suspended in 5% CMC p.o., for 7 days after inducing hyperlipidemia. The ninth group was administered with the standard hyperlipidemic drug Atorvastatin "Ator" at 10mg/kg bw p.o. for 7 days after inducing hyperlipidemia [11].
- F. Collection of blood sample: Food was withdrawn 12 hours prior to the blood sample collection. On the 8th day, blood was collected by cardiac puncture under chloroform anaesthesia. The blood samples were placed in dry and clean centrifuge tubes without anticoagulant and allowed to clot at room temperature. They were centrifuged for 20 minutes at 3000rpm. Then sera were collected for the lipid profile and other biochemical parameters [12].
- G. Laboratory analysis: Laboratory analysis was carried out to determine the phytochemical compositions of the plant. The quantitative determination of phenol was evaluated using the method described by Edeoga *et al.*, (2005) [13], flavonoids was determined using the aluminium chloride method as described by Chang *et al.*, (2002) [14], alkaloid was determined using the method described by Harborne, (1976) [15], saponin was determined using the method described by Oloyed, (2005) [16] and tannin was determined using the method described by Krishnaiah *et al.*, (2009) [17].
- H. Statistical analysis and calculations: The results were expressed as Mean±SEM of triplicate determination using the Statistical Package for Social Science (SPSS) Version 19 and one-way analysis of variance (ANOVA), followed by Duncan post-hoc test. Result were considered to be significant when p values were less than 0.05 (p<0.05). The LDL-c and VLDL-c concentrations were calculated using standard protocol equations by Friedawald [18]

III. RESULTS AND DISCUSSION

A. *Phytochemical analysis:* The result of phytochemical analysis on table 1 showed high concentrations of phenols (166.83±2.21mg/100g), alkaloids (95.53±1.01mg/100g),

saponins $(33.80\pm0.48$ mg/100g) and flavonoids $(38.02\pm1.60$ mg/100g).

The content of phenol 166.83±2.21mg/100g falls within the range of 46mg/100g-439mg/100g for various species of *Brassica* vegetables as reported by Lee, (2007) [19]. Phenols have been reported to have various physiological functions including antioxidant, antimutagenic, antitumor and free radical scavenging activities [20].

The alkaloid content 95.53±1.01mg/100g is higher compared to 1.16±0.09mg/100g and 0.99±0.00mg/100g in two eggplant varieties reported by Agoreyo *et al.*, (2012) [21], but lower when compared to the range of 0.81g/100g-1.8g/100g obtained in some green leafy vegetables in South East, Nigeria [22].

The saponin content 33.80±0.48mg/100g is higher compared to 5.34±0.31mg/100g and 11.63±0.29mg/100g in eggplant varieties [21], but lower when compared to 11.20±0.31g/100g in *Vernonia amygdalina* "bitter leaf", 9.40±0.28g/100g in Amaranthus *tricolor* "green amaranth", and 12.00±0.09g/100g in Telfairia occidentalis "Ugu" [23]. Alkaloids and saponins are known to elicit antimicrobial abilities and defend plants against microbial and pathogenic attacks [24]. Their analgesic, anti-inflammatory, anti-hypertensive and anti-microbial properties had been reported [25]. Saponins have been reported to posses' cholesterol-lowering effects and are also alleged to function in cancer protection by breaking down the cholesterol-rich membranes of cancer cells [26].

The total flavonoid content of 33.02±1.60mg/100g is comparable and within range of 102µg/g-944µg/g reported for various species of Brassica vegetables [27]. Dunja et al., (2011) [28] also reported similar value of total flavonoids composition ranging from 0.08±0.0mg/ml-3.28±0.12mg/ml of same vegetable juice extracted at different developmental stages. Flavonoids are well known for their antioxidant activity, protecting human, animal and plant cells against the damaging effects of free radicals. Due to this remarkable including their anti-inflammatory hypolipidemic, they are being used in numerous medical cancer-prevention treatments associated to cardiovascular system protection, including prevention of oxidative damage [6, 29].

The tannin content of 2.84±0.60mg/100g is higher compared to 1.50mg/100g in green cabbage, 1.57mg/100g in red cabbage, 1.57±0.36mg/100g in Chinese cabbage [30], but lower than 7.40±0.14mg/100g in Balanite aegyptiaca 'desert date' and 4.83±0.15 mg/100g in Vitex donianan 'black plum' [31]. Onyeka and Nwambekwe, (2007) [22] reported high levels of tannins in some locally cultivated fresh green vegetables in Nigeria with values ranging from 0.13g/100g to 0.28g/100g. Tannins impose an astringent taste in foods thereby affecting palatability. They have been reported to possess anti-nutrient effects by forming complexes with essential nutrients including enzymes of the digestive tract, thereby suppressing the availability and utilization of essential nutrients [31, 32]. However, tannin compounds have been reported to posses antibacterial [33], antiviral and antiparasitic effects [34].

