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Effect of *Ananas comosus* L. Dried Cuts and Leave Extract on hypercholesterolemic Rats

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Abstract

Worldwide, millions of people are suffering from chronic illnesses and health ailments including hypercholesterolemia and hyperglycemia. Current study was carried out to elucidate the comparative effect of *Ananas comosus* L. dried cuts (ADC) and leaf extract (ALE) on overall body weight, lipid profile and atherogenic indices (AI) in hypercholesterolemic rats. Phytochemical screening tests demonstrated that ALE exhibited far better results against total phenolic content and DPPH assay as compared with ADC. In efficacy study, moderate and higher doses of ADC (6% and 9%) and ALE (0.6 gm/Kg and 0.9 gm/Kg) were administered to hypercholesterolemic rats for 40 days. Concluded results were analyzed statistically and compared with normal and hypercholesterolemic rats group. Significant reduction in overall body weight gain percentage, feed efficiency ratio, TC, LDL-c, VLDL-c, TG, organs weight (liver and kidney), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and AI was observed with the exception of HDL-c that increased significantly after applying ADC and ALE treatments solely in hypercholesterolemic rats in comparison to control (hypercholesterolemic) group. Since ALE possess TPC and antioxidant load higher than ADC so consequently lipid metabolizing activity of ALE was seen to be higher than that of ADC. Both treatments of ALE revealed even better results in reducing plasma lipid level, overall body weight gain and atherogenicity risks factors as compared with ADC (6% and 9%) treatments. Hence, it may be concluded that ALE treatments seemed to be more efficient in controlling and managing hypercholesterolemia without affecting vital organs or causing any toxicity issues in hypercholesterolemic rats.

Key words: *Ananas comosus* L, atherogenic indices, efficacy study, hypercholesterolemia, hyperlipidemia.

Introduction

Hypercholesterolemia and hyperlipidemia are widespread health ailments refers to elevated levels of cholesterol, low-density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides and very low density lipoproteins (VLDL). Excessive fat deposition in circulatory vessels causes plaque formation, blockage in artery walls and ultimately brain stroke or heart attack (Stapleton *et al*., 2010). Herein Pakistan, 25% population is overweight and obese whereas ratio of hyperlipidemia is 37.1% and numbers are expected to rise (Jafar *et al*., 2006; Abid *et al*., 2012). Usually lipid profile varies in different populations due to differences in socio-environmental conditions, genetic makeup, eating habits, diversity in age/gender and decreased fatty acid metabolism due to deficiency of carnitine and resistance towards insulin. Hyperlipidemia and hypercholesterolemia leads to in complications like cardiovascular diseases (CVD), diabetes and hypertension (Hopkins *et al*., 2003; Stapleton *et al*., 2010). Hyperlipidemia and hypercholesterolemia can be managed by controlling the type and amount of lipids in diet, exercising and utilizing lipid lowering diet. Medical system is facing a challenge in the cure of hypercholesterolemia without any side effects (Islam *et al*., 2011).

Ananas comosus L. (commonly known: pineapple, family: bromeliaceae) is a tropical and subtropical fruit (20% of produce), mainly cultivated in China, Australia, Hawaii, Malaysia, Thailand, Mexico, Kenya and South Africa. Generally, *A. comosus* is not produced in Pakistan, but is cultivated on a very small scale by using vegetative propagation technique.

Cayenalisa, Green selacia, Red spanish, Perola and Perolera (Bartholomew *et al*., 2002) are some of the well-known varieties of *A. comosus* fruit, containing insoluble and soluble fiber, bromelain compound (Ketnawa *et al*., 2012), phytochemicals, vitamin C (63 mg/100 ml *A. comosus* fruit juice) and glycoproteins that have a key role in lowering risks

