

A study on the use of Apitherapy as a Hepatoprotective tool

A thesis

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Liver

Liver is the largest and important organ in the human body that plays a role in multiple physiological processes, it aids in digestion (bile production), metabolism, protein synthesis and general detoxification. It also produces bile, used in digestion to emulsify fats. Some of this bile is stored in the gallbladder, and abnormal liver enzyme levels can thus indicate a problem with the gallbladder. The liver also plays a role in the immune system, as phagocytes are dispersed throughout the liver tissue (american liver foundation)

1. Liver disease:

Surveillance studies in the United States document an annual incidence of newly diagnosed chronic liver disease of 72 per 100,000 population (Lim and Kim, 2008) Liver disease accounts for over 27,000 deaths per year in the United States (1.1% of all deaths).

Liver disease is categorized both by the cause and the effect:

Infection, Injury, exposure to drugs or toxic compounds, autoimmune process and genetic defect (such as hemochromatosis).

These causes can lead to hepatitis, cirrhosis, stones that develop and form blockages, fatty liver and in rare instances liver cancer.

2. Patterns of Hepatic Injury:

The liver has a relatively limited repertoire of cellular and tissue responses to injury, regardless of cause. The most common are:

- Hepatocyte degeneration and intracellular accumulations.
- Hepatocytes necrosis and apoptosis.
- Inflammation.
- Regeneration.
- Fibrosis

Clinically, a few common syndromes which include hepatic failure, cirrhosis, portal hypertension, and disturbances of bilirubin metabolism causing jaundice and cholestasis which is detected only by abnormal laboratory tests, liver injury and healing may also occur without clinical detection. Hence, individuals with hepatic abnormalities who are referred to hepatologists most frequently have chronic liver disease.

3. Hepatitis

Acute or chronic hepatitis caused by biological factors, most commonly due to infection by one of several organisms' viruses, bacteria or parasites. Hepatitis viruses named in the order of their discovery as hepatitis A, B, C, D, and E. (center for disease control and prevention)

3.1 Viral Hepatitis :

Including:

HAV (Hepatitis A virus) which discovered in 1973, is a small, nonenveloped, positive-strand RNA which can be cultured in vitro(Feigelstock *et.al.*, 1998) HAV is spread by ingestion of contaminated water and foods. Donated blood is not specifically screened for this virus. HAV itself does not seem to be cytopathic. Cellular immunity, particularly CD8+ T cells, plays a key role in hepatocellular injury during HAV infection (Martin and Lemon ,2006).

HBV(Hepatitis B virus) is still the most common cause of acute and chronic viral hepatitis spread by exposure to blood, through sexual relations, and from mother to baby. It can produce acute hepatitis with recovery and clearance of the virus, nonprogressive chronic hepatitis, progressive chronic disease ending in cirrhosis, fulminant hepatitis with massive liver necrosis, and an

asymptomatic carrier state. HBV-induced chronic liver disease is an important precursor for the development of hepatocellular carcinoma (Pungpapong *et. al.*, 2007).

HCV(Hepatitis C virus) Spread mainly by exposure to contaminated blood including sharing of needles or other works used in consuming drugs. Hepatitis C is less common cause of acute hepatitis than hepatitis B but the majority of the people who contract it become chronically infected, able to spread the infection to others, and usually have chronic damage to the liver.

HDV (Hepatitis D virus) is a unique RNA virus that is dependent for its life cycle on HBV infection together with HBV infection occurs only in (Koytak *et.al.*, 2007).

HEV Hepatitis E virus is an enterically transmitted, water-borne infection that occurs primarily in young to middle-aged adults. HEV is a zoonotic disease with animal reservoirs, such as monkeys, cats, pigs and dogs (Purcell and Emerson, 2008).