B. Antihyperlipidemic effect of the plant extracts: The administration of triton X-100 caused significant (p<0.05) increase in the levels of serum total cholesterol (TC), Triglycerides (TG), Low density lipoprotein cholesterol (LDL-c), Very low density lipoprotein cholesterol (VLDL-c)

c) respectively and significant (p<0.05) decrease in High density lipoprotein cholesterol (HDL-c) level as compared with normal control group (Figure 1-5). Treatment with different extracts of *Brassica oleracae* var. *capitata L*. caused significant (p<0.05) decrease in the levels of TC, TG, LDL-c, VLDL-c respectively and significant (p<0.05) increase in HDL-c level compared to the hyperlipidemic control group (Figure 1-5). Standard anti-hyperlipidemic agent-Atorvastatin at 10 mg/kg body weight was also able to elicit significant (p<0.05) decrease the elevated serum TC, TG, LDL-c, VLDL-c and significant (p<0.05) increase the HDL-c level compared to hyperlipidemic control group (Figure 1-5).

At different doses of the extracts, it was observed that Methanolic extract at 500mg/kg body weight, 300mg/kg body weight and 100mg/kg body weight reduced serum cholesterol level by 41.3%, 38.3% and 31.5% respectively, while petroleum ether extract at 500mg/kg body weight, 300mg/kg body weight and 100mg/kg body weight reduced serum cholesterol level by 40.9%, 31.7% and 25.3% compared to the hyperlipidemic control group (Figure 1). It was also observed that Methanolic extract at 500mg/kg body weight, 300mg/kg body weight and 100mg/kg body weight reduced triglyceride level by 56.7%, 56.3% and 48.7% respectively, while petroleum ether extract at 500mg/kg body weight, 300mg/kg body weight and 100mg/kg body weight reduced triglyceride level by 40.1%,

The increase in HDL-c level by the Methanolic extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw was observed to be 115%, 92.6% and 24% respectively, while petroleum ether extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw increased the HDL-c level by 94.5%, 61.5% and 14.8% compared to the hyperlipidemic control group (Figure 3).

39.3% and 36.7% compared to the hyperlipidemic control

group (Figure 2).

Figure 4 showed that Methanolic extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw reduced LDL-c level by 44%, 38.8% and 28.4% respectively, while petroleum ether extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw reduced LDL-c level by 48.5%, 33.9% and 23.4% compared to the hyperlipidemic control group.

The reduction in VLDL-c level by Methanolic extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw was observed to be 56.7%, 56.3% and 48.7% respectively, while petroleum ether extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw was observed to reduce the level of VLDL-c by 40.1%, 39.3% and 36.6% compared to the hyperlipidemic control group (Figure 5).

The reduction in serum cholesterol, triglyceride, LDL-c, VLDL-c by the extracts was observed to be dose dependent and the increase in HDL-c by the extracts was also observed to be dose dependent (Figure 1-5). This is in agreement with Islam *et al.*, (2011) [8] who reported hypolipidemic effect of different fractions of *Brassica oleracae* on alloxan induced diabetic rats.

The cholesterol-lowering activity of the extracts may be as a result of the rapid catabolism of LDL-cholesterol to cholesterol through its hepatic receptors for final elimination in the form of bile acids [35]. Decreased triglyceride level after treatment with extracts may be due to increased activity of the endothelium bound lipoprotein lipase which

hydrolyzes the triglyceride into fatty acid or due to inhibition of lipolysis so that fatty acids do not get converted to triglyceride. Lipoprotein lipase found in the endothelial walls of vessels converts VLDL into LDL with the release of triglyceride; and the hydrolytic breakdown of LDL-cholesterol to cholesterol which is converted to bile acids. The increased level of HDL-c after treatment with the extracts may be due to the increased activity of lecithin: cholesterol acetyl transferase which incorporates free cholesterol from LDL into HDL thereby increasing the concentration of HDL-c. Also the lipid lowering role of the extracts could be attributed to the fact that they contain constituents that are able to inhibit enzymes such as hydroxyl-methyl-glutaryl-CoA (HMG-coA) reductase which participates in the *de novo* synthesis of cholesterol [8].

