Biochemical Effects of *Arctium Lappa* Extract on Mice Pancreatic Cancer Induced By Ethenol

Suzan Shawky[1], Ismail I. Hegazy[2], Ahmed Monir[3], Mohamed Adel[3], Asmaa Amin[3] and Yara Elsherbini[3]

[1]Department of Biochemistry, Faculty of Applied Medical Sciences, October 6 University, Egypt
[2]Prof bio chemistry of Faculty of Medicine, AlAzhar University, Egypt
[3]Department of medical laboratory Faculty of Applied Medical Sciences, October 6 University, Egypt
a.m.eldmrdash@gmail.com

Abstract: The aim of this paper was to study the bio chemical effects of *A. lappa* leaves and root extract (AE) on pancreatic cancer -induced by ethanol in male mice. The present work was done to elucidate the possible protective and anticancer effect of *Arctium lappa* (burdock). Mice received intraperitoneally IP injections of (80%) ethanol (0.2 ml\l /kg BW) induced pancreatic cancer mice. as well as For treatment and for proflective was administered intraperitoneal injection of *Arctium lappa* (0.2 and 0.1 ml\l /kg BW) daily, respectively for 14 days showed a significant Changes of body weight (gm) of mice in different groups were measured at 14 days and (improve) amylase activity, The treatment also resulted in a significant increase in plasma C-peptide, decrease in fasting blood glucose level and increase LDL. The *Arctium lappa* exerted rapid protective effects against lipid peroxidation by scavenging of free radicals there by reducing the risk of pancreatic complications. *Arctium lappa* extract decrease gene expression(TNF, IL2 and BCl2). And histopathological examination was determined *Arctium lappa* extract tend to inhibit cancer progress and control alcohol toxicity on liver and pancrease tissue.
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Key words: *Arctium lappa* (burdock), pancreatic cancer, ethenol, amylase, C-peptide, Glucose, gene expression.

1. Introduction

Arctium lappa and *Arctium minus*, commonly known as burdock, are introduced, feral, biennial species found throughout the United States. Native to Europe, these plants have been valued for centuries for their medicinal properties, it is a common medicinal herb in China, Europe, North America and Asia. It was used for the treatment of many health complains. Many active chemical groups were isolated from *Arctium lappa*; include volatile oils, lignans, sesquiterpene lactones, polyynes, polysaccharides, phytosterols, tannins, flavonoids, amino acids, trace elements and many other contents. Pharmacological studies showed that *Arctium lappa* exerted many pharmacological effects including enhancement of sexual behavior, anti-fatigue, antidiabetic, antioxidant, anticancer, *Arctium lappa* extract has important role in anticancer and antipeloriferative effect (**1).**

Arctium lappa extract has Anti-inflammatory, gastroprotective, hepatoprotective and antimicrobial effect according to presentstudies which reviewed the chemical constituents and the pharmacological and therapeutic of *arctium lappa* extract **(2).**

The pancreas contains exocrine and endocrine cells. The endocrine cells secrete insulin, glucagon, and somatostatin, whereas exocrine cells are involved in the secretion of digestive enzymes. Pancreatic cancer (PC) is lethal malignancy and approximately, 95% of PC has an exocrine cell origin. It is very difficult to diagnose at an early stage due to the lack of symptoms and deep retroperitoneal of pancreas. This PC type is commonly known as pancreatic ductal adenocarcinoma (PDAC), with a 5-year survival rate of ~7.2% in the United States (US) (**3)**. PC has become the third leading cause of cancer-related deaths with an estimated new case of 55,440 and deaths of 44,330 in 2018 (**4)**PC is frequently diagnosed at an advanced stage, when the cancer has metastasized to distant organs like the liver, lung, lymph node and peritoneal cavity (**5)**. It provides a window of opportunity to diagnose and treat PC if it is detected at an early stage **(6).** To date, efforts are being made in multiple directions to develop early diagnostic test for PC including histopathological tests on fine needle aspirates, serological tests, imaging (computed tomography/magnetic resonance imaging), and analysis of genetic mutation markers **(7)**. Heavy alcohol consumption has been known to be a major cause of chronic pancreatitis and a risk factor for type 2 diabetes mellitus, both of which are linked to pancreatic cancer. It has been established that an extensive normal interaction exists between the exocrine and endocrine pancreas, as well as in inflammatory processes and carcinogenesis. Alcohol and its metabolites (acetaldehyde and fatty acid ethyl esters) can alter metabolic pathways involved in the inflammatory response and carcinogenesis, dysregulation of proliferation and apoptosis. These