3.2.Bacterial Hepatitis

Extrahepatic bacterial infections, particularly sepsis, can induce mild hepatic inflammation and varying degrees of hepatocellular cholestasis. The latter effect is attributable to the effects of pro-inflammatory cytokines released by Kupffer cells and endothelial cells, in response to circulating endotoxin. Several bacteria can infect the liver directly, including *Staphylococcus aureus* in the setting of toxic shock syndrome, *Salmonella typhi* in the setting of typhoid fever, and *Treponema pallidum* in secondary or tertiary syphilis. Alternatively, bacteria may proliferate in a biliary tree especially when outflow is compromised by partial or complete obstruction. The intra-biliary bacterial composition reflects the gut flora, and the severe acute inflammatory response within the intrahepatic biliary tree is called *ascending cholangitis*.

3.3 Parasitic and helminthic hepatitis

Parasitic and helminthic infections are major causes of morbidity worldwide, and the liver is frequently involved. These diseases include malaria, schistosomiasis, strongyloidiasis, cryptosporidiosis, leishmaniasis, echinococcosis, and infections by the liver flukes *Fasciola hepatica* and *Clonorchis sinensis*. Usually this kind of infection resulted in *Liver abscesses*, a form of liver infection that is common in developing countries, deserve special mention. They are usually caused by echinococcal and amebic infections, and less commonly, by other protozoal and helminthic organisms. In developed countries liver abscesses are uncommon; the incidence of amebic infections is low and is usually present in immigrants from endemic regions. Most such abscesses are pyogenic, representing a complication of a bacterial infection elsewhere. The organisms reach the liver by (1) the portal vein, arterial supply, ascending infection in the biliary tract (ascending cholangitis), direct invasion of the liver from a nearby source, or (5) a penetrating injury. The majority of hepatic abscesses mostly resulted from portal spread of intra-abdominal infections (e.g., appendicitis, diverticulitis, colitis). With improved management of these conditions, spread now occurs primarily through the biliary tree or the arterial supply in patients suffering from some form of immune deficiency (e.g., old age with debilitating disease, immunosuppression, or cancer chemotherapy with marrow failure). In these settings, abscesses may develop without a primary focus elsewhere.

3.3. a Pathology: With acute hepatitis, hepatocyte injury takes the form of diffuse swelling “**ballooning degeneration**”, so the cytoplasm looks empty and contains only scattered eosinophilic remnants of cytoplasmic organelles. An inconstant finding is **cholestasis**, with bile plugs in canaliculi and brown pigmentation of hepatocytes. The canalicular bile plugs result from cessation of

the contractile activity of the hepatocyte pericanalicular actin microfilament. Several patterns of hepatocyte cell death are seen.

Rupture of the cell membrane leads to cell death and focal loss of hepatocytes. The sinusoidal collagen reticulin framework collapses where cells have disappeared, and scavenger **macrophage aggregates** mark sites of hepatocytes.

3.3.b Apoptosis caused by anti-viral cytotoxic (effector) T cells. Apoptotic hepatocytes shrink, become intensely eosinophilic, and have fragmented nuclei; effector T cells may still be present in the immediate vicinity. Apoptotic cells are rapidly phagocytosed by macrophages and hence might be difficult to find, despite a brisk rate of hepatocyte injury.

In severe cases of acute hepatitis, confluent necrosis of hepatocytes may lead to bridging necrosis connecting portal-to-portal, central-to-central, or portal-to-central regions of adjacent lobules. Hepatocyte swelling and regeneration compress sinusoids, and the more or less radial array of hepatocyte plates around terminal hepatic veins is lost.

Pathology of chronic liver damage is the deposition of fibrous tissue which starts with portal tracts fibrosis, then periportal septal fibrosis followed by linking of fibrous septa (bridging fibrosis). In clinical practice, several systems have been used to score the severity and progression of liver damage due to HBV and HCV infection (Goodman., 2007) In each system the key elements are inflammation and hepatocyte destruction (grade), and the severity of fibrosis (stage).

