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Effect of Pineapple Leaves Extract (PLE) on Lipid Profile, Glucose, Insulin Concentration and Atherogenic Index in Hypercholesterolemic Rats

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ABSTRACT

The aim of this research to study the effects of oral intake of pineapple leaves extract (PLE) at three dosages (250, 500 and 750 mg/kg b. wt.) for 4weeksonhypercholesterolemic rats on serum levels of total cholesterol (TC), triglycerides (TG), atherogenic index (AI), lipoprotein fractions, Glucose and insulin concentration were performed. Thirty male Wister Albino rats were distributed into five groups as follows: group1: negative control group, group 2: positive control (hypercholesterolemia rats) group, groups 3, 4 and 5 fed on High cholesterol and high fat diet (HCFD) and treated with orally doses of PLE of 250, 500 and 750mg/kg b. wt., respectively. Results indicated that, the total content of phenolic compound of pineapple leaves extract was 22.28 mg/100 ml. The phenolic compounds were separated based on their functional groups into 25 fractions and identified 24 compounds from them by HPLC. The oral intake of PLE to hypercholesterolemia rats for 4 weeks significantly decreased serum levels of TC, TG, low density lipoproteins cholesterol (LDL-c), very low density lipoproteins cholesterol (VLDL-c), atherogenic index (AI) and Glucose concentration when compared to the control positive group. Levels of high density lipoprotein cholesterol (HDL-c) and insulin concentration were significantly increased as compared to the control positive group. There is a potent antiatherogenic and antioxidant effect of PLE on hypercholesterolemic rats.

Key words: Pineapple leaves, rats hypercholesterolemia, total cholesterol (TC), triglycerides (TG), atherogenic index (AI), phenolic compound.

Introduction

Polyphenols are phytochemicals present in plants that contribute to their antioxidant and different pharmacological activities. Phenolic or polyphenolic compounds are plant metabolites widely spread throughout the plant kingdom. Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending plants against pathogens and stress in general (Yordi *et al.,* 2012).

Beneficial effects of polyphenols on human health are partly explained by their antioxidant activity. Because of the antioxidative property, it is suggested that polyphenols may delay or prevent the onset of diseases such as cancer, diabetes induced by free radicals. They also inhibit low density lipoprotein (LDL) oxidation and platelet aggregation and are reported to have negative correlation with incidences of coronary heart disease. Several medicinal plants contribute their activities due to the presence of flavonoids and polyphenols (Shetgiri *et al*., 2010).

Ananas comosus (L.) (AC) also commonly named Pineapplepossess a wide array of pharmacological properties such as antibacterial activity, antihyperlipidemic activity, Antidysuria activity, antitumor activity. (Kalpana *et al*., 2014 and Kataki, 2010).

The leaves has been using as anthelmintics, aborfacient and emmenagogue and are useful in abortion, helminthiasis, amenorrhaea, dysmenorrhoea, whooping cough, antidyspepsia and anti - diarrhea agent (Islam*et al*., 2011).

Extract of AC indicated the presence of carbohydrates, alkaloids, saponins, sterols/terpenes, flavanoids, tannins and phenolic compounds and proteins and amino acids. Presence of flavanoids, phytosterol, glycosides and phenols, phenolic compounds and tannins were very prominent. Most

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antioxidant activities of plant sources are derived from phenolic-type compounds **(**Kataki, 2010 and Kalpana *et al*., 2014).

Cholesterol is essentially insoluble in water; it is transported in blood and in protein particles (Berg *et al.,* 2007). The American Heart Association (AHA) describes the existence of two types of cholesterol :- (1) high density lipoproteins (HDL-c) which is considered good cholesterol, because it has the ability to carry excess cholesterol back to the liver for recycling, and (2) low density lipoproteins (LDL-c) which is the major carrier of cholesterol in humans. Low density lipoprotein (LDL-c), bad cholesterol, is harmful when its concentration is elevated in the blood. The ratio between these two types of cholesterol LDL/ HDL is defined an atherogenic index which is an important predictor of heart disease and atherosclerosis. The normal level of cholesterol that should be found in the blood varies between 140 and 200 mg per deciliter (mg/dL). Elevated blood total cholesterol (hypercholesterolemia) occurs when its concentration become higher than 240 (mg/dL) (AHA, 2011)

Hypercholesterolemia is a major socioeconomic problem in common individuals as well as health professionals due to the strong correlation between cardiovascular diseases and lipid abnormalities (Venkadeswaran *al et*., 2014). Hyperlipidaemia is manifested as hypercholesterolemia and/or hypertriglycerolemia.