of diseases like hyperlipidemia, hypercholesterolemia, hyperglycemia, dysuria (Sripanidkulchai *et al*., 2000), dyslipidemia, CVD and certain other health ailments (Hale *et al*., 2005; Xie *et al*., 2005;Nawaz *et al*., 2012). Considering phytochemistry, *A. comosus* fruit and leaves not only contain variety of phytochemicals as polyphenols, saponins, alkaloids, terpenes, sterols, flavonoids and tannins, but also possess anti-oxidative and anti-hypercholesterolemic properties. Mechanism of lowering elevated cholesterol and lipid profile is concerned with bromelain compound (catalytic enzymes: thiolendopeptidases and peroxidases) and phytochemicals that triggers lipoprotein lipase enzymes and inhibits the 3-hydroxyl-methyl glutaryl coenzyme A (HMGCoA) reductase that ultimately lower fatty acid or lipid molecules synthesis. Phenolic compounds like p-coumaric acid lower lipid levels by reducing and protecting LDL cholesterol from oxidation (Xie *et al*., 2007; Saeed *et al*., 2016).

The present study aimed to assess the overall body weight gain, lipid profile, atherogenic indices (AI) and normal functioning of liver and kidneys of rats suffering from hypercholesterolemia by the effect of *A. comosus* dried cuts and leaves extract in an efficacy study model.

Materials and methodology

Procurement of raw material

Proposed study project was started at National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad (UAF), Pakistan. Good quality *A. comosus* (variety: Fresh Premium) were purchased from local market. Chemicals and standard reagents required for experiments were purchased from a scientific store in Faisalabad. For serum analyses, all of commercial diagnosis kits (ELISA kits) were purchased from another scientific store in Faisalabad. Rats for experimental study were obtained from “Animal room” of Doctor of Veterinary Medicine (DVM) faculty, UAF.

Preparation of samples

A. comosus fruit passed through various handling and processing steps to obtain *A. comosus* dried cuts (ADC) and *A. comosus* leaves extract (ALE).

Preparing *A. comosus* dried cuts (ADC)

Firstly, *A. comosus* fruit (Fresh Premium) underwent initial preparatory steps such as peeling, coring and cutting to make smaller sized triangular cuts. Afterwards, fruit cuts were placed in an aluminum tray and dried by hot air-drying technique using constant temperature (70^{o}C) and fixed air velocity (1.5 m/s) for 5-7 hours (Ramallo and Mascheroni, 2012). The dried cuts were then stored in air tight jars until further analysis.

Preparing ethanolic extract of *A. comosus* leaves extract (ALE)

A. comosus leaves were first washed out extensively with distilled water and then dried under the sunlight. Dried leaves were ground in an electrical grinder and the leaves powder obtained consequently was soaked in an ethanol solvent (80%) for 4 days at room temperature and this suspension was filtered out later (Kemasari *et al*., 2011). To evaporate solvent and obtain thickened ALE, filtrate was condensed with rotary evaporator at 45^{o}Cand the semisolid extract thus obtained was stored in shade (darker place) until further use.

Analysis of total phenolic content (TPC) and DPPH assay

Quantification of total phenolic content (TPC) and DPPH assay in ADC and ALE was measured by using method of Yuris and Siow, (2014).

Experimental rats

Healthy male albino rats (36 rats) were purchased and housed in an animal room under standard conditions of light (dark/light cycle: 12 hours), temperature (20-23^{o}C) and humidity (63-64%) level. All the rats weighed 175±10 gm on average. Firstly, the rats were fed on normal feed and water *ad libitum* for 1 week before starting the efficacy study and no other supplement was given [Table 1(a) and 1(b)]. All experimental studies conducted on rats were approved by Office of Research Innovation and Commercialization (ORIC), UAF (Reference. no. 1138/ORIC/14-02-2018). Rats were kept under observation and maintained according to prescribed guidelines and requirements of internationally accepted principles for laboratory animal use.

