RESEARCH ARTICLE

Editorial Process: Submission:12/07/2022 Acceptance:04/18/2023

Can *Echinococcus Granulosus* Infestation Prevent Pancreatic Cancer? An In vivo Experimental Study

Serhat Doğan[1], Murat Çakir[2], Adil Kartal[2], Haydar Öztaş[3]*, Pembe Oltulu[4]

Abstract

Hydatid cyst is a zoonotic infestation caused by *Echinococcus granulosus*, and it is known that some parasites found in humans cause cancer in humans or some may have a protective effect against cancer. Cancer is one of the most serious health problems of today and it has been shown in some studies that parasites such as *Echinococcus granulosus* can have an inhibitory effect. The aim of this study was determined as whether *Echinococcus granulosus* has an inhibitory effect on exocrine pancreatic cancer with the help of the azaserine-rat model used in different cancer studies. Material and Methods: During experimental process a total of 45 male Wistar rats used, 14-day-old male Wistar rats were divided into groups according to the experimental protocol, administered azaserine injection protocol or kept as a control group without azaserine injection. Animals are grouped as Group 1, Control Group (group not treated with Azaserine and not injected with protoscolex.) (E-A-) (n=7); Group 2, Group injected with (IP) Azaserine only (30mg/kg) (E-A+) (n=8);Group 3, Group injected (IP) with protoscolex suspension of 1 cc only (E+A-) (n=15);Group 4, Group injected both Azaserine (IP) and protoscolex suspension (IP) (E+A+) (n=15). Atypical Acinar Cell Foci (AACF) load in the exocrine pancreas of each rat was measured quantitatively with the help of a video image analyzer and the AACF load was calculated with the help of a mathematical model. **Results:** Findings showed that the Atypical Acinar Cell Foci (AACF) burden was statistically significantly lower in the Azaserine+ protoscolex (Azaserine-injected-protoscolex-implanted) rat group compared to the other groups, suggesting that Echinococcosis in the azaserine-rat model could inhibit the development of precursor foci of neoplastic changes in the exocrine pancreas. **Conclusion:** The most significant aspect of our study is that it contributes new insights into the controversy that Echinococcosis suppresses pancreatic cancer.

Keywords: *Echinococcus granulosus*- azaserine- rats- pancreatic cancer

Asian Pac J Cancer Prev, **24 (4)**, 1307-1312

Introduction

Hydatid cyst is a zoonotic infestation caused by *Echinococcus granulosus* (EG). It is widely seen in the Mediterranean countries, the Middle East, South America, New Zealand, Australia and South Eastern Asia (Karaman et al., 2011; Lynen et al., 2011). In addition to this, cancer is one of the most significant healthcare problems today. There are also articles reporting that parasites can lead to or inhibit cancer in humans (Akgül et al., 2003; Alvarez-Errico et al., 2001). However, it is not known exactly what kind of histopathological change parasites bring about in the pancreas in vivo animal experiments (Rhim and Stanger, 2010; Noya et al., 2013).

It is assumed that pancreatic ductal adenocarcinoma (PDAC) completes its developmental process from a precursor lesion into cancer through multiple stages. Pancreatic intraepithelial neoplasia (PanIN) is a precursor lesion frequently detected in the pancreas of pancreatic cancer patients (Longnecker and Curphey, 1975; Rhim and Stanger, 2010; Vincent et al., 2011; Ji et al., 2009; Gourgou-Bourgade et al., 2011). It has been found that preneoplastic structures in the pancreas were seen in 80% of these cases (Hruban et al., 2008). KRAS oncogene is a well-known mutant gene that leads to pancreatic cancer. KRAS oncogene leads to the inactivation of tumor suppressor genes CDKN2A, TP53, DPC4 and BRCA2.