Hypercholesterolemia is a metabolic condition that determines the onset of chronic degenerativediseases such as atherosclerosis (Bertges *et al.*, 2011). Hypercholesterolemia is a condition where there is an aberrantly elevated concentration of cholesterol in the blood (Bamimore, 2013). Defined as excessively high plasma cholesterol levels, has emerged as a strong risk factor for cardiovascular disease (CVD).

Epidemiologic studies support the view that a high consumption of fruit and vegetables has commonly been associated to a reduction of the risk of cardiovascular diseases. These beneficial effects have been partly attributed to the compounds which possess antioxidant activity. The major antioxidants of vegetables and fruits are vitamins C and E, carotenoids, and phenolic compounds, especially flavonoids (Mahmoud*et al*., 2013).

Saxena and Panjwani (2014) evaluated the cardioprotective effects of hydro alcoholic extract of *Ananas comosus* (HEAC), on Isoproterenol (ISO) induced myocardial infarction in Albino Wistar rats.

The aim of this study was performed to investigate the effect of oral intake of pineapple (*Ananas comosus* L.) leaves extract (PLE) at three dosage levels (250, 500 and 750mg/kg dw.t) on hypercholesterolemia male rats after 4 weeks of treatment on serum levels of total cholesterol (TC), triglycerides (TG), atherogenic index (AI), lipoprotein fractions , Glucose and insulin concentration

Materials and Methods

Materials:

Pineapple (Ananas comosus L.) leaves:

Fresh pineapple leaves (*Ananas comosus* L.) used in this study was purchased from a local market, Jeddah, Kingdom of Saudi Arabia

Cholesterol (white crystalline powder) Bile acid and phenolic compounds were purchased from Sigma-Chemical Company St. Lowis, USA.

Saturated fat was purchased from local market.

Enzymatic glucose kits, colorimetric kits for serum total Cholesterol, serum Triglycerides (TG) and High Density Lipoprotein Cholesterol (HDL-c) were obtained from Human Gesellschaft for Biochemical, Germany. Glucose and insulin ELISA kits were purchased from Cayman Chemical Company, Ann Arbor, MI, USA.

Animals:

A total number of thirty male albino rats of Wistar strain weighed 180-200g each, were obtained from the experimental Animal Unit of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia.

Methods:

Preparation of the Basal Diet:

The basal diet for rats was prepared using AIN-93 according to Reeves *et al*., (1993). The basal diet consists of the following: Protein (Casein) 20%; Sucrose 10%;Corn Oil 4%; Choline Chloride 0.2%; Vitamin mixture 1%; Salt mixture 3.5%; Fibers (Cellulose) 5% and the remainder is Corn Starch up to 100%.

Induction of hypercholesterolemia:

Induction of hypercholesterolemia was induced by feeding the rats on basal diet plus 1% (w/w) cholesterol according to Alissa *et al*., (2004), 0.2% (w/w) bile salts according to Lamb *et al*., (1999) and 20% (w/w) saturated fat (Santillo *et al*., 1996) for four weeks.

Preparation of pineapple leaves extract (PLE):

The leaves of pineappleswas separated, cleaned and dried in oven at 40°C, then powdered in a grinder, then stored in an airtight container at 5°C until further use (Kalpana *et al*., 2014).

Experimental Design of Rats:

The experiment was performed on thirty male mature Wistar rats. Animals were distributed randomly into five equal groups, six rats each. Rats were housed in standard plastic cages at a room temperature (24± 2 °C), with fixed 12-hour lighting system. All rats were allowed to free access to basal diet and water for one week before starting the experiment for acclimatization. After acclimatization period, the rats were allocated in to the following groups:

*Group (1):*Rats were fed on the basal diet only, kept as a negative control group (Con -ve) and received oral gavages' of distilled water, for eight weeks.

The other four groups (n=24) rats were fed on experimental diet for four weeks. After this period, blood samples were taken for measuring total cholesterol level. Rats with blood cholesterol level ≥200 mg /dl were considered to be hypercholesterolemia (Iqbal *et al*., 2011).These rats were distributed into the following groups:

Group (2): Rats were fed on experimental diet only, kept as a positive control group (Con +ve) and received oral gavages of distilled water for four weeks.

Group (3): Rats were fed on experimental diet and orally PLE in a dose of 250 mg/kg body weight (b. wt.) for four weeks.