Inducing hypercholesterolemia
After 1 week of observation, rats of selected groups were made hypercholesterolemic through administration of high fat diet (HFD) by following the protocols of dietary models of rats suggested by Matos *et al*., 2005.
Efficacy study model
Rats were distributed randomly into 6 groups with 6 rats (n=6) in each group. Duration of efficacy study was 40 days.
1st Group: Contained the normal healthy rats that were given normal feed and water as placebo only for 40 days.
2nd Group: Contained hypercholesterolemic rats, fed on normal feed but rats were neither provided by *A. comosus* dried cuts (ADC) nor *A. comosus* leaves extract (ALE).
3rd Group: Contained hypercholesterolemic rats which were given *A. comosus* dried cuts (ADC 6%) for 40 days.
4th Group: Contained hypercholesterolemic rats which were fed *A. comosus* dried cuts (ADC 9%) for a period of 40 days.
5th Group: Contained hypercholesterolemic rats which were fed on *A. comosus* leaves extract (ALE 0.6 g/kg body weight) for 40 days.
6th Group: Contained hypercholesterolemic rats fed on *A. comosus* leaves extract (ALE 0.9 g/kg body weight) for 40 days.
Measuring body weight gain percentage (BWG %)
To assess the overall feeding pattern and growth performance of rats, the average feed intake and body weight gain (BWG) were recorded throughout the study to calculate feed efficiency ratio (FER) by following the method of Adeyemi *et al*., 2015.

$$\boldsymbol{FER} = \frac{Body\ weight\ gain}{Feed\ intake}$$

Assessment of blood parameters
At the end of efficacy study; rats were fasted over a night (almost 12-13 hours) after which they were sacrificed to collect the blood samples in separate test tubes that contained anti-coagulants. The samples were then placed in a refrigerator to store them till further use for analytical purpose.
Plasma lipid profile was checked to determine the concentrations of total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-c), low density lipoprotein (LDL-c) and very low-density lipoprotein (VLDL-c) in mg/dL. Liver enzymes in U/L [alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP)] were also analyzed by using enzymatic colorimetric method with commercial diagnostic kits (Musial *et al*., 2015).
Biological safety tests
Assessment of relative weight of organs (liver and kidney)
When rats were sacrificed and carefully dissected, the organs (liver and kidney) were confiscated. Then these organs were washed extensively under drop line of cold saline solution and dried well with filter paper. These organs were not blemished with any adhering blood particle. Afterwards, the organs were weighed (Saeed *et al*., 2016). Theoretical finding of each organ for relative weight was done by the formula:

$$\boldsymbol{Relative\ weight\ of\ organ} = \frac{Organ\ weight\ of\ animal}{Final\ weight\ of\ animal} \text{ x } 100$$

Assessment of AI (atherogenic indices) To evaluate the risk assessment of CVD and atherosclerotic events associated with cholesterol and lipoprotein levels, certain indices like TC/HDL-c ratio, LDL-c/HDL-c ratio and AI were calculated. AI was calculated by the Friedwald formula (Vuyyuru *et al*., 2012):

$$\boldsymbol{A.I} = \frac{[TC] - [HDL]}{[HDL]}$$

Statistical analysis
Results obtained from all parameters (physical and blood) were expressed as mean ± SD (standard deviation). The values were analyzed statistically by one-way ANOVA followed by Tukey's test using Statistix (8.1) software. P < 0.05 values were considered to be statistically significant.
Results and discussion
Total phenolic content (TPC) and DPPH assay
Quantities of total phenolic content (TPC) and DPPH assay of *A. comosus* dried cuts and leaves extract are shown in Table 1. TPC observed in ADC and ALE was 37.43 and 23.01 mg GAE respectively. Whereas DPPH assay demonstrated that ADC and ALE possess antioxidant capacity as 5.29 and 9.74 mg AA equivalent per 100 gm sample. Same research findings are