various metabolic effects of alcohol can lead to or interact with other risk factors (genetic, dietary, environmental, and lifestyle factors) that result in acute and chronic pancreatitis and diabetes mellitus and, ultimately, affect the multistep process of carcinogenesis toward the development of pancreatic cancer **(8)**.

2-Materials and Methods

2.1- Materials

Ethanol was purchased from bio diagnostic company and Arctium were purchased from Harraz company were obtained from china In the form of leaves and burdock (*A. lappa*) root.

2.2Animals

25 Male albino mice (18-25 g) (age 2-3 months old) were obtained from animal house of Cancer Epidemiology, National Cancer Institute, Cairo University. Mices were fed on a standard diet and free access to tap water. They were kept for one week to be acclimatized to the environmental conditions.

2.3-plant

Preparation of ethanol extract of burdock (*A. lappa*) leaves and root. 1.5 mg/kg arctium (dissolved in the ethanol). The levolization was carried out at ARRI (Animal Reproduction Research Institutes) at -40 degree using labcolco freeze dryer USA.

2-4-Experimental Design

25 mice were divided into 2 main groups the 1st group included the normal healthy rats comprised of 10 mice while the 2nd group was experimentally induced pancreatic cancer group and comprised of 15 mice (five mice in each group).

The 1st group included the normal healthy mice were divided into 2 subgroups as follows:

Group 1: Given saline served as negative control.

Group 2: Normal healthy control rats; administered with arctium (**0.2 ml\l** /kg BW) for 14 days.

The 2nd group was experimentally induced pancreatic cancer group and comprised of Pancreatic cancer group was experimentally induced by injection with ethanol i.p.p. dose of absolute Ethanol **(80 ml\l)** Dissolved in **(20 ml\l)** distilled water for 7 days with dose was **(0.2 ml\l)**. After injection; animals had free access to food and waste rendoscopic ultrasound examination were obtained after 7 days of ethanol injection to ensure the induction of pancreatic cancer was determined comment of MRI.

Pancreatic cancer mice were classified into 3 groups 15 mice (five mice in each group). As follows:

Groups 3: Considered as a positive control for pancreatic cancer mice dose (0.2 ml /kg BW)

Groups 4: Considered as arctium extract treated for pancreatic cancer mice (0.2ml/kg BW) daily for 14 days.

Group 5: pancreatic cancer mice administered with arctium dose 0.1/ml BW) daily for 14 days.

2.5-sampling

I-blood sample

Blood samples were collected at the end of 14 days of experiment. pancrease tissue and liver were obtained after 14days of administration. Blood samples were divided into 3portions:-1-1st portion containing EDETA blood samples to used for determination of plasma C-peptide according to the method of Bonger, and Garcia-Webb, (1984), and amylase according to the method of **Rinderknecht (9).**

2nd portion containing sodium florid as anticoagulante for plasma separation to assayed blood glucose level according to the method of **Trinder (10).**

3th portion containing blood samples left 10 minutes to clot and centrifuged at 3000 rpm for serum separation, to use for MDA according to method of. Mihara **(11)**

II-Tissue sample

A- Frozen tissue for gene expression (TNF, IL1 and BCL2).

B- 10 % buffer formalin for biopsy of liver and pancreas for histopathological examination.

3. Results and Discussion

Table 1 showed the body weight of control and experimental groups of rats. A significant decrease in body weight was observed in ethenol induced pancreatic mice (Group 3) when compared to the control group of mice (Group I) ($P \leq 0.05$) arctium administered mice (Groups 4 and 5) showed progressive increase in body weight ($P \leq 0.05$).

Table 2 showed the amylase level slightly decrease with non-significant in positive group (group 3) than negative (group 1). while Administration of arctium in treated group and proflactive group tends to Significant increase between (group 4) than positive (group 3), and proflective (group 2) significant increase than negative (group 1), positive (group 3).