Group (4): Rats were fed on experimental diet and orally PLE in a dose of 500 mg/kg body weight (b. wt.) for four weeks.

Group (5): Rats were fed on experimental diet and orally PLE in a dose of 750 mg/kg body weight (b. wt.) for four weeks.

Total cholesterol (TC) was assessed by using enzymatic colorimetric kit as described by Allain *et al*. (1974).

Total Phenolic Content in PLE were determined and identified by High-performance liquid chromatography (HPLC) according to the method reported by Mattila *et al.* (2000).

Enzymatic colorimetric GPO-PAP kit was used for measured triglycerides (TG) as described by Fossati and Prenape (1982).

An enzymatic colorimetric kit was used for the determination of high density lipoprotein cholesterol (HDL-c) as described by Lopes-Virella *et al*. (1977).

The Atherogenic index was determined according to the method described by (Mertz, 1980).

Serum glucose was measured by enzymatic GOD / POD kits according to the method by (Trinder, 1969).

Insulin was estimate using enzyme linked immune sorbent assay ELISA according to the method by Clark and Hales (1994).

Statistical analysis:

Statistical analysis was done by using (SPSS) Statistical Package for the Social Sciences for Windows, version 22 (SPSS Inc., Chicago, IL, USA). Collected data was presented as mean± standard error (SE). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to (Armitage and Berry, 1987). All differences were consider significant if $P < 0.05$.

Results and Discussion

Quantification and identification of total phenolic content in pineapple leaves extract PLE:

Phenolic compounds in fruit and vegetables play a strong role as antioxidant to chelate metal ions involved in the production of free radicals, that confer health benefits to humans (El-Sello, 2011) and Yapo *et al* . (2011).The quantification of total phenolic content of pineapple leaves extract (mg/100ml sample) were presented in Table 1, it was recorded 22.28 mg/100 ml.

Table 1: Total phenolic content of pineapple leaves extract PLE.

Sample	Total phenolic compounds (mg/100ml)
PLE	22.28± 1.54

Mean ± SE of triplicate measurement.

Identification of phenolic compound in pineapple leaves extract (*Ananas Comosus* L.) was done by HPLC are shown in Table 2. Phenolic compounds are very important in PLE constituents, by virtue of their antioxidant activity by chelating redox- active meta ions, inactivating lipid free radical chains and preventing hydroperoxide The phenolic compound were separated based on their functional groups into 25 fractions and identified 24 compounds from them by HPLC. The obtained results indicated that, pineapple leaves extract contains four phenolic compounds as the major content. It was Pyrogallol (628.84 ppm), Benzoic (510.12 ppm), e-vanillic (321.50 ppm) and Salycilic (253.56 ppm).These results are in agreement with Takaeidi *et al* (2014) on Date seeds which known as important sources of phenolic acids consisting of hydroxylated derivatives of benzoic acid (gallic acid, protocatechuic acid, p-hydroxybenzoic acid and vanillic acid) and cinnamic acid (caffeic acid, p-coumaric acid, ferulic acid, m-coumaric and o-coumaric acid).

Table 2: Identification of phenolic compounds inpineapple leaves extract PLE.

Identification of Phenolic compounds	Concentration (ppm)	Identification of Phenolic compounds	Concentration (ppm)
Pyrogallol	628.84	Caffeine	40.20
Benzoic	510.12	Ellagic	40.46
e-vanillic	321.50	Protocatchuic	36.40
Salycilic	253.56	Alpha-coumaric	34.85
Chlorogenic	81.59	Iso- ferulic	28.41
Epicatechein	72.18	Coumarin	27.59
P-OH-benzoic	63.29	Reversetrol	26.49
Vanillic	58.34	4- Amino-benzoic	25.08
Ferulic	52.93	3,4,5- methoxy- cinnamic	24.31
Caffeic	42.82	P-coumaric	24.18
Catechein	42.23	Gallic	16.98
Catechol	41.10	3-OH- Tyrosol	10.24

Effect of different doses from pineapple leaves extract PLE on the serum level of total cholesterol (TC) and triglycerides (TG):

Effect of oral intake of different doses from pineapple leaves extract PLE on the serum level of total cholesterol (TC) and triglycerides (TG) in hypercholesterolemia male ratsarerecorded in Table 3.Results of biochemical analyses revealed that hypercholesterolemia male rats (positive control group) had a

significant (P<0.05) increase in total cholesterol (TC) by 176.47 % and triglycerides (TG) by 77.39 % compared to normal rats (negative control group).