discussed by Lu *et al*., 2014. Researchers illustrated anti-oxidative capacity of 26 pineapple genotypes of Chinese origin in which TPC exists in a range from 31.48 to 77.55 mg GAE/100 gm and DPPH assay range from 3.68 to 22.85µmol TE/gm fresh weight. Similarly, Li *et al*., 2014 found out anti-oxidative features of pineapple peel and they also reported that pineapple's peel possess 31.76 mg GAE TPC in 100 gm dry extract and as 0.037 gm ascorbic acid (AA) DPPH assay per gram of sample. Since TPC observed more in ADC compared with ALE but the antioxidant capacity showed that DPPH assay of ALE was more than that of ADC, which could be associated with increased anti-hypercholesterolemic activity of pineapple leaves extract. Yuris and Siow, 2014 demonstrated that phenolic compounds of pineapple fruit extract showed a slow reaction rate with DPPH radicals, eventually leading to lower readings of ascorbic acid equivalence antioxidant capacity (AEAC). Moreover, phenolic compounds in fruits and vegetables exhibit key role as anti-oxidative agents and possess metal ions chelating activities. Since metal ions produce free radicals, so this chelating activity is considered important for inhibiting and provoking free radicals production (Hijazi *et al*., 2016) which ultimately confers health benefits to human beings.

Table 1: TPC and DPPH assay of *A. comosus* dried cuts and leaves extract

Groups	TPC (mg GAE/100 gm sample)	DPPH assay (mg AA/100 gm sample)
***A. comosus* dried cuts (ADC)**	37.43±3.10	5.29±0.69
***A. comosus* leave extract (ALE)**	23.01±2.08	9.74±1.1

Body weight gain percentage (BWG %) and feed efficiency ratio (FER)

Body weight gain percentage was premeditated by finding the differences between average initial and final weight of rats in percentage during efficacy study. Statistical results showed significant reducing effect of ADC and ALE treatments on BWG% and FER when compared with hypercholesterolemic rats group (Table 2). Mean values of BWG% and FER showed considerable differences in rats treated with ADC and ALE when compared with hypercholesterolemic group. However, no significant difference was found between ALE 0.6 gm and ALE 0.9 gm treated rat groups for BWG% and FER mean values compared with normal group. Similarly, no significant difference was seen between ADC 6% and ADC 9% treated rats groups. Highest mean values of BWG% and FER were obtained in rats of hypercholesterolemic group due to high fat diet (HFD) and Al-Sowyan *et al*., (2009) also revealed that the increased amount of body weight and adipose tissues produced as result of administrating HFD. Rats treated with ADC (6% and 9%) presented the lowest mean values of BWG% and FER compared with ALE (0.6 gm and 0.9gm) treated groups. Reason is that *A. comosus* leaves seemed to be ineffective in inhibiting exogenous lipid (cholesterol) absorption in intestines (Xie *et al*., 2007) as compared to ADC. These research findings are in line with Al-Hamedan *et al*., 2010, who illustrated that the rats treated with *Lepidium sativum* L. (LS) extract, LS 5% and LS 10% powder showed reduced tendency of weight gain as well as low values of FER and lipid profile as compared with rats of control group. This might be due to presence of bromelain compound in pineapple dried cuts which reduces body's capacity to gain weight. Pineapple leaves extract also showed significantly lowered levels in body weight gain and feed efficiency ratio (Saeed *et al*., 2016). Basically, soluble fiber content of viscous consistency is present largely in fruit mesocarp (passion fruit) or fruit peels (pineapple fruit), that is involved in changing gastric emptying time, delaying in absorption time of nutrients like simple carbohydrates and increased feeling of fullness or satiety (Musial *et al*., 2015). All these play an important role in lowering overall weight gain capacity of body by binding excess fats and nutrients with bile salts in intestine, thus lowered absorption and stimulate excretion out of the body.

Table 2: Mean values of BWG% and FER of experimental rat groups

Parameters / Groups	Avg. Feed Intake	Body Weight		Body Weight Gain	Feed Efficiency Ratio
	(g/d)	Avg. Initial weight (g)	Avg. Final weight (g)	(BWG %)	(FER)
Normal group	23.67±2.50	174.50±2.25	260.52±4.44	49.30±5.50	0.090±0.002
Hypercholesterolemic group	25.76±3.50	172.10±3.75	393.77±11.1	128.80±22.01	0.215±0.004
ADC 6%	23.17±0.21	171.70±5.50	214.40±3.14	24.87±3.20	0.046±0.001