Brassica oleracae var. capitata L. contain significant amount of bioactive compounds including phenols, flavonoids, saponins and alkaloids (Table 1). Phenols can bind to cholesterol and bile acids, and thus facilitate their removal via the faeces; hence the uptake of lipids from the intestine to the blood is reduced [36]. Saponins and fibres are important therapeutically as they have been reported to elicit hypolipidemic effects [37, 38]. This could also be seen as a possible cause for the reduction in lipid concentrations of the experimental rats. Flavonoids have been reported to elicit diverse biological properties including hypolipidemic effect resulting from their antioxidant activity [8]. It may therefore be suggested that the effect of Brassica oleracae var. capitata L. on lipid concentrations was as a result of the synergy between the nutrient composition (high fiber and low fat content) and several bioactive compounds which are present in substantial amount.

C. Hepatoprotective effect of the plant extracts: The results (Figure 6-8) showed significant (p<0.05) elevation in SGOT, SGPT and Alkaline phosphatase activities in triton treated groups indicating that triton may have induced liver injury compared to the normal control group. Many chemicals and drugs have been reported to cause liver injury resulting in the leakage of cellular enzyme like SGOT, SGPT and ALP into the serum, hence resulting in their increased concentration [39]. Treatment with different extracts of Brassica oleracae var. capitata.L. at various doses caused significant decrease (p<0.05) in the activity of marker enzymes when compared with the hyperlipidemic rats.

Figure 4.6 showed that Methanolic extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw reduced the activity of the enzyme (SGPT) by 50.8%, 49.2% and 44.6% respectively, while petroleum ether extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw reduced SGPT activity by 51.8%, 49% and 45.8% compared to the hyperlipidemic control group.

The reduction in the activity of SGOT by the Methanolic extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw was observed to be 54.9%, 54.6% and 52% respectively, while petroleum ether extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw reduce SGOT activity by 55%, 54.4% and 52.3% compared to the hyperlipidemic control group (Figure 7).

Also Methanolic extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw was able to reduce the activities of Alkaline phosphatase by 49%, 48% and 45.8% respectively, while

petroleum ether extract at 500mg/kg bw, 300mg/kg bw and 100mg/kg bw reduced Alkaline phosphatase activity 49.6%, 48% and 46.5% compared to the hyperlipidemic control group (Figure 8).

All doses used in the assessment of enzymes activity in this study were observed to elicit significant effect on the enzymes, however Petroleum ether extract and Methanolic extract at 500mg/kg bw were observed to elicit the highest effect by reducing the activity of SGPT by 51.8% and 50.8%, SGOT by 55% and 54.9% and Alkaline phosphatase by 49.5% and 49% respectively. This is in agreement with Islam et al., (2011) [8], who reported the reduction in SGOT and SGPT activity of different fractions of Brassica oleracae in diabetic rats. It is also in agreement with Ahmed et al., (2012) [40] who reported the reduction in activity of SGPT, SGOT and Alkaline phosphatase by ethanolic extract of Brassica oleracae L. var capitata against Simvastatin induced hepatotoxicity rats. The decrease observed in the activities of SGPT, SGOT and Alkaline phosphatase following the administration of the extracts in the animals may be due to the protective effect of Brassica oleracea var. capitata L. extracts on liver cells probably by the restoration of liver cell membrane permeability [41]. This protective effect is seen in the reduction of the enzymes present in the serum which have also been reported in several experimental studies [8, 40]. Brassica oleracae vegetables have been reported to contain substantial amounts of sulphur containing compounds-glucosinolates and S-methyl cysteine sulfoside, phenols, flavonoids and ascorbic acid which have been proposed to possess' hepatoprotective activities [42].

Table I: Phytochemical compositions of Brassica oleracae var. capitata L.

Parameter	Composition (mg/100g)
Alkaloids	95.53±1.01
Flavonoids	38.02±1.60
Phenols	166.83±2.21
Saponins	33.80±0.48
Tannins	2.84 ± 0.60

Values are expressed as mean \pm standard error of mean (SEM) of triplicate determinations.

