BioCore Exploring Scientific Community

International Journal of Biotechnology and Bioengineering

Research Article ISSN 2475-3432

Propolis -Induced IL-22 Ameliorated the Inflammatory Response in Experimental Acute Pancreatitis in Male Rats.

Tharwat A.G.Eldin*

**Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal university,Dammam, Saudi Arabia.*

Abstract

Bee propolis which is a natural resinous product collected by honey bees from plants, is used as folk medicine since ancient time. propolis has been shown to produce various beneficial biological and pharmacological effects due its immunomodulatory , anti-inflammatory, antioxidant and antimicrobial properties which have been investigated extensively in the last decade. A total number of 30 male albino Wistar rats 150-250 gm were conducted in the present study .They divided into 3 equal groups 10 rats each: (1)The negative controls ,they did not receive any treatment, (2)The positive controls with experimental acute pancreatitis(AP) by L-Arginine injections), they did not receive any treatment (3) Group with AP and treated with Propolis .The following investigations were done to test the anti-inflammatory and anti-oxidant effects of propolis : (1) proinflammatory cytokines : (IL-1β, IL-6, TNF-α) and anti-inflammatory cytokine: IL-22.(2) serum lipase and Amylase.(3) thiobarbituric acid-reactive substances (TBARS): to detect lipid peroxidation . Propolis showed a strong anti-inflammatory and anti-oxidant effects by attenuation of the severity of acute inflammation of the pancreatic tissues of rats with L-arginine-induced AP through inhibition of the secretions of proinflammatory cytokines and also a novel result of this study was the finding that propolis significantly increased levels of IL-22, which had not been tested in previous studies .Therefore, propolis and IL-22 could be considered as a potential treatment for AP in humans,which needs more investigations for side effects and tolerance.

Keywords: Propolis – Acute pancreatitis-L-Arginine-TBARS-Interleukins – Amylase -Lipase.

Corresponding author: Tharwat A.G.Eldin

Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal university, Dammam, Saudi Arabia.

E-mail: taeldin@iau.edu.sa

Citation: **Tharwat A.G.Eldin. (2020),** Propolis -Induced IL-22 Ameliorated the Inflammatory Response in Experimental Acute Pancreatitis in Male Rats. Int J Biotech & Bioeng. 6:7

Received: November 12, 2020
Accepted: November 16, 2020
Published: November 26, 2020

Introduction:

Pancreatitis is a major gastrointestinal problem worldwide. Acute pancreatitis (AP) is a sudden pancreatic inflammation accompanied by an excessive reactive oxygen species production that provokes inflammation. maldigestion and abdominal pain [1].

In the majority of cases, it is mainly caused by gallstones or heavy alcohol use while other causes include medications, infections, trauma, metabolic disorders and surgery. In up to 30% of people with acute pancreatitis, the cause is unknown(idiopathic) [2]. Despite the development of new therapeutic and diagnostic approaches, the clinical course of acute pancreatitis (AP) is associated with significant high mortality rates [3].

Experimental studies in animal models were focused on the molecular pathway, including proinflammatory cytokines, and shedding light on the pathophysiologic mechanisms of AP[4]. Increased levels of proinflammatory cytokines such as interleukin (IL)-1, IL-6 and tumour necrosis factor-alpha (TNF-α) aggravate AP by increasing vascular permeability [5].The disease is usually associated with Oxidative damage to pancreatic cells with the release of pancreatic digestive enzymes into the pancreatic interstitium and to the systemic circulation with increased proinflammatory cytokine production[6].These cytokines act locally with the released pancreatic enzymes to aggravate acute pancreatitis [7]. Oxidative stress is regarded as a major pathogenic factor in acute pancreatitis. In human acute pancreatitis, the

increased levels of lipid peroxide in pancreatic tissue and subnormal levels of antioxidant vitamins in the blood were reported [8]. Among several animal models of experimental pancreatitis that exhibit the biochemical, morphological, and pathophysiological similarities to various aspects of human pancreatitis, L-arginine pancreatitis was shown to be one of the best-characterized , more selective and widely used experimental models [9]. L- Arginine produces large amounts of reactive oxygen species (ROS) activates oxidant-sensitive nuclear transcription factor NF-κB and thus induces cytokine expression in freshly isolated pancreatic acinar cells without inflammatory cells in vitro [10].