ADC 9%	24.01±0.19	173.50±4.25	210.05±5.55	21.06±5.02	0.038±0.003
ALE 0.6 g/kg b.w.	25.16±1.03	172.80±2.25	275.78±1.22	59.59±7.09	0.102±0.001
ALE 0.9 g/kg b.w.	23.02±0.02	173.10±4.75	271.42±7.41	56.79±6.11	0.106±0.002

Lipid Profile

After cessation of efficacy study, rats of all groups were sacrificed to obtain serum samples and assess the levels of TG, TC, HDL-c, LDL-c and VLDL-c. After analyses, mean values were tabulated as shown in Table 3 (a) and 4(b).

Total cholesterol (TC) and triglycerides (TG)

Mean values of plasma lipids were observed to be significantly reduced by ADC and ALE treatments effect as compared with hypercholesterolemic group (Table 3a).Rats of ALE(0.6 gm and 0.9 gm) treated groups indicated significant reduction in TC and TG level compared with ADC(6% and 9%) treated groups and hypercholesterolemic group. Mean values of TC and TG level showed no significant difference in between ALE 0.6 gm and ALE 0.9 gm treated rats for TC and TG mean values compared with normal group. Similarly mean values for TC and TG had no significant difference between ADC 6% and ADC 9% treated rats. However, notable differences were found in mean values of TC and TG in rats of ADC and ALE treated groups when compared with hypercholesterolemic group. Highest mean values of TC and TG were seen in hypercholesterolemic group because of HFD. Rats treated with ALE (0.6 gm and 0.9 gm) indicated the lowest mean values of TC and TG level compared with ADC treated rat groups and hypercholesterolemic group. This could be attributed to phytochemicals such as flavonoids present in pineapple leaves extract that play a key role in inhibiting activity of lipid metabolizing enzymes i.e. HMGCoA reductase is inhibited up to 20 to 49% and provoked endogenous lipid (cholesterol and triglycerides) synthesis. These flavonoids also accelerate lipoprotein lipase enzymes (up to 200% to 400%) that stimulate fat burning process (Xie *et al*., 2007). These research findings are in agreement with Bahnasy and Yassin (2015) who illustrated that obese-hyperlipidemic rats showed significantly lowered levels of TC and TG when fed on diet with added dried pumpkin and dried pineapple. The tabulated results of the study are also in line with the results of Hijazi *et al*. (2016) who illustrated that phenolic compounds contained in pineapple leaves extract including caffeic acid, p-Coumaric acid and chlorogenic acid, exhibit hypercholesterolemic and hypolipidemic features by lowering levels of TC and TG in plasma, heart, liver and adipose tissues. Islam *et al*., 2011 revealed that phytonutrients (flavonoids), dietary fibers (hemicelluloses) and soluble fibers (pectin and oat) are helpful in lowering TC and TG levels.

Table 3(a): Mean values of TC and TG of experimental rat groups

Parameters / Groups	Total Cholesterol (TC) (mg/dL)	Triglycerides (TG) (mg/dL)
Normal group	142.50±3.01	65.45±2.10
Hypercholesterolemic group	223.13±9.22	129.34±8.43
ADC 6%	183.62±5.59	84.17±4.41
ADC 9%	176.02±6.64	88.53±4.65
ALE 0.6 g/kg b.w.	174.81±3.67	81.10±3.20
ALE 0.9 g/kg b.w.	161.50±4.50	76.34±5.39

High density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c)