Loss of chromosomes, gene amplification and telomere contraction are usually observed anomalies in pancreatic cancer cases in which activation of this gene is involved (Vincent et al., 2011). It has been reported that antigenic properties of tumor and EG infestation were similar (Alvarez et al., 2001). In another study, it has been demonstrated that mucin-like peptides occurring due to

*[1]Malatya Turgut Özal University, Faculty of Medicine, Department of General Surgery, Malatya, Turkey. [2]Necmettin Erbakan University, Meram Medical School, Department of Surgery, Konya, Turkey. [3]Necmettin Erbakan University, Ahmet Keleşoğlu Faculty of Education, Department of Biology, Konya, Turkey. [4]Necmettin Erbakan University, Meram Medical School, Department of Pathology, Konya, Turkey. *For Correspondence: haydar_oztas@yahoo.com.tr*

EG induce the increase of antitumor activity (Noya et al., 2000). EG is claimed to have antigenic properties similar to cancerous cells. This trait is thought to be protective against the development of cancer (Noya et al., 2013). The aim of this study was determined as whether *Echinococcus granulosus* has an inhibitory effect on exocrine pancreatic cancer with the help of the azaserine-rat model used in different cancer studies. It was main subject of research that whether experimentally induced *Echinococcus granulosus* in rats' pancreas may inhibit pancreatic cancer precursor focus (AACF).

Materials and Methods

The study was carried out following the approval of the Experimental Animals Ethics Committee of the Experimental Medical Research and Implementation Center (Ethics Committee Approval No 2013-207). 45 male Wistar albino rats raised in Experimental Medical Research and Implementation Center were used. The study lasted 6 months. Male inbred Wistar strain rats were obtained from N. Erbakan University Animal House and kept five animals to a cage under standard conditions (room temperature 23 C; lighting 7 am-7pm), on sawdust bedding. Standard diet and tap water were supplied ad libitum. As experimental materials, azaserine (98% purity), hematoxylin, eosin were obtained from Sigma Chemical Co. Ltd (USA) acetone, formalin, HCl, methanol, and chloroform were obtained from Sigma-Aldrich Chemicals (USA).

Experiment protocol

In this study, using the azaserine-rat model previously developed by Longnecker and Curphey (1975), it was investigated whether *Echinococcus granulosus*, a common internal parasite in humans and animals, has an inhibitory effect on the development of AACF, which is known as a potential precursor of neoplastic changes in the exocrine pancreas.

Pancreatic cancer rat model

Azaserine, which is a chemical well known to have a mutagenic feature in the induction of cancer in the rat pancreas, was employed in order to induce hyperplastic differentiation in acinar cells of subjects (Noya et al., 2000). The azaserine-rat model, developed by Longnecker and Curphey (1975) to examine experimental exocrine pancreatic changes in rats, has recently been used by many researchers to investigate the origin of pancreatic acinar cell foci (AACF) (Khoo et al., 1991; Yıldız et al., 2013, 2019; Kalıpcı et al., 2012; 2013; Yener et al., 2013). In order to initiate neoplastic differentiation induced by means of EG infestation in the liver of rats, the rats were intra-peritoneally (IP) injected with 30 mg/kg azaserine for three weeks successively, dissolved in 0.9 % NaCl solution on the day of injection. After for three consecutive weeks azaserine injected and untreated rats randomly divided into experimental groups as below; Group 1: Control Group (group not treated with Azaserine and not injected with protoscolex.) (E-A-) (n=7); Group 2: Group injected with (IP) Azaserine only (30mg/kg) (E-A+) (n=8);Group 3: Group injected (IP) with protoscolex suspension of 1 cc only (E+A-) (n=15); Group 4: Group injected both Azaserine (IP) and protoscolex suspension (IP) (E+A+) (n=15).

At the end of the 6-month period the rats in each experiment group were weighed on precision scales. Before sacrification, all animals were anesthetized with 50mg/kg (sc) Ketamine HCl and 15 mg/kg xylasin HCL (sc) and the rats were sacrificed by cervical dislocation. Each experimental animal was laparotomies. Pancreatic tissue was found and dissected. The entire rat pancreas was excised at autopsy and all adherent fat, mesentery and lymph nodes were carefully trimmed off. The wet weight of each pancreas was recorded before fixation in 10% buffered neural formalin. Before immersion in fixative solution each pancreas was spread out on a piece of porous paper to ensure maximal trans-sectional area for subsequent sectioning. Routine hematoxylin and eosin stains were applied to sections of 5 μm thicknesses from the prepared blocks with a rotary microtome (Thermo Scientific, Shandon Finesse 325). Histological sections were stained with haematoxylin & eosin and were examined by a light microscopy (ZEISS Axio Lab. A1). Foci in the sections were identified according to established criteria (Rao et al., 1982; Roebuck et al., 1984). According to the criteria's of Longnecker (1987) carcinogen-induced pancreatic lesions were atypical acinar cell foci (AACF). Therefore, AACF has been evaluated as a possible precursor cancer cell growth in rat exocrine pancreas.