Table 3: Effect of oral intake of different doses from pineapple leaves extract PLE on the serum levels of total cholesterol (TC) and triglycerides (TG) in hypercholesterolemia male rats.

Parameters / Groups	TC (mg/dl)	TG (mg/dl)
Group (1) Negative control	143.83 ± 3.49^{c}	74.50 ± 1.80^{c}
Group (2) Positive control	397.66±9.50^{a}	132.16 ± 2.35^{a}
Group (3) PLE 250 mg / kg.b.w.	146.66 ±1.25^{b}	78.83 ± 1.79^{b}
Group (4) PLE 500 mg / kg.b.w.	142.50±3.64 ^{c}	76.83 ± 2.34 ^{c}
Group (5) PLE 750 mg / kg.b.w.	143.00 ± 2.04 ^{c}	79.66± 2.30^{b}

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Values with similar or partially similar superscripts are non-significant.

Oral intake of PLE at the tested doses 250, 500 and 750 mg/kg b. wt., significantly (P<0.05) decreased serum TC by 63.11, 64.16 and 64.03 % respectively compared to hypercholesterolemia male rats (positive control group).

Oral intake of 250,500 and 750 mg/kg b. wt., of PLE to hypercholesterolemia male rats significantly (P<0.05) reduced serum TG levels by 40.35, 41.86 and 39.72 % respectively when compared to hypercholesterolemia male rats (positive control group).These results were consistent with (Islam *et al*. (2011), Ma *et al* . (2007) and Cho *et al*. (2010) who said that the content of both caffeic acid and chlorogenic acid in ethanolic extract of AC might be lowered the concentrations of TG (in plasma, liver and heart) and TC (in plasma, adipose tissue and heart) concentrations. Many studies have mentioned the anti-hyperglycemia and anti-dyslipidemic properties of PLE due to its content of phenolic acids including p-Coumaric acid , Caffeic acid and Chlorogenic acid significantly reduce plasma TC and TG in obese rats (Cho et al. 2010) , and Chai et al. (2013).

Effect of oral intake of different doses from pineapple leaves extract PLE on the serum level of high density lipoprotein cholesterol (HDL-c) in hypercholesterolemia male rats are illustrated in Table 4. The hypercholesterolemia male rats(positive control group) had a significant (P< 0.05) decrease in HDL-c serum when compared with the normal rats (negative control group) by 38.88 %.

Table 4: Effect of oral intake of different doses from pineapple leaves extract PLE on the serum levels of lipoprotein fractions in hypercholesterolemia male rats:

Parameters / Groups	HDL-c (mg/dL)	LDL-c (mg/dL)	VLDL-c (mg/dL)
Group (1) Negative control	45.00 ±1.82 ^{a}	86.06 ±3.97^{c}	14.90 ±.36^{c}
Group (2) Positive control	27.50 ± .99^{d}	337.26±5.18^{a}	26.23± .499^{a}
Group (3) PLE 250 mg / kg.b.w.	36.00 ±1.09 ^{c}	92.60±2.46^{b}	15.40 ± .47 ^{c}
Group (4) PLE 500 mg / kg.b.w.	42.00 ±.96^{b}	84.46±3.69 ^{c}	15.16± .33 ^{c}
Group (5) PLE 750 mg / kg.b.w.	45.66 ±1.60^{a}	89.50±2.92^{b}	15.90 ± .44^{b}

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Values with similar or partially similar superscripts are non-significant.

Oral intake of 250, 500 and 750 mg/kg b. wt., of PLE to hypercholesterolemia male rats significantly (P<0.05) increases serum HDL-c level by 30.90, 52.72 and 66.03 % respectively when compared to hypercholesterolemia male rats (positive control group).

Hypercholesterolemia male rats (positive control group) had a significant (P<0.05) increase in serum level of low density lipoprotein cholesterol (LDL-c) when compared to the normal rats (negative control group) by 291.88 %. Oral intake of PLE at three dosage levels significantly (P<0.05) decreased serum levels of LDL-c when compared to hypercholesterolemia male rats (positive control group). The decreases in serum levels of LDL-c in rats given PLE at doses 250, 500 and 750 mg/kg b. wt., groups were 72.54, 74.95 and 74.46 % respectively.

Concerning serum levels of very low density lipoprotein cholesterol (VLDL-c), the results revealed that hypercholesterolemia male rats (positive control group) had a significant (P<0.05) increase in serum level of VLDL-c when compared with normal rats (negative control group) by 76.04 %.