Compared to rats of hypercholesterolemic group, rats were treated with ADC and ALE showed a declining trend in mean values of LDL-c and VLDL-c (Table 3b). ALE (0.6 gm and 0.9 gm) treated rat groups showed elevated levels of HDL-C and reduced levels of LDL-c and VLDL-c compared with ADC (6% and 9%) treated rats group and hypercholesterolemic group. There existed no significant difference between the mean values of HDL-c, LDL-c and VLDL-c level for ALE 0.6 gm and ALE 0.9 gm treated rat groups. Similarly, mean values of HDL-c, LDL-c and VLDL-c level revealed no significant difference in between ADC 6% and ADC 9% treated rat groups. However, distinguishable differences were found in mean values of HDL-c, LDL-c and VLDL-c levels in rats treated with ADC and ALE when compared with hypercholesterolemic group. Lowest mean values of HDL-c and highest mean values of LDL-c and VLDL-c were found in hypercholesterolemic group. ALE (0.6 gm and 0.9 gm) treatments revealed highest mean values of HDL-c level and lowest mean values of LDL-c and VLDL-c level when compared with hypercholesterolemic and ADC treated rat groups. Similarly, increasing trend in all lipoprotein levels was detected by Daher *et al*., 2005 with the use of pineapple fruit juice on lipid profile. Scientists reported that chronic consumption of pineapple juice increases the rate of metabolism, reduction of lipoprotein particles like LDL-c from blood. Moreover, polyphenols possesses improved lipid profiling effect by cholesterol excretion and increased HDL-C formation (Cho *et al*., 2012). Tabulated results (Table 4b) are also in agreement with findings of Xie *et al*., 2005 which suggested that pineapple leaves had greater influence on lipoproteins level as HDL-C seemed to increase by up to 66.2%and LDL-C decreased up to 47.9% which is the indication of antidyslipidemic and anti-oxidative potential of pineapple leaves. Furthermore, blood lipoproteins become more susceptible towards oxidation when HFD or high cholesterol diet is administered. Oxidized lipoproteins cause intravascular deposition or plaque formation in blood vessels and are not eliminated easily out of blood. *A. comosus* leaves extract reduces lipid peroxidation in blood plasma and organs like liver, brain and kidneys. So, pineapple could be more beneficial against atherosclerotic disorders.

Table 3(b): Mean values of HDL-C, LDL-C and VLDL-C of exp. rat groups

Parameters / Groups	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal group	48.66±2.55	56.34±1.32	17.10±0.70
Hypercholesterolemic group	33.36±5.12	99.70±1.94	25.10±1.01
ADC 6%	63.01±2.06	68.47±0.71	20.81±0.88
ADC 9%	57.36±1.10	62.75±1.07	19.08±0.78
ALE 0.6 g/kg b.w.	69.76±3.39	61.05±0.59	16.08±0.93
ALE 0.9 g/kg b.w.	66.01±3.21	58.55±0.92	15.01±1.23

Biological safety assessment

Assessment of liver and kidneys organ weight and their secretary enzymes is considered obligatory to check the normal physical and biochemical functioning after experimental study on rats. Biological safety assessment includes various tests and inspection protocols but herein study, liver and kidney weight, ALT, AST, ALP and atherogenic index (A.I) were measured.

Organs weight (liver and kidney)

Results as shown in table 4 elucidate that liver and kidneys weight (gm) in hypercholesterolemic rats group was not significantly influenced by ADC and ALE treatments. Although results revealed no considerable differences in obtained mean values of liver and kidney weight (gm) for rats treated with ADC and ALE compared with normal groups but hypercholesterolemic rats group indicated significantly higher mean values compared with other groups. Liver and kidney weight in rats treated with ALE (0.6 and 0.9 gm) had a lower mean value compared with ADC (6% and 9%) and hypercholesterolemic group. These research findings are in agreement with those of Xie *et al*. (2014) which report that polyphenols contained in pineapple leaves (mainly p-coumaric acid) decreases lipid accumulation in liver by accelerating liver fat metabolism (fat oxidation mechanism) via up regulating CPT-1 mRNA and protein expression in $HepG_2$ Cells in HFD fed mice. Vuyyuru *et al*. (2012) described a slight decrease in organs weight after administrating *A. comosus* leaves extract in albino rats as compared to HFD fed rats. This could be attributed to increased excretion of fecal-bile components (that increase cholesterol conversion into bile acids and salts) and leave extract interfering activity with endogenous cholesterol synthesis (biosynthesis) in liver which competes with cholesterol binding sites ultimately prevents fat accumulation in liver. Al-Hamedan (2010) revealed curative and protective effect of *L. sativum* seed extract on body organs (liver and kidney) after examining reduced level of liver cholesterol and total lipids upon administrating *L. sativum* seed extract in hypercholesterolemic rats Bahnasy and Yassin (2015) demonstrated that feeding rats on hyperlipidemic and hypercholesterolemic diet to relatively more weight gain of organs (liver and kidney) as compared to rats on normal diet.