Propolis is a natural resinous compound collected by bees from the gum of various plants Propolis has attracted global attention for its wide range of pharmacological and biological properties, making it a potentially promising therapeutic agent. The efficacy of propolis depends mainly on the presence of flavonoids, primarily caffeic acid phenethyl ester (CAPE), which provide an anti-inflammatory and anti-oxidant effects.[11]. Many studies have shown that the anti-inflammatory activity of propolis inhibit the activation of cyclooxygenase (COX)-2 gene expression, suppress enzyme activities of COX-1 and COX-2 and inhibit the release of arachidonic acid from cell membranes. [12]. IL-22, a member of the IL-10 family, is a cytokine secreted by several types of immune cells such as T helper (Th) 22, Th1, and Th17 cells, γδ T cells, natural killer T cells, and innate lymphoid cells. It is a principal component in mucosal barrier defense, tissue repair, epithelial cell survival, and proliferation [13]. In cases of AP, IL-22 production was found to be increased while, the receptor expression is restricted to leukocytes and epithelial cells, respectively [14]. IL-22 is recognized today as a key player in the antimicrobial , anti-inflammatory defense, regeneration, and protection against damage [15]. The aim of the present study was to investigate the possible therapeutic effects of propolis against destructive and oxidative changes of acute pancreatitis induced by L-Arginine .Also , to find out the mechanism of action of propolis as anti-inflammatory agent. The aim was reached via measurement of the following parameters :

Serum pancreatic enzymes(lipase and amylase), Serum biochemical inflammatory mediators (interleukins): IL-1B, IL-6 and the anti-inflammatory cytokine IL-22., Serum TNF- α, and Serum Lipid peroxidation products (TBARS) .

Methods :

The present study was conducted at Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. A total of 30 male albino Wistar rats weighing 150–250 g were obtained from the university's animal house for this study. All rats were maintained in a room at a constant temperature of 22 ± 1°C with 12 hour light/dark cycles and had free access to standard laboratory food pellets and water.

Rats were equally divided into three groups. Group-1 was the undiseased untreated negative control group, group 2 consisted of the diseased untreated positive controls and group 3 was the diseased and treated group.Rats of group 2 and 3 were injected with L-arginine to induce AP. Two intraperitoneal (IP) injections of L-arginine (Sigma-Aldrich Chemical, Merck KGaA, Darmstadt, Germany) at a dose of 250 mg/100 g of body weight (BW) prepared in isotonic saline (20% 0.15 M sodium chloride) were administered at a one-hour interval to induce AP[9]. The rats in group 3 were treated orally with Brazilian green propolis alcohol extract (Uniflora Apicultores Associados Ltda, Olímpia, Brazil) 100 mg/kg of BW after two hours of L-arginine injection and daily for seven days.[16] This dose was previously shown to be anti-inflammatory dose [17]. AP was diagnosed clinically as rats became sluggish and lethargic with anorexia and weight loss as well as the serum levels of lipase and amylase. The condition was most severe 72 hours after the L-arginine-injections . The treatment regimens were stopped after seven days and 12 hours before the rats were anaesthetised with an IP injection of ketamine (50 mg/kg of BW; Alfasan International BV, Woerden, the Netherlands). Rats were euthanised and blood was collected from the abdominal aorta by means of a vacutainer .The levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α were assessed by enzyme-linked immunosorbent assay (ELISA), kits supplied from Bio-Rad Laboratories Inc., Hercules, California, USA [18]. The levels of IL-22 were measured by ELISA Kit supplied from R&D Systems, Minneapolis, Minnesota, USA[19] .Amylase and Lipase : by ELISA kits using an ELISA reader[20]. lipid peroxidation product level : thiobarbituric acid-reactive substances (TBARS): to detect lipid peroxidation which is a well established mechanism of cell membrane injury.The measurement of TBARS was done by Cayman chemical TBARS assay kit[21].