Quantitative estimation of Atypical Acinar Cell Foci (AACF) load in pancreatic preparations stained with hematoxilen-eosin can provide useful information about precancerous lesions in the pancreas of rats used for experimental purposes. For this purpose the total area of exocrine pancreatic tissue was measured from each pancreas by means a video image analyzer (ZEISS Axio Lab.A1.) This system includes a microscope, video camera and data capture software. The same instrument was used to count acidophilic AACF via measure their transactional area. The observed data were proceed numerically by a computer software package (ZEISS Axio Lab.A1), which uses an algorithm based on the mathematical formula of Campel et al., (1982). As modified Pugh and his coworkers (1993). The total number of foci in whole pancreas was derived by multiplying the weight of pancreas. The number of foci, mean values and either standard errors of means or ranges were determined for all data. Non-parametric statistical analyses were performed using the Kruskal-Wallis one-way analysis of variance and Mann-Whitley U-test.

Echinococcus granulosus infestation

Live protoscoleces extracted from the cyst of animals with cystic hydatid to be sacrificed were brought to the laboratory in eau de roche in cold chain and secondary hydatidosis was formed by means of 1 ml IP injection. The vitality of protoscoleces was confirmed by means of hematoxylin–eosin stain (Burgu, 1975). After the protoscoleces were detected on the preparations, they were stained with eosin dye and it was decided whether

they were alive or not according to the color change. The protoscoleces that were not stained were considered live (Figure 1a), those that were stained were considered dead (Figure 1b). The dead cell was stained since its cell membrane was not fully functional and thus the dye could permeate the membrane (Tezok et al., 1970; Kazez et al., 2000).

Results

The main body weights of experimental group are shown at Table 1. The differences were observed between only azaserine initiated group and others. There was a decrease in the mean body weight of Azaserine control with compared Untreated controls and Echinococus implanted rats. The mean weight of experimental animals at the end of the sixth month was 347.5 gr. A statistically significant loss of body weight was observed in the subjects in Group 2 (E-A+) compared to other groups ($p<0.05$) (Figure 2). The mean pancreas weight of the groups was 0.86 gram. There was no significant difference in the pancreas weights of the groups ($p>0.05$) (Figure 3). The number of AACF was higher only in the group injected with Azaserine (Group 2). The number of atypical acinar cell foci (AACF) was significantly different ($p< 0.05$) in the preparates developed in the pancreases of the group injected with Azaserine (Group 2) and the group injected with Azaserine + Protoscolex (Group 4) (Figure 4).

Acidophilic AACF have been identified in the pancreas section in azaserine treatment groups. In the majority of these lesions the cells have a zymogen-rich cytoplasm and basal nucleus, round to oval in shape, which is slightly larger than normal and contains a prominent nucleolus (Figure 5a, b). The individual cells are usually smaller than normal parenchymal cells, resulting in an appearance of increased cellularity. Acidophilic foci are generally composed of larger acini then normal acinar cells. The number of AACF in Group 2 was established to be 25 on average for each preparate. The number of AACF in Group 4 (A+E+) was observed to be merely 6. However, no AACF foci were detected in Group 1 (Control Group) and Group 3 (E+ A-) (Figure 4). This led us to assume at least that EG infestation played no role in the development

Table 1. The Main Body and Pancreatic Weights of Experimental Groups

	Control (n=7)	Azeserine (n=8)	Echinococcus (n=15)	Azaserine + Echinococcus (n=15)
Mean Body weights (gr)	353.2857±2,13809	348.0±2,50713 *	353.0±2,61861	351.4667±3,13657
Mean pancreatic weights (g)	0.8614±0,02968	0.8613±0,02031	0.8621±0,02293	0.8607±0,02056