Table 3 showed the fasting plasma glucose and C-peptide levels in control and experimental groups of mice. There was a significant increase in plasma glucose and significant decrease in C-peptide levels of pancreatic cancer positive control group compared with normal control rats ($P \leq 0.05$). Administration of arctium tends to bring down the blood glucose and increase significantly of plasma C-peptide levels compared with untreated pancreatic cancer mice ($P \leq 0.05$).

Table 4 showed the MDA level high significantly increase in positive group (group 3) than negative (group 1). While Administration of arctium in treated group especially (group 5) tends to Significant

decrease ($P \leq 0.05$) than positive (group 3), and proflective (group 2) slightly decrease than negative (group 1), positive (group 3). the SOD activity in Positive pancreatic cancer mice (Group 3) was significantly decrease ($P \leq 0.05$) than negative normal mice (group 1), while Administration of *Arctium lappa* extract in treated group especially (group 5) tends to Significant increase ($P \leq 0.05$) than positive (group 3), and proflective (group 2) slightly increase with non-significant than negative (group 1).

Table 5 showed that the TNF gene expression, BcL2 and IL1 in control and experimental groups of mice. There was high significant increase in TNF levels in positive group than negative group. In pancreatic cancer mice administration of *Arctium lappa* extract especially (group 5) showed significant reduction ($P<0.05$) of TNF gene expression, BcL2 and IL1 compared with pancreatic cancer positive control mice. Administration of *Arctium lappa* extract as prophylactic group slightly increase than normal group in TNF and IL1 while decrease in BCL2.

Effect of arctium on body weights of mice in different groups

Parmeter (gm)	Main group	Groups	Mean+_std
Body weight	1st health group	negative	27+2.0
		prophylactic	26+2.0
	2nd pancreatic cancer	positive 0.2	14+1.0
		Positive &T 0.1	24+1. 6
		Positive & T0.2	23.5+1.5

Effect of arctium on Amylase in different group

Parameters	Main group	**Groups**	**Mean+_sd**
Amylase	1st health group	Negative	2572.5+_345.5
		proflective	2671.5+_350,5
	2nd pancreatic cancer	Positive 0.2	2493.0+_203.,5
		Positive & T0.1	2884.5+_113,5
		Positive & T0.2	2496.3+_205,.9

Effect of arctium on Random blood sugar and c-peptide indifferent group

parameter	Main group	group	Mean+_std
Random blood sugar	Health group	Negative	88.5+22.50
		proflective	124.5+26.50
	Pancreatic cancer	Positive 0.2	137.5+13.50
		Positive & T0.1	128.0+13.00
		Positive & T0.2	96.7+11.15
C-peptide	Health group	Negative	0.045+0.015
		proflective	0.015+0.005
	Pancreatic cancer	Positive 0.2	0.055+0.015
		Positive & T0.1	0.140+0.050
		Positive & T0.2	0.080+0.030

Effect of arctium on MDA (mol/ml) and SOD (U/mghb) in different group

Parameters	Main Groups	Groups	Mean ±SD
MDA (mmol/ml)	Ist Health gp.	I-negative	730.13±0.021
		2- Prophylactive	684.27±0.025
	2nd Pancreatic cancer	3-Positive 0.2	1265.37±0.026
		4- positive & T 0.1	1035.59±0.0238
		5- positive & T 0.2	927.78±0.08
SOD (U/mgHb)	Health gp.	I-negative	3.42±0.035
		II- Prophylactive	3.61±0.03
	Pancreatic cancer	I-Positive 0.2	1.45±0.021
		II- positive & T 0.1	2.54±0.031
		III- positive & T 0.2	3.15±0.015

Effect of arctiumongene expression in different group

Parameters	Main groups	Groups	Mean ±SD
TNF-α	Health gp.	I-negative	1.03±0.01
		II- Prophylactive	2.56±0.015
	Pancreatic cancer	I- Positive 0.2	10.84±0.025
		III- positive & T 0.1	5.3±0.015
		IV- positive & T 0.2	9.38±0.025
Bcl2	Health gp.	I-negative	1.03±0.015
		II- Prophylactive	0.41±0.038
	Pancreatic cancer	I- Positive 0.2	16.45±0.04
		II- positive & T 0.1	9.26±0.012
		III- positive & T 0.2	11.89±0.036
IL1	Health gp.	I- negative	1.01±0.031
		II- Prophylactive	1.36±0.01
	Pancreatic cancer	I- Positive 0.2	3.46±0.025
		II- positive & T 0.1	1.52±0.025
		III- positive & T 0.2	2.85±0.015