Furthermore the persistent high levels of triglycerides in blood cause pain and swelling/inflammation of such organs in hyperlipidemic patients. In addition to that, Xie *et al*. (2005) demonstrated that *A. comosus* leaves extract attenuates organs weight by reducing production of lipid peroxidation products like malondialdehyde (MDA) in liver, brain and kidney, thus preventing their tissues from oxidative damage. Saeed *et al*. (2016) examined that the pineapple leaves extract (in low or moderate doses) protects liver parenchyma cells from fat deposition and subsequent inflammatory reactions.

Table 4: Mean values of relative weight of organs (liver and kidney) of exp. rat groups

Parameters / Groups	Liver (gm)	Kidney (gm)
Normal group	2.21±0.67	0.69±0.06
Hypercholesterolemic group	3.77±0.89	0.99±0.19
ADC 6%	2.98±0.60	0.82±0.03
ADC 9%	2.90±0.43	0.85±0.04
ALE 0.6 g/kg b.w.	2.44±0.21	0.70±0.04
ALE 0.9 g/kg b.w.	2.49±0.39	0.79±0.02

Liver enzymes

No significant change in level of liver enzymes such as ALP, AST and ALT was seen in rats treated with ADC and ALE as compared to normal group (Table 5). This increased activity of liver enzymes (ALP, AST and ALT) is apparently due to hepatic tissue damage (mild hepatocellular injury) in hyperlipidemic rats after fatty infiltration of liver and ultimately results in leakage from liver cytosol into circulating plasma. These research findings are in line with the results of Kalpna *et al*. (2014). They reported hepatoprotective and non-toxic effects of *A. comosus* leaves after observing decreased levels of blood aminotransferases (AST and ALT) and ALP in rats treated with *A. comosus* leaves. Musial *et al*. (2015) suggested that ingestion of pineapple fruit peel and passion fruit mesocarp in mice and rats did not induce any signs of liver toxicity because fruit peel and mesocarp did not cause any change in serum AST and ALT concentrations. Ajani *et al*. (2012) from their study suggested that administrating pineapple wine (moderate consumption) may not lead to liver damage, as they found considerable decreased concentrations of ALP, AST and ALT in experimental rats after administrating pineapple wine orally for 3 months. Hepatic tissue damage or inflammation in liver may be better described by ALT concentrations. Kemasari *et al*., 2011 concluded from its study that ingestion of *M. inidica* leaves extract brought back ALT enzyme to normal level, thus exhibited medicinal properties through its phenolic contents, flavonoids and polyphenols. Rathod *et al*., 2009 reported that *C. gigantea* flower and leaf extracts restores hepatic damaging in diabetic rats, when observed the reduced level of SGOT, SGPT and ALP in diabetic rats.

Table 5: Mean values of AST, ALT and ALP of exp. rat groups

Parameters / Groups	ALP (U/L)	AST (U/L)	ALT (U/L)
Normal group	91.11±3.41	84.16±6.55	68.24±5.29
Hypercholesterolemic group	131.28±5.25	116.24±9.49	93.80±11.45
ADC 6%	95.42±7.74	91.37±8.41	80.11±8.43
ADC 9%	82.01±3.28	82.44±4.76	76.33±9.23
ALE 0.6 g/kg b.w.	84.63±8.01	77.53±5.20	69.82±3.54
ALE 0.9 g/kg b.w.	77.49±5.39	78.15±6.12	62.43±6.61