Oral intake of PLE at 250, 500 and 750 mg/kg b. wt., for 4weeks produced significant (P<0.05) decreases in serum levels of VLDL-c by 41.28, 42.20 and 39.38 % respectively, when compared to the hypercholesterolemia male rats (positive control group).These results were consistent with the studies of (Wang *et al*. (2011) and Mariee *et al*. (2012).

Effect of different doses from pineapple leaves extract PLE on the serum glucose and insulin levels:

Effect of oral intake of different doses (250, 500 and 750 mg/kg b. wt.,) from pineapple leaves extract PLE on the serum glucose levels in hypercholesterolemia male rats are shown in Table 5.The hypercholesterolemia male rats (positive control group) had a significant (P<0.05) increase in serum glucose level when compared to the normal rats (negative control group) by 42.7 %. Oral intake of PLE at three dosage levels significantly (P<0.05) decreased serum glucose level when compared to hypercholesterolemia male rats (positive control group). The decreases in serum glucose level in rats given PLE at doses 250, 500 and 750 mg/kg b. wt., groups were 30.73, 31.90 and 29.11 % respectively. The obtained results are consistent with (Zhang *et al*. (2007).

Table 5: Effect of oral intake of different doses from pineapple leaves extract PLE on the serum glucose and insulin levels in hypercholesterolemia male rats:

Parameters / Groups	Glucose (mg/dl)	Insulin (uU /ml)
Group (1) Negative control	100.66 ±.76^{b}	18.16 ± .47^{b}
Group (2) Positive control	143.66 ± 2.83^{a}	10.83 ±.74^{c}
Group (3) PLE 250 mg / kg.b.w.	99.50± 2.51^{b}	18.50 ± .61^{b}
Group (4) PLE 500 mg / kg.b.w.	97.83 ± 1.60^{c}	19.16 ±.60^{a}
Group (5) PLE 750 mg / kg.b.w.	101.83 ± 1.74^{b}	17.83± .79^{b}

Data are presented as means ± standard deviation, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Values with similar or partially similar superscripts are non-significant.

Effect of oral intake of different doses (250, 500 and 750 mg/kg b. wt.,) from pineapple leaves extract PLE on the insulin levels in hypercholesterolemia male rats are shown in Table 5. The obtained data indicated that hypercholesterolemia male rats (positive control group) had a significant (P< 0.05) decrease in serum of hypercholesterolemia male rats when compared with the normal rats (negative control group) by 40.36 %. Oral intake of 250, 500 and 750 mg/kg b. wt., of PLE to hypercholesterolemia male rats significantly (P<0.05) increases insulin level by 70.82, 76.91 and 64.63 % respectively when compared with hypercholesterolemia male rats (positive control group).The present data was confirmed by the results of (Craig and Beck (1999) who reported that the presence of Phytochemicals- bioactive compounds- found in plants works synergistically with nutrients and dietary fibers to protect against diseases.

Effect of different doses from pineapple leaves extract PLE on Atherogenic indexes (AI):

Atherogenic indexes (AI) of hypercholesterolemia male rats treated with three doses (250, 500 and 750 mg/kg b. wt.) from pineapple leaves extract PLE are presented in Table 6.

Concerning Atherogenic index (AI) of rats, the results showed that hypercholesterolemia male rats had a significant ($P< 0.05$) increase in Atherogenic index (AI) as compared to normal rats (negative control group) by 663.15 % respectively.

Oral intake of 250, 500 and 750 mg/kg b. wt., of PLE to hypercholesterolemia male rats significantly ($P<0.05$) decreases Atherogenic index (AI) by 78.31, 83.44 and 79.08 % respectively when compared to hypercholesterolemia male rats (positive control group).

Table 6: Effect of oral intake of different doses from pineapple leaves extract PLE on Atherogenic index (AI) in hypercholesterolemia male rats:

Parameters / Groups	Atherogenic index (AI)
Group (1) Negative control	$1.71\pm.16^{d}$
Group (2) Positive control	$13.05\pm.30^{a}$
Group (3) PLE 250 mg / kg.b.w.	$2.83\pm.12^{b}$
Group (4) PLE 500 mg / kg.b.w.	$2.16\pm.07^{c}$
Group (5) PLE 750 mg / kg.b.w.	$2.73\pm.11^{b}$

Data are presented as means ± standard error, (n = 6 for each group).
Values with different superscripts within the column are significantly different at P< 0.05.
Values with similar or partially similar superscripts are non-significant.

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