Histopathologic examination of liver and pancrease

Histopathological examination showed that, liver tissue in pancreatic cancer group (positive) exhibited foci of inflammatory cells in between hepatocytes and surrounding a central vein, necrosis and degenerative changes of hepatocytes hyperplaseia (photo 2). Administration of *Arctium lappa* extract due to activation of hepatocytes (some hepatocytes are slightly vacuolated) with normal nuclei and sinusoids with non-pancreatic cancer and pancreatic cancer mice (photo 1). Pancreatic tissue in pancreatic cancer (positive group) showed anaplasia and inflammation in (photo 3) while Administration of Arctium *lappa* extract due to mild inflammation with degeneration in (photo 4).

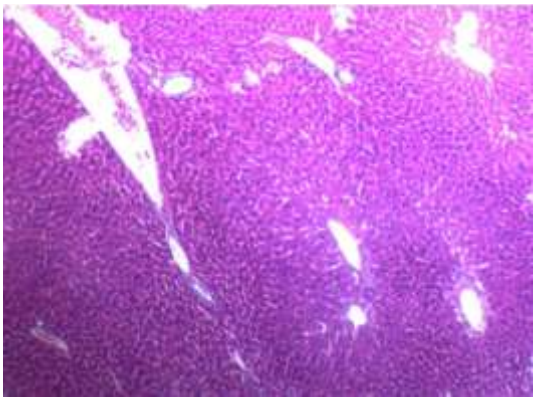

Photo 1.liver tissue in positive group

Histopathological finding in positive group hyperplasia of epithelial lining bile duct and fibrosis in the wall

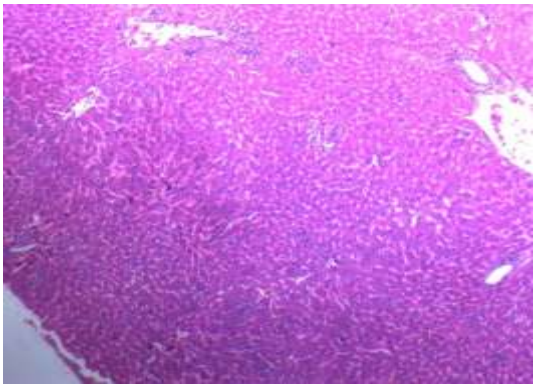

Photo 2.liver tissue with arctium treated group.

Liver of mice from positive treated group showing the normal histological structure of hepatic lobule (H & E X 400).

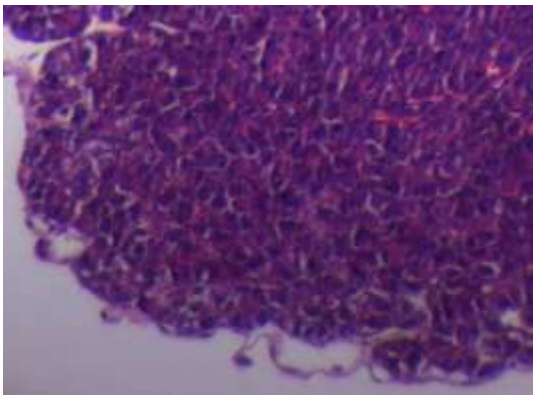

Photo 3.pancrease tissue in positive group

Photo 3 anaplasia of pancreatic tissue (H & E X 400).