Atherogenic indices

Measuring of AI seemed to be a potent indicator of cardiac risk factors in heart diseases and atherosclerotic events. Higher the AI, higher the chances of developing risks for CVD (cardiovascular diseases), CHD (coronary heart disease) and brain stroke. A.I was measured in all normal and hypercholesterolemic rat groups after treating them with different doses of

ADC and ALE as mentioned in Table 6. Outcomes for TC/HDL-c ratio, LDL-c/HDL-c ratio and A.I clarify certain differences between *A. comosus* (ADC and ALE) treatments and hypercholesterolemic rats group. ADC and ALE treatments reverted A.I back in hypercholesterolemic rats to the levels seen in rats of normal group or even below that level. ALE (0.6 and 0.9 gm) revealed significant decreased levels of A.I compared with ADC (6% and 9%) treated groups. ADC treated groups showed no significant differences compared with normal group. Hypercholesterolemic group represented higher level of A.I amongst all these groups. These outcomes are in agreement with the results mentioned by Hijazi *et al.* (2016). who elucidated that ingesting pineapple leaf extract (PLE) leads to significant decreased A.I in PLE treated rats compared with hypercholesterolemic (+ve) rats group. Higher serum lipid profile and high levels of BMI (body mass index)/obesity are positively correlated with higher AI (Kanthe *et al*., 2012). Elevated total cholesterol in blood plasma referred to potent modifiable risk factor of CVD and reduced serum total cholesterol was associated with lowering of lipoproteins fractions particularly LDL-c (Vuyyuru *et al*., 2012). According to Muruganandan *et al*. (2005) the diminution of lipid profile or triglycerides in plasma may contribute to decreased A.I by changing mechanism of glucose-fatty acid cycle. Their suggestion was based upon a study illustrating that daily intake of mangiferin (10 and 20 mg per kg) for 28 days reduces lipid profile plus mangiferin also diminishes lipid peroxidation biomarker namely MDA (malondialdehyde) in organs like kidney, liver and heart indicating its antioxidant features. Ajiboso *et al*. (2016) explicated that *C.procera* leaves as potent substance may be helpful in preventing and managing of CHD (coronary heart diseases) by correcting derangement of lipid/fat metabolism. Al-Hamedan (2010) reported that events of developing risks of fatal IHD (ischemic heart disease)and heart attack may be related to reduce by *L. sativum* seed powder or extract that contained antioxidant components like phenolic compounds, α-linolenic acid and amino acids.

Table 6: Mean values of A.I of exp. rat groups

Groups \ Parameters	T.C Ratio HDL-C	LDL-C Ratio HDL-C	Atherogenic Index(A.I)
Normal group	2.92±0.51	1.15±0.32	1.92±0.39
Hypercholesterolemic group	6.68±0.68	2.98±0.41	5.68±0.62
ADC 6%	2.91±0.47	1.08±0.29	1.91±0.22
ADC 9%	3.06±0.42	1.09±0.37	2.06±0.19
ALE 0.6 g/kg b.w.	2.50±0.30	0.87±0.41	1.50±0.10
ALE 0.9 g/kg b.w.	2.44±0.50	0.88±0.33	1.44±0.20

Conclusion

Owing to therapeutic potential and physiological health promoting benefits, world is moving towards organic foods and focusing more on use of ethnobotanical agents as curative measures with minimal side effects. Current study revealed that ADC and ALE had meaningful effect on decreasing overall body weight, lipid profile and A.I in hypercholesterolemic rats. Whereas; ALE showed far better results for phytochemical screening assay compared with ADC. Moreover; efficacy study clarified that ALE treatments had higher beneficial impact than ADC treatments in controlling hypercholesterolemia in rats by accelerating its lipid metabolizing activity in blood plasma and body tissues.

Suggestions & Recommendations

Functional and nutraceutical foods must be included in normal daily meal plan that provides health benefits beyond to that of nutritional purposes. Use of micronutrient rich foods and extracts confront oxidative stress and regulates metabolic disorders and helps to cure non-communicable diseases. A person chooses more ingredients closed to nature will have better and active lifestyle.

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