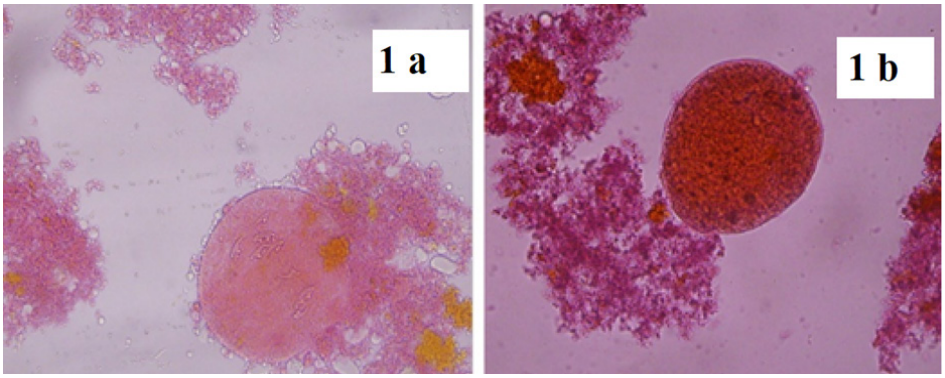

Figure 1. a, Live protoscoleces ; b, dead protoscoleces

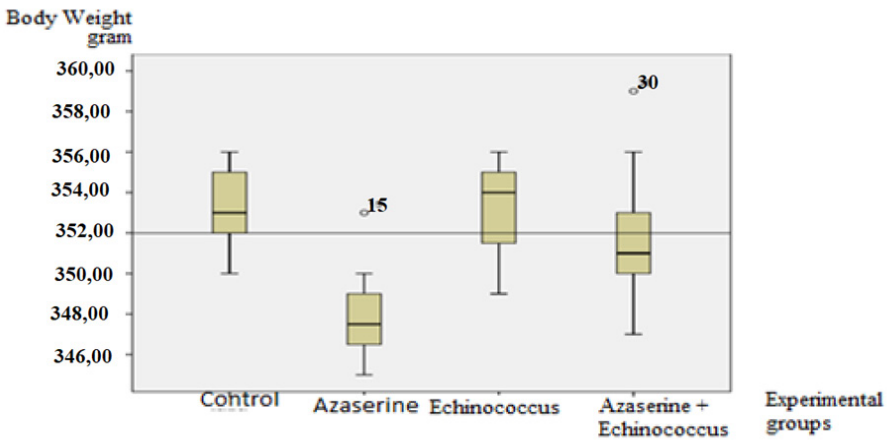

Figure 2. The Mean Body Weights of Experimental Groups (g).

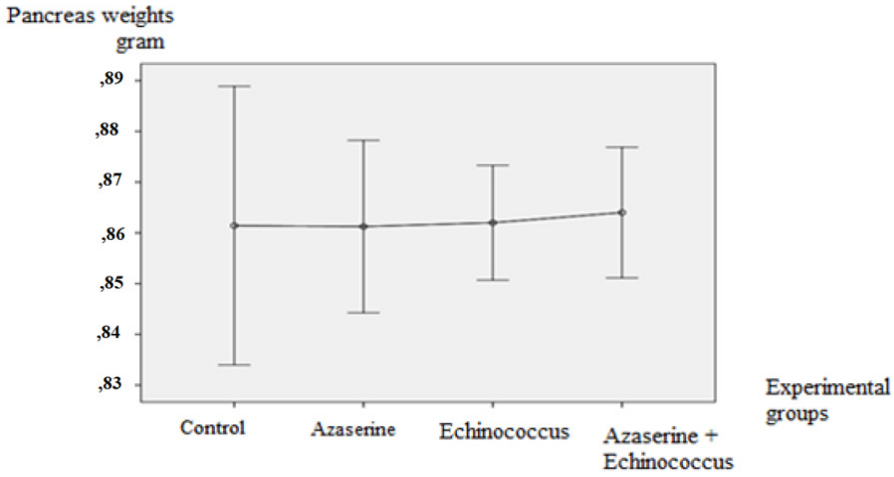

Figure 3. The Mean Pancreatic Weights of Experimental Groups (g).

of pancreatic cancer. There was a clearly reduction AACF ration in Azaserine + Echinococcus group (Group 4).The rate of the mean area of AACFs with respect to the total area of pancreas in the group treated with Azaserine (Group 2) was found to be 10.23% while the same rate was 1.61% in the Azaserine + Echinococcus group (Group 4). There was a significant difference between the two ($p<0.05$). In the Azaserine group (Group 2) the total area of AACF was measured to be 27,765,107 μm^2 ($p<0.05$), while in the Azaserine + Echinococcus group (Group 4) the total area of AACF was 4,360,084 μm^2. There existed a statistically significant difference between