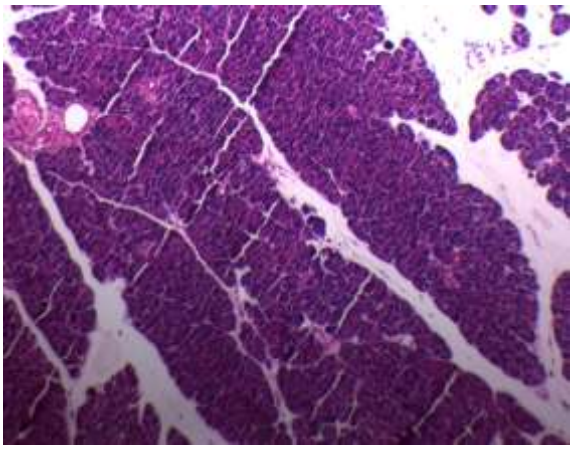

Photo 4.pancreatic tissue with arctium extract treated group

Photo 4 Pancreace of mice from *Arctium lappa* extract treated (group5) showing the mild chronic inflammation and degeneration of histological structure of pancreace (H & E X 400).

The purpose of this study was to investigate the inhibitory effects of A. lappa extract (ALE) on pancreatic cancer.

This study showed the effect of extract *Arctiumlappa* extract on body weight, amylase enzyme activity, Plasma glucose, insulin, c-peptid, oxaditive and anti-oxidant pancreatic, gene expression and histopathological examination. In normal group as proflective and in pancreatic cancer mice which induced by 80% ethanol In, this present study showed that induced Alcohol due to changes pancrease function and liver and pancrease tissue that agree with **Lowenfels and Maisonneuve (12) & Michaud, Minerva (13)** alcoholis important factor to determinant pancreatitis, a risk factor of pancreatic cancer.

Some studies noted a statistically significantly higher risk of pancreatic cancer studies have examined the association between alcohol consumption at the time of enrolment into the study and pancreatic cancer risk **(14;15).**

In, this present study in (table 1) showed that extract of burdock *Arctium lappa* root increase body weight in mice. Numerous studies have examined whether there is an association between alcohol consumption and the risk of other cancers. Evidence is accumulating that alcohol is associated with increased risks of melanoma and of prostate and pancreatic cancers ethenol induced cancer is characterized by severe loss in body weight **(16)**and this was also seen in the present study *Arctium lappa* administration controlled this loss in body weight. However, it did not normalize the body weight completely as it remained lesser than normal control mice. The decrease in body weight observed in pancreatic cancer and diabetic (increase blood glucose level) might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source **(17)**. *Arctium lappa* supplement increases protein synthesis. Thus, it is beneficial in building muscles. Moreover, it has effects on en body weight or composition in individuals with diabetes. **Hallmark et al. (18) & Mendez et al. (19)** reported that non-enzymatic glycation of albumin was the potential to alter its biological structure and function. It is mainly due to the formation of a Schiff base between amino-group of lysine (and sometimes arginine) residues and excess glucose molecules in blood to form glycoalbumin. Hypoalbuminemia is one of the factors responsible for the onset of ascites related to liver fibrosis **(20)**.In present study (table 2) ethanol group did not affect amylase activity although ethanol disordered the pancreatic plasma membranes and cause cancer. The activity of amylase related to physical factors as carbohydrates diet not at glucose level and quantity of alcohol and duration this agree with **Perry *et al*. (21)** amylase activity evolved as an adaptation to dietary habits mainly carbohydrat and consume alcohol lead to decreased amylase level according to **Nina Enberg (22).** The Chronic ethanol ingestion due to lowered the basal rate of amylase secretion that agree with the present study.bioactive compounds of phenolic nature as arctiumlappa that exhibit anti-amylase activity **(23)**. Expert opinion: Pancreatic alpha-amylase inhibitors from traditional plant extracts are a promising tool for diabetes treatment. The extracts from different parts of burdock have long been considered to be good for health. They help enhance the body's immune system and improve metabolic functions **(24).**

This present study (table3) agree with **Akram et al. (25)** that *Arctium lappa* extract decrease plasma glucose, increase insulin-c-peptid, ($p<0.01$). Administration of burdock root ethanolic extract (BRE) significantly decreased blood glucose and increased insulin level in diabetic rats compared to the control group. gamma-glucoside-fructose ester, also known as inulin, can help to regulate blood glucose levels. Inulin, a natural carbohydrate present in the root of burdock, can act on cell surface receptors to keep the blood glucose level constant, therefore improving the tolerance to high glucose level. Also, the production of short chain fatty acids is also increased **(26).**

Reduced insulin sensitivity also participates in lipid metabolism and hyperglycemia. Hypertriglyceridemia was demonstrated to be connected to liver disorder and increased FFA levels, which are responsible for increased insulin-peptide levels **(27).**

The present results in (table4) reveal the treatment of pancreatic cancer mice with *Arctium lappa* strengthens the endogenous antioxidant defenses from ROS and restores the optimal balance by neutralizing the reactive species. They are gaining a great importance by virtue of their critical role in disease prevention. *Arctium lappa* is known to have antioxidant and antidiabetic effects in traditional medicine.