Continued loss of hepatocytes and fibrosis results in cirrhosis which is characterized by irregularly sized nodules separated by broad scars, and called

post-necrotic cirrhosis. However, this term is not specific to viral etiology, and is applied to all forms of cirrhosis in which the liver shows large, irregular-sized nodules with broad scars. In addition to viral hepatitis, autoimmune hepatitis, hepatotoxins (carbon tetrachloride, mushroom poisoning), pharmaceutical drugs (acetaminophen, α -methyldopa), and even alcohol can give rise to cirrhotic livers with irregular-sized large nodules. In about 20% of cases the inciting cause of the cirrhosis cannot be determined, and these are labeled as **cryptogenic cirrhosis**. Thus, the morphology of the end-stage cirrhotic liver is often not helpful in determining the basis of the liver injury.

4. Schistosomiasis

Schistosomiasis is the second most prevalent tropical liver disease in the world (Engles *et al.*, 2002) and (Charles and King, 2009) it is also known as bilharzia in honor of Theodore Bilharz, who first identified the etiological agent for *Schistosoma hematobium* in Egypt 1851. *Schistosoma mansoni* infects about 83.31 million people worldwide (Crompton, 1999), causing intestinal schistosomiasis. (The all types of schistosomiasis caused by all *Schistosoma* species infects over 200 million people (Oliveira *et. al.*, 2004), (Barrie *et.al.*, 2012).

4.1. Geographical distribution

Schistosoma mansoni originated in Africa but was carried to South America, with the slave trade; it is transmitted by snails of the genus *Biomphalaria* snails. *Schistosoma haematobium* which causes urinary bilharzia is transmitted by snails of the species *Bulinus*, which inhabit less permanent water bodies (Ross *et al.*, 2002).

Schistosomes are atypical trematodes in that the adult stages have two sexes (dioecious) and are located in blood vessels of the definitive host (Jauréguiberry *et.al*, 2005 and Barrie *et.al*, 2012). Most other trematodes are

hermaphroditic and are found in the intestinal tract or in organs, such as the liver. The life cycle of schistosomes includes two hosts: a definitive host (i.e. human) where the parasite undergoes sexual reproduction and a single intermediate snail hosts where there are a number of asexual reproductive stages (Tchuenté *et.al.*, 2004).

The main form of human schistosomiasis is caused by 5 species of flatworm in the genus *Schistosoma*, within the class trematode. The 5 species are as follows: *Schistosoma japonicum*, *Schistosoma hematobium*, *Schistosoma mansoni*, *Schistosoma intercalatum* and *Schistosoma mekongi*. The worm is also called blood flukes because they live in the vascular system of humans and other vertebrates (Babbage *et.al.*, 2012).

4.2. Epidemiology:

Globally, schistosomiasis is a major source of morbidity and mortality. The unique schistosomal life cycle limits endemic areas to tropical and subtropical zones, but these areas exist around the world and may even increase with some agricultural practices. Although freshwater lakes and streams are usually identified as the source of the disease, man-made reservoirs and irrigation systems are increasingly implicated in some countries. Indeed, geographic spread continues because of water resource engineering issues in developing countries and the migration of infected populations.

Intestinal schistosomiasis caused by *S. mansoni* occurs in 52 nations, including Caribbean countries (i.e., Saint Lucia, Antigua, Montserrat, Martinique, Guadeloupe, Dominican Republic, Puerto Rico), Eastern Mediterranean countries, South American countries (i.e., Brazil, Venezuela, Surinam) and most countries in Africa (John *et.al.*, 2008).

More than 207 million people in at least 74 countries have active schistosomal infection (WHO, 2012, Barnabas *et.al.*,2012).Approximately 60% of the populations have disease symptoms, including organ-specific complaints and problems related to chronic anemia and malnutrition from the infection; more than 20 million are severely ill. Along with the preexisting programs for the annual treatment of farmers and cattle, efforts were made to optimize animal grazing sites, sewage management, drinking water supplies, and health education with regard to schistosomiasis (Wang *et.al.*, 2009).

Nevertheless, the human cost of schistosomal infections remains high, and the disease contributes to co morbidity with other infections, including hepatitis, human immunodeficiency virus (HIV), and malaria, in endemic regions (Kallestrup *et.al.*, 2006).