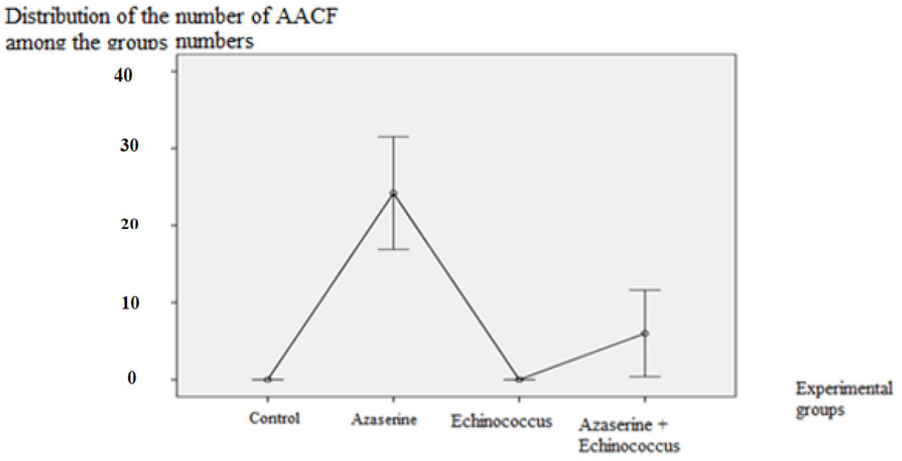

Figure 4. Distribution of the Mean Number of AACF among the Groups

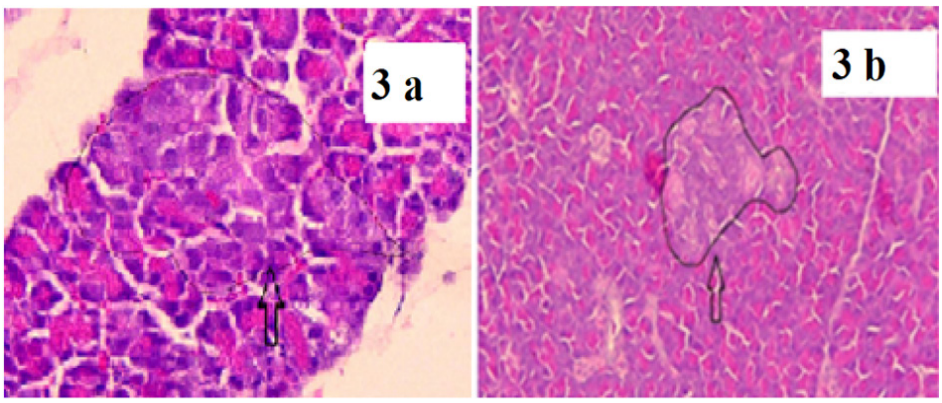

Figure 5. Atypical Acinar Cell Foci (AACF) in Azaserine (Group 2) Group, marked (in x50 magnification) (3a), AACF in Azaserine + Echinococcus group (Group 4) marked (in x50 magnification) (3b).

the two ($p< 0.05$).

Discussion

Alterations that occur in acinar cells and ducts in the development of pancreatic ductal adenocarcinoma (PDAC) is critical in the development of pancreatic cancer (Rhim and Stanger, 2010). However, while altered acinar cells induce PanIN, Kras oncogene in vivo can also lead to pancreatic cancer (Ji et al., 2009; Gourgou-Bourgade et al., 2011). Longnecker et al., point out in a study that some of the cancers detected in human pancreas stem from acinar cells (Zurlo et al., 1982). In a study Flaks et al. argue that pancreatic cancer, for the most part, derives from acinar cells that mutate at the outset and transform into ducts or ductal structures and finally induce pancreatic cancer (D.E. et al., 1991). Azaserine (o-diazo acetyl-L-serin) is an antimetabolite that can be isolated from Streptomyces cultures. It has a chemical with mutagenic property according to Salmonella typhimurium test (Longnecker et al., 1979). It was first used for inducing pancreatic cancer in rats in 1975 (Longnecker et al., 1979). In many subsequent studies, the azaserine-induced rat model appears as a dependable model (Longnecker and Curphey, 1975; O'connor et al., 1989). We also used this model in our study and observed that the number of atypical cells increased in the group in which we used azaserine.