The main mechanism of burdock's anti-decrepitude effect involves improvement of SOD activity and reduction of MDA. Aburdock could be decrease of oxidative stress by increasing superoxide dismutase (SOD) activity and by decreasing malondialdehyde (MDA). Antioxidants has also been found in the *Arctium lappa* root. Antioxidants and antidiabetic compounds have also been found in the *Arctium lappa* root. In the seeds, some active compounds possess anti-inflammatory effects and potent inhibitory effects on the growth of tumors such as pancreatic carcinoma.

Many studies have addressed the importance of antioxidants as for the control of the abnormalities in diabetic tissue **(28; 29)**

Arctigenin, an active compound found in the seeds of burdock, has the ability to eradicate nutrient-deprived cancer cells **(30).** It inhibit cancer growth by improvement antioxidant according to **Zheng et al. (2)**

In, this present study (table 5) showed that and agree with several studies have shown its biological activities as an anti-inflammatory agent, there have been no reports on *A. lappa* with regard to regulatory role in inflammasome activation. The activation of caspase-1, which generates the biologically active cytokines IL-1β and IL-18. Active caspase-1 can also induce a kind of cell death known as pyroptosis through the cleavage of the cytosolic substrate gasdermin **(31).**

Tuli *et al*. (32)these results indicate that *Arctium lapp* extract mediates apoptosis via Bcl-2. In order to achieve these changes, burdock root extract appears to induce differential expression of genes in mice.

Component of *A. lappa* in the majority of studies Arctigenin (AR)

Exhibits potent anti-inflammatory activities by inhibiting inducible nitric oxide synthase (iNOS) via modulation of several cytokines. Due to its potent anti-inflammatory effects, AR may serve as a potential therapeutic compound against both acute inflammation and various chronic diseases. AR are suggested to promote its ability to serve as a therapeutic agent as well as an ideal bioactive marker for *A. lappa*.

Arctigenin exhibits anti-inflammatory effects by inhibiting the exudation and recruitment of leukocytes into inflamed tissues via reducing the release/production of inflammatory mediators and also exhibits neuro protective activity through reducing surplus ROS production and down regulating the mitochondrial membrane potential administration of *Arctiumlappa* extract as proflactive, prophylactic group slightly increase than normal group in TNF and IL1 while decrease in BCL2.

Chronic tissue inflammation involving the generation of cytokines (e.g., interleukin-6 and tumor necrosis factor-alpha). Natural *A. lappa* fruit extract significantly improves the metabolism of the dermal extracellular matrix and leads to a visible wrinkle reduction in vivo. anti-inflammatory (i.e., reduction of interleukin-6 and tumor necrosis factor-alpha).

In conclusion, the present study demonstrates the antiglycemic, antioxidant effects of *Arctiumlappa*. The present data reveal that *Arctiumlappa*-treatment to ethanol–pancreatic cancer mice produce a remarkable amelioration of body weight, plasma glucose, C-peptide and amylase and gene expression (TNF, BCL2 and IL2) Thus, and also this study showed that the burdock aqueous extract exhibits hypolipidemic activity in hyperlipidemic mice. This activity is practically that of a triple-impact antioxidant, hypolipidemic, and hepato tissue protective. The burdock can defend cells from fat accumulation and oxidative stress. These effects are clearly derived from its high levels of flavonoids and polyphenolics. The safely promising therapeutic dose used in the currents study, can be effective in treatment and enhanced levels of *Arctiumlappa* may use as candidate Inantipancreatic cancer drugs.

A. lappa potential anti-inflammatory effects and might be developed as a therapeutic application - associated inflammatory disorders.

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