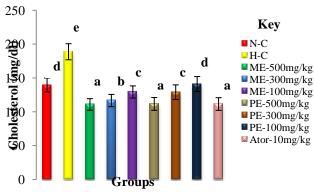


Figure 1: Effect of *Brassica oleracae* extracts on Serum Cholesterol level of Normal control "N-C", Hyperlipidemic control "H-C" and Hyperlipidemic treated Rats. Values are expressed as Mean \pm SEM of triplicate readings. Bars with different alphabet indicate significant (p<0.05) difference of the control of the cont

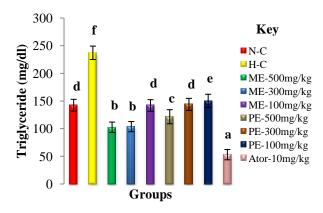


Figure 2: Effect of *Brassica oleracae* extracts on Serum Triglyceride level of Normal control "N-C", Hyperlipidemic control "H-C" and Hyperlipidemic treated Rats. Values are expressed as Mean ± SEM of triplicate readings. Bars with different alphabet indicate significant (p<0.05) difference.

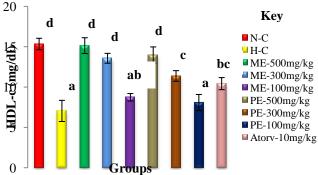


Figure 3: Effect of *Brassica oleracae* extracts on Serum HDL-c level of Normal control "N-C", Hyperlipidemic control "H-C" and Hyperlipidemic treated Rats. Values are expressed as Mean \pm SEM of triplicate readings. Bars with different alphabet indicate significant (p<0.05) difference.

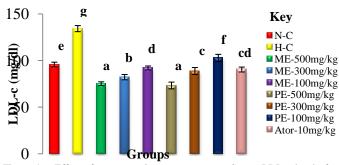


Figure 4: Effect of *Brassica oleracae* extracts on Serum LDL-c level of Normal control "N-C", Hyperlipidemic control "H-C" and Hyperlipidemic treated Rats. Values are expressed as Mean \pm SEM of triplicate readings. Bars with different alphabet indicate significant (p<0.05) difference.

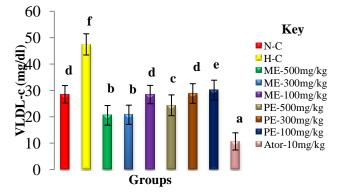


Figure 5: Effect of *Brassica oleracae* extracts on Serum VLDL-c level of Normal control "N-C", Hyperlipidemic control "H-C" and Hyperlipidemic treated Rats. Values are expressed as Mean \pm SEM of triplicate readings. Bars with different alphabet indicate significant (p<0.05) difference.

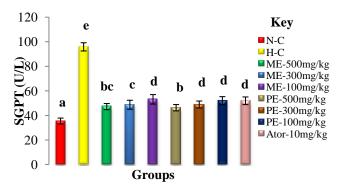


Figure 6: Effect of *Brassica oleracae* extracts on Serum glutamate pyruvate transaminase (SGPT) activity of Normal control, Hyperlipidemic control and Hyperlipidemic treated Rats. Values are expressed as Mean \pm SEM of triplicate readings. Bars with different alphabet indicate significant (p<0.05) difference.

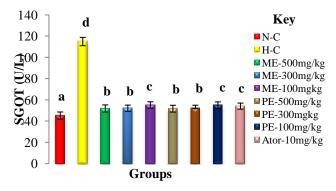


Figure 7: Effect of *Brassica oleracae* extracts on Serum glutamate oxaloacetate transaminase (SGOT) activity of Normal control "N-C", Hyperlipidemic control "H-C" and Hyperlipidemic treated Rats. Values are expressed as Mean \pm SEM of triplicate readings. Bars with different alphabet indicate significant (p<0.05) difference.

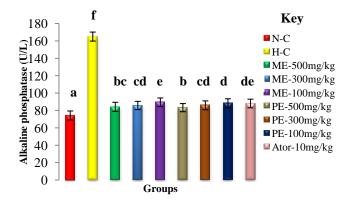


Figure 8: Effect of *Brassica oleracae* extracts on Alkaline phosphatase activity of Normal control "N-C", Hyperlipidemic control "H-C" and Hyperlipidemic treated Rats. Values are expressed as Mean \pm SEM of triplicate readings. Bars with different alphabet indicate significant (p<0.05) difference.

CONCLUSION

The results obtained showed that *Brassica oleracea* var. *capitata L.* possess' both antihyperlipidemic and hepatoprotective effect in hyperlipidemic rats, which may be due to the synergy between the several bioactive constituents present in the extracts. Thus, extracts of the plant could be explored as an ethno-medicine for hypolipidemic and hepatoprotective activity.

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