Epidemiological studies demonstrate that there is a correlation between some parasites such as EG and development of cancers (Springer, 1997). Neoplastic cells induced in experimental animals are known to be inhibited by some bacteria (Listeria monocytogenes, Cornebacterium parvum) and parasites (Toxoplasma gondii, Besnoitia jellison). It is argued that these pathogens stimulate the immune system and thus inhibit neoplastic development. The fact that nonspecific macrophage activity resists the tumor with the immune response which is formed against parasitic infestation as well as the systemic inhibition of angiogenesis supports this observation (Hunter et al., 2001). In our study we demonstrated that the immune response against EG inhibits the development of AACF in the pancreas. There was a statically significant difference (Figure 5). The fact that the total area of AACF was 27,765,107 μm^2 ($p<0.05$) in the Azaserine group (Group 2), while the total area of AACF in the group injected with Azaserine+Echinococcus (Group 4) was 4.360.084 μm^2 also supports antitumor activity.

Results obtained from surgical procedures in areas where EG infestations are common established that there was a negative correlation between the existence of hydatid cysts and development of cancers (Akgül et al., 2003). Interestingly, several studies have reported that antigenic properties of tumor and EG infestation were similar (Alvarez et al., 2001; Knapen, 1980). Mucin-like peptides derived from EG increases anti-tumor activity. There are also studies in literature which put forth that EG suppresses the immune system rather than cancer itself (Turhan et al., 2015). The results of our study demonstrate just the opposite of the thesis set forth in that study. Anti-tumor activity that appears against EG infestation inhibits the development of cancer. The number of AACF in the rats which were injected with only azaserine was 25, while the number of AACF in the subject injected with Echinococcus and azaserine was 6, which was statistically significant. Thus we believe that we have established anti-tumor activity in EG infestation. We assume that subjects do not have to have cysts in their peritonea or livers for the existence of anti-tumor activity.

Even if the protoscoleces in the suspensions injected are dead, the subject's immunity activates itself against the antigenic structure of protoscolex. We have observed this fact within the scope of our pilot study. However, we used protoscoleces suspension whose vitality was proved. In our study we have also observed that immunity that develops against scolex and hydatid fluid of *Echinococcus granulosus* suppresses cancer-precursor atypical cell foci. The limitations of this study include the fact that EG-specific antigenic structure has not been serologically established.

In conclusion, findings from this experiment It has been shown in the well-known azaserine-rat model that azaserine-induced neoplastic changes in the exocrine pancreas can be inhibited by the parasitic *Echinococcus granulosus* in humans and animals. This supports the clinical and epistemological findings (Karaman et al., 2011; Turhan et al., 2015). The most significant aspect of present study is that it may contribute new insights into the controversy that *Echinococcus granulosus* suppresses pancreatic cancer.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

References

Akgül H, Tez M, Ünal A, et al (2003). Echinococcus against cancer: why not?. *Cancer*, **98**, 9.

Alvarez-Errico, Medeiros A, Miguez M, et al (2001). O-glycosylation in *echinococcus granulosus*: indentification and characterization of the carcinoma - associated to antigen. *Exp Parasitol*, **98**, 100-9.

Burgu A (1975). Secondary cyst of *Echinococcus granulosus* proloskolex in white mice The effect of radiation on their ability to form. *AÜ Vet Fac J*, **22**, 137-48.

Campbell HE, Pitit HC, Potter BR, Laishes BA (1982). Application of quantitative stereology to evaluation of enzyme altered foci in rat liver. *Cancer Res*, **42**, 465.

Khoo DE, Flaks B, Oztas H, Williamson RC, Habib NA (1991). Effects of dietary fatty acids on the early stages of neoplastic in duction in therat pancreas. Changes in fatty acid composition and development of atypical acinar cell nodules. *Int J Exp Pathol*, **72**, 571–80.

Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, et al (2011). FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med*, **364**, 1817-25.