Statistical analysis :was performed using Statistical Package of Social Science (SPSS), Version 21 (IBM Corp., Armonk, New York, USA). Data are presented as means ± standard error of the mean. One-way analysis of variance followed by Tukey's multiple comparison post-hoc test was used to compare the means. Statistical significance was set at $P < 0.05$.

All experiments were performed in accordance with the recommendations of the national guidelines for the care and handling of laboratory animals. The experimental protocol was approved by the Local Animal Ethics Committee .

Results :

From **table(1)** : the means±S.D of serum concentrations of **IL-1-B** of the 3 studied groups were 36.393±pg/ml, 116.13±4.28 and 41.43±4.01 respectively, showing a statistically significant increase in group 2 (positive control) than the other groups indicating a significant effect on the propolis treated group which was found near the negative control.

From **table(1)**: the means± S.D of serum concentrations of **IL-6** of the 3 studied groups were 39.021±3.13 and 85.4±3.39 and 45.2±2.05 respectively, showing a statistically significant increase in group 2 (positive control) than the other groups indicating a significant effect on the propolis treated group which was found near the negative control.

From **table(1)** : the means± S.D of serum concentrations of **IL-22** of the 3 studied groups were 0.1927±0.020 , 0.1565±0.023 and 0.3053±0.056 respectively, showing a significant increase in group 3 (propolis treated) than the other groups indicating a significant effect on the propolis treated group .

From **table(1)**: the means± S.D of serum concentrations of **TNF- α** of the 3 studied groups were 59.748±1.40, 96.9±2.51and 48.8±1.51 respectively, showing a significant increase in group 2 (positive control) than the other groups indicating a significant effect on the propolis treated group which was found insignificantly lower than the

	Negative Control G1	L-Arginine(Positive Control) G2	Propolis treated G3	P value
IL-1β pg/ml	36.393±3.07	116.13±4.28*	41.43±4.01	<0.05
IL-6 pg/ml	39.021±3.13	85.4±3.39*	45.2±2.05	<0.05
IL-22 pg/ml	0.1927±0.020	0.1565±0.023	0.3053±0.056*	<0.05
TNF-α pg/ml	59.748±1.40	96.9±2.51*	48.8±1.51	<0.05

Table(1) shows the means± S.D of the proinflammatory cytokines(IL-1-B , IL-6 and TNF- α) and anti-inflammatory IL-22 in the three studied groups.

	Negative Control	Arginine(Positive Control)	Propolis treated	P value
Serum amylase U/L	150.4±3.22	1535.9±4.78***	154.9±2.66	<0.001
Serum lipase U/L	49.2±2.9	605.2±3.11**	60.8±2.45	<0.01
Serum TBARS umol/L	53.9±3.12	94.9±2.45*	41.8±1.8	<0.05

Table(2) shows the means± S.D of serum amylase, lipase and TBARS in the three groups.

negative control group .

From **Table (2)**: the means±S.D of serum concentration of **Amylase** of the 3 studied groups were 49.2±2.9, 605.2±3.11 and 60.8±2.45 respectively, showing a very highly statistically significant increase in group 2 (positive control) than the other groups indicating a significant effect on the propolis treated group which was found insignificantly higher than the negative control.

From **Table (2)**: the means±S.D of serum concentration of **lipase** of the 3 studied groups were 150.4±3.22, 1535.9±4.78 and 154.9±2.66 respectively, showing a highly statistically significant increase in group 2 (positive control) than the other groups indicating a significant effect on the propolis treated group which was found insignificantly higher than the negative control.

From **Table (2)**: the means±S.D of serum concentration of **TBARS** for the 3 studied groups were 53.9±3.12, 94.9±2.45 and 41.8±1.8 respectively, showing a statistically significant increase in group 2 (positive control) than the other groups indicating a significant effect on the propolis treated group which was found insignificantly lower than the negative control.

Discussion :

Previous studies have used experimental AP induced by L-arginine to study the effect of various therapeutic agents and the pathophysiologic mechanisms of the disease.[22] The present study aimed to determine the effects of propolis on immune mediators and its anti-inflammatory and antioxidant effect in rats with AP. The effect of propolis was evidenced by a decrease in proinflammatory cytokines (TNF-α,IL-1-B , and IL-6), increase the levels of anti-inflammatory cytokine IL-22, decreased levels of pancreatic enzymes and the protective strong antioxidant effect through the significant decrease of TBARS in the propolis treated group which was detectd before to ameliorate the oxidative stress in the pancreas of experimentally diabetic rat [16].

Interestingly, an important and novel result of the present study was the finding that propolis significantly increased the levels of the anti-inflammatory cytokine IL-22, which had not been tested in the previous studies.This result added more infomations about the mechanism of the anti-inflammatory action of propolis which still not fully understood. A study was suggested that propolis has an anti-inflammatory effect through the inhibition of the release of histamine, prostaglandins and leukotrienes.[23] Another study reported that the anti-inflammatory properties of propolis are due to CAPE as it exerts its anti-inflammatory actions by suppressing the inflammatory enzyme activities of COX-1 and COX-2 and inhibiting the release of arachidonic acid from cell membranes [24]. The benificial effect of propolis on experimental acute pancreatitis induced by cerulein was studied before , a significant beneficial effect of propolis was found in this study[22]. So it is necessary to test this natural agent again in a L-arginine induced pancreatitis which is more similar to the human acute pancreatitis to support this finding which was done in the present study which showed non-significant minimal change in IL-22 levels between groups 1 and 2, but there was a significant increase of IL-22 in group 3 (propolis treated group) which may be mediated via

the signal transducer and activator of transcription STAT3 signaling pathway in which exogenous recombinant IL-22 protected mice from L-arginine-induced AP[25]. The favourable effect of IL-22 is thought to depend on the extent of AP inflammation. In mild cases of induced AP, administration of exogenous IL-22 successfully aborted the development of the disease, while it ameliorated the more severe cases [15]. Furthermore, over-expressed animal models of IL-22 were resistant to AP development [26] .It was reported that the administration of the anti-IL-22 anti-body, to block the receptors, endogenously aggravated pancreatic injury which supports the essential role of IL-22[27]. Collectively, these findings strongly suggest that IL-22 plays a vital role in AP prevention and amelioration. Furthermore, IL-22 may mediate a protective effect against L-arginine-induced AP via activation of the STAT3 signaling pathway, which can suppress apoptosis by inducing downstream genes, including Bcl-xL and Bcl-2[26].

Conclusion :

Propolis attenuated the severity of inflammation of the pancreatic tissues of rats with L-arginine-induced AP through the induction of secretion of the anti-inflammatory cytokine IL-22. Therefore, propolis and IL-22 could be considered as a potential treatment for AP in humans,which needs more investigations for side effects and tolerance.

Conflict of Interest :

The authors declare no conflicts of interest.

Funding

Financial support was received from King Abdulaziz City of Science and Technology, Riyadh, Saudi Arabia.

References :

1-Forsmark CE, Vege SS, Wilcox CM.(2016), Acute pancreatitis. N Engl J Med; V : 375 p: 1972-1981.

2- Banks PA, Freeman ML.(2006) , Practice Parameters Committee of the American College of Gastroenterology. Practice guidelines in acute pancreatitis. Am J Gastroenterol. ;101:2379–400.

3-Banks PA, Conwell DL, Toskes PP.(2010), The management of acute and chronic pancreatitis. Gastroenterol Hepatol (NY) v : 6 (2 Suppl 3): 1-16.

4- Kusske AM, Rongione AJ, Reber HA. (1996) , Cytokines and acute pancreatitis Gastroenterology.V:110:639–42.

5- Gukovskaya AS, Gukovsky I, Zaninovic V, Song M, Sandoval D, Gukovsky S, et al.(1997), Pancreatic acinar cells produce, release, and respond to tumor necrosis factor-alpha. Role in regulating cell death and pancreatitis. J Clin Invest. ;100:1853–62.

6- Pooran N, Indaram A,Singh P, Bank S.(2003) ,Cytokines (IL-6, IL-8, TNF): Early and reliable predictors of severe acute pancreatitis.J Clin Gastroenterol. 37:263–66.

7-Whitcomb DC. (2006), Clinical practice. Acute pancreatitis N Engl J Med 354: 2142-2150

8- Sweiry J, Mann G.(1996), Role of oxidative stress in the pathogenesis of acute pancreatitis. Scandinavian Journal of Gastroenterology.;31(sup219):10-5.

9- Dawra R, Saluja AK. L.(2012), arginine-induced experimental acute pancreatitis. Pancreapedia: Exocrine Pancreas Knowl Base. doi: 10.3998/panc.2012.6.

10-G. Biczo, E.T. Vegh, N. Shalbueva. et al.(2018),Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models. Gastroenterology,V: 154 pp. 689-703.

11- Greenaway W, Scaysbrook T, Whatley FR.((1990), The composition and plant origins of propolis: A report of work at Oxford. Bee World. ;71:107–18.

12- Rossi A, Ligresti A, Longo R, Russo A, Borrelli F, Sautebin L.(2002), The inhibitory effect of propolis and caffeic acid phenethyl ester on cyclooxygenase activity in J774 macrophages. Phytomedicine.V:9(6):530–5.

13- Kotenko SV.(2001), Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rbeta]) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. J. Biol. Chem. V;276:2725–2732.

14- Shioya, M., Andoh, A., Kakinoki, S., Nishida, A. & Fujiyama, Y.(2008), Interleukin 22 receptor 1 expression in pancreas islets. Pancreas 36, 197–199 .

15- Huan C, Kim D, Ou P, Alfonso A, Stanek A.(2016), Mechanisms of interleukin-22's beneficial effects in acute pancreatitis. World J Gastrointest Pathophysiol.V : 7:108–16.

16- Al-Hariri M, Eldin TG, Abu-Hozaifa B, Elnour A. (2011),Glycemic control and anti-osteopathic effect of propolis in diabetic rats. Diabetes Metab. Syndr. Obes. V;4:377–84.

17- Park EH, Kahng JH.(1999), Suppressive effects of propolis in rat adjuvant arthritis. Arch Pharm Res. V: 22:554–8.

18. Frossard JL, Hadengue A, Pastor CM.(2001), New serum markers for the detection of severe acute pancreatitis in humans. Am J Respir Crit Care Med. V:164:162–70.

19. Shukla R, Santoro J, Bender FC, Laterza OF. (2013), Quantitative determination of human interleukin 22 (IL-22) in serum using Singulex-Erenna® technology. J Immunol Methods. V :390:30–4.

20- Treacy J, Williams A, Bais R, Willson K, Worthley C, Reece J, et al.(2001), Evaluation of amylase and lipase in the diagnosis of acute

pancreatitis. ANZ journal of surgery V:71(10):577-82.21-Draper H, Hadley M. (1990), Malondialdehyde determination as index of lipid peroxidation. Methods in enzymology. V:186:421-31.

21- Feng D, Park O, Radaeva S, Wang H, Yin S, Kong X, et al.(2012), Interleukin-22 ameliorates V
V :8:249–57.

22- Büyükberber M, Savaş M, Bağci C, Koruk M, Gülşen MT, Tutar E, et al. (2009),The beneficial effect of propolis on cerulein-induced experimental acute pancreatitis in rats. Turk J Gastroenterol. V :20 P:122–8.

23-Hu F, Hepburn HR, Li Y, Chen M, Radloff SE, Daya S.(2005), Effects of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models. J Ethnopharmacol. V:100:276–83.

24- Michaluart P, Masferrer JL, Carothers AM, Subbaramaiah K, Zweifel BS, Koboldt C, et al.(1999), Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase- 2 in human oral epithelial cells and in a rat model of inflammation. Cancer research. V;59:2347–52.

25- Sonnenberg GF, Fouser LA, Artis D. 1(2010), Functional Biology of the IL-22-IL-22R Pathway in Regulating Immunity and Inflammation at Barrier Surfaces. Advances in immunology. V:107:1.

26-. Qiao YY, Liu XQ, Xu CQ, Zhang Z, Xu HW.(2016), Interleukin-22 ameliorates acute severe pancreatitis-associated lung injury in mice. World J Gastroenterol. V;22:5023–32.

27-Xue J, Nguyen DT, Habtezion A.(2012), Aryl hydrocarbon receptor regulates pancreatic IL-22 production and protects mice from acute pancreatitis. Gastroenterology. V;143:1670–1680.