4.3.Life cycle

The geographic distribution and etiology of schistosomiasis reflect the unique life cycle of *Schistosoma* species. After the eggs of the human-dwelling parasite are emitted in the faeces and into the water, the ripe miracidium hatches out of the egg within 24 hrs. The hatching happens in response to temperature, light and dilution of faeces with water. The miracidium searches for a suitable freshwater snail (*Biomphalaria glabrata*, *Biomphalaria straminea*, *Biomphalaria tenagophila*, *Biomphalaria alexandrina* or *Biomphalaria sudanica*) to act as an intermediate host and penetrates it (Gatlin *et al.*, 2009). Following this, the parasite develops via a so-called mother-sporocyst and daughter-sporocyst generation to the cercaria. The purpose of the growth in the snail is the numerical multiplication of the parasite. From a single miracidium result a few thousand cercaria 4-6 weeks after infection, every one of which is capable of infecting man.

The cercaria emerges from the snail during daylight and they propel themselves in water with the aid of their bifurcated tail, utilizing oral and ventral suckers, actively seeking out their final host. When they recognize human skin, they penetrate it within a very short time. This occurs in three stages, an initial attachment to the skin, followed by the cercaria creeping over the skin searching for a suitable penetration site, often a hair follicle, and finally penetration of the skin into the epidermis using proteolytic secretions from the cercarial post-acetabular, then pre-acetabular glands. On penetration, the head of the cercaria transforms into an endoparasitic larva, the schistosomule, developing double-lipid-bilayer teguments that are highly resistant to host immune responses and incorporate host proteins, including major histocompatibility complexes (MHCs) and blood group antigens, into their integuments, their metabolism shifts to glycolysis. Each schistosomule spends a few days in the skin and then enters the circulation starting at the dermal lymphatics and venules. Here they feed on blood, regurgitating the haem as haemozoin (Oliveira *et. al.*, 2000) The schistosomule migrates to the lungs (5–7 days post-penetration) and then moves via circulation through the left side of the heart to the hepatoportal circulation (>15 days) where, if it meets a partner of the opposite sex, it develops into a sexually mature adult and the pair migrate to the mesenteric vein (Kalhor L. and Kalhor H. *et.al.*, 2011), such pairings are monogamous (Beltran and Boissier, 2008).

Male schistosomes undergo normal maturation and morphological development in the presence or absence of a female, although behavioral, physiological and antigenic differences between males from single-sex, as opposed to bisex, infections have been reported. On the other hand, female schistosomes do not mature without a male. Female's schistosomes from single-sex infections are underdeveloped and exhibit an immature reproductive system. Although the maturation of the female worm seems to be dependent on the

presence of the mature male, the stimuli for female growth and for reproductive development seem to be independent from each other.

The adult female worm resides within the adult male worm's gynaecophoric canal (couple), which is a modification of the ventral surface of the male forming a groove. The paired worms move against the flow of blood to their final niche in the mesenteric circulation where they begin egg production (>32 days). The *S. mansoni* parasites are found predominantly in the small inferior mesenteric blood vessels surrounding the large intestine and caecal region of the host. Each female lays approximately 300 eggs a day (one egg every 4.8 minutes), which are deposited on the endothelial lining of the venous capillary walls (Philip *et.al.*, 2004). The eggs move into the lumen of the host's intestines and are released into the environment with the feces.

Eggs that are not shed outside successfully may remain in the tissues or be swept back to the portal circulation ,from the mesenteric vessels, or to the pulmonary circulation from the vesicular vessels via the inferior vena cava. Eggs can end up in the skin, brain, muscle, adrenal glands, and eyes. As the eggs penetrate the urinary system, they can find their way to the female genital region and form granulomas in the uterus, fallopian tube, and ovaries. CNS involvement occurs because of mobilization of eggs from the portal mesenteric system to the brain and spinal cord via the paravertebral venous plexus (Corachan., 2002) and (Houston.*et.al.*, 2004).

4.4.Pathology:

Schistosome eggs, which may become lodged within the host tissues, are the major cause of pathology in schistosomiasis. Some of the deposited eggs reach the outside environment by passing through the wall of the intestine; the rest are swept into the circulation and are filtered out in the periportal tracts of

the liver resulting in periportal fibrosis. Onset of egg lying in humans is sometimes associated with an onset of fever (Katayama fever) (Leshem *et.al.*, 2008).

This "acute schistosomiasis" is not, however, as important as the chronic forms of the disease. For *S. mansoni* , "intestinal" and "hepatic schistosomiasis", associated with formation of granulomas around trapped eggs lodged in the intestinal wall or in the liver, respectively (Barrie *et.al*, 2012). The hepatic form of the disease is the most important, granulomas here giving rise to fibrosis of the liver and hepatosplenomegaly in severe cases. Initially, the inflammatory reaction is readily reversible. In the latter stages of the disease, the pathology is associated with collagen deposition and fibrosis resulting in organ damage that may be only partially reversible. *In vivo* microscopy revealed in addition to these lesions, dilatation and sacculation of sinusoids. These lesions were associated with varying degrees of reduction of blood flow due to schistosomules (El-Banhawy *et al.*, 2007)(Aly and Hamed, 2006).

Granuloma formation is initiated by antigens secreted by the miracidium through microscopic pores within the rigid egg shell, and there is strong evidence that the vigorous granulomatous response, rather than the direct action of parasite egg antigens, is responsible for the pathologic tissue manifestations in schistosomiasis (Boros, 1989). In addition to the embryos in the un-capsulated eggs which released toxins that damage the liver (Reynolds *et al.*, 2002).The granulomas formed around the eggs impair blood flow in the liver and consequently induce portal hypertension. With time in case of chronic schistosomiasis, collateral circulation is formed and the eggs disseminate into the lungs, where they cause more granulomas, pulmonary arteritis and, later, cor pulmonale (Lapa *et.al.*, 2009). A contributory factor to portal hypertension is Symmers' fibrosis, which develops around branches of the portal veins. This fibrosis occurs only many years after the infection and apparently is caused in

part by soluble egg antigens and various immune cells which react to them. Enlargement of the spleen and especially liver will continue resulting development of the arteriovenous shunts in the liver. Ascites of the liver and esophageal varices may proceed. Among sever cases hepatomegaly and enlargement of the spleen are found in high number of cases (Talaat and Miller, 1998) and (Lapa *et.al.*, 2009). The enlargement of the spleen may be attributed to the direct deposition of the eggs in that organ or due to inflammatory and fibrotic reactions in the splenic host that are the main factors responsible for obstruction to portal venous flow which its major consequence is splenomegaly, in histopathological examination, congestion was evident in sinusoids of red pulp and lymphoid follicles (White pulp) were enlarged. This marked congestion in red pulp showed evidence of haemorrhages (Aly and Hamed, 2006).

Granuloma size is consistent with levels of IL-13, which plays a prominent role in the reduction of granuloma formation and granuloma size. IL-13 receptor $\alpha 2$ (IL-13R $\alpha 2$) binds IL-13 with high affinity and blocks the effects of IL-13. Thus, this receptor is essential in preventing the progression of schistosomiasis from the acute to the chronic (and deadly) stage of disease. Synthetic IL-13R $\alpha 2$ given to mice has resulted in significant decreases in granuloma size, implicating IL-13R $\alpha 2$ as an important target in schistosomiasis (Mentink-Kane *et.al.*, 2004).

Total protein was reduced in bilharzial infection. This could be attributed to hepatocellular damage caused by parasite toxins. The main fraction of total protein content is albumins and the reduction in total protein may be due to reduction in albumin fraction level that in turn may be result from decrease anabolism or increase catabolism; hence, malnutrition and/or malabsorption may contribute to decrease biosyntheses of albumin (Rizk *et al.*, 2000, El-Ansary *et al.*, 2007). The significant decrease in total protein is mainly due to increase in

messenger RNA degradation which is the possible cause for the hypoalbuminemia of murine schistosomiasis (Metwally *et al.*, 1990).

4.5.Evasion of Host Immunity

Adult and larval worms must migrate through the host's blood circulation and avoid the host's immune system. The worms have many tools that help in this evasion, including the tegument, antioxidant proteins, and defenses against host membrane attack complex (MAC) (Wilson and