Hruban RH, Maitra A, Goggins M (2008). Update on pancreatic intra epithelial neoplasia. *Int J Clin Exp Pathol*, **1**, 306-16.
Hunter CA, Yu D, Gee M, et al (2001). Cutting Edge: Systemic Inhibition of Angiogenesis Underlies Resistance to Tumors During Acute Toxoplasmosis. *J Immunol*, **166**, 5878-81.
Lynen I, Sogut O, Guldur ME, et al (2011). Primary hydatid cyst: an unusual cause of a mass in the supraclavicular region of the neck. *J Clin Med Res*, **3**, 52–4.
Ji B, Tsou L, Wang H, Gaiser S, et al (2009). Ras activity levels control the development of pancreatic diseases. *Gastroenterol*, **137**, 1072-82.
Kalıpcı E, Ozdemir C, Oztas H (2013). Assessing eco-toxicological effects of industrial 2,4-D acidiso-octylester herbicide on rat pancreas and liver. *Biotech Histochem*, **88**, 202-7.
Kalıpcı E, Yener Y, Yıldız H, Oztas H (2012). Investigationof possible ecotoxic effects of acrylamide on liverwiththeazaserine-rat model. *Pol J Environ Stud*, **21**, 1243-7.
Karaman E, Yilmaz M, Ada M, Yilmaz RS, Isildak H (2011). Unusual location of primary hydatid cyst: soft tissue mass in the parapharyngeal region. *Dysphagia*, **26**, 75–7.
Kazez K, Sahin M, Vatansev C, et al (2000). Experimentally developed secondary Echinococcosis in pleural and peritoneal cavities and utility of serological tests during the follow-up. *Turk J Med Sci*, **30**, 101–7.
Khoo DE, Flaks B, Oztas H, Williamson RCN, Habib NA (1991). Effects of dietary fatty acids on the early stages of neoplastic induction in the rat pancreas, Changes in fatty acid composition and development of atypical acinar cell nodules. *Int J Exp Pathol*, **72**, 571-80.
Knapen FV (1980). Echinococcus granulosus infection and malignancy. *Br Med J*, **281**, 195–6.
Longnecker DS, Curphey TJ (1975). Adenocarcinoma of the pancreas in azaserine-treated rats. *Cancer Res*, **35**, 2249-58.
Longnecker DS, Lılja HS, French J, et al (1979). Transplantation of azaserine – induced carcinomas of pancreas in rats. *Cancer Lett*, **7**, 197-202.
Noya V, Bay S, Festari MF, et al (2013). Mucin-like peptides from Echinococcus granulosus induce antitumor activity. *Int J Oncol*, **43**, 775-84.
O'connor P, Roebuck BD, Peterson J, et al (1989). Effect of dietary omega-3 and omega-6 fatty acids on development of azaserine-induced preneoplastic lesions of rat pancreas. *J Nat Cancer Inst*, **8**, 858-63.
Pugh TD, King JH, Koen H, et al (1983). Reliable stereological method for estimating the number of microscopic hepatocellular foci from their transections. *Cancer Res*, **43**, 1261-8.
Rhim AD, Stanger BZ (2010). Molecular biology of pancreatic ductal adenocarcinoma progression: aberrant activation of developmental pathways. *Prog Mol Biol Transl Sci*, **97**, 41-78.
Springer GF (1997). Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis and immunotherapy. *J Mol Med (Berl)*, **75**, 594-602.
Tezok UF, Kılıçturgay K, Toppare S, Aktaş H (1970). Studies on experimental hydatid cyst formation and treatment. 1. Secondary cyst implantation in experimental animals, *Microbiol Bult*, **4**, 207-14 .
Turhan N, Esendagli G, Ozkayar O, et al (2015). Co-existence of Echinococcus granulosus infection and cancer metastasis in the liver correlates with reduced Th1 immune responses. *Parasite Immunol*, **37**, 16–22.
Vincent A, Herman J, Schulick R, Hruban RH, Goggins M (2011). Pancreatic cancer. *Lancet*, **378**, 607-20.
Yener Y, Kalıpcı E, Oztas H, Aydin AD, Yıldız H (2013). Possible neoplastic effects of acrylamide on rat exocrine pancreas. *Biotech Histochem*, **88**, 47-53.
Yıldız H, Oztas H, Yıldız D, Koc A, Kalıpcı E (2013). Inhibitory effects of acetylsalicylic acid on exocrine pancreatic carcinogenesis. *Biotech Histochem*, **88**, 132-7.
Yıldız H, Kalipci E, Oztas H, Yıldız D (2019). Effects of acetylsalicylic acid on rats: an ın vivo experimental study in azaserine-rat model. *J Cancer Res Ther*, **15**, 231-6.
Zurlo J, Roebuck BD, Rutkowski JV, Curphey TJ, Longnecker DS (1982). Effect of pyridoxal deficiency on pancreatic DNA damage and nodule induction by azaserine. *Proc Am Assoc Cancer Res*, **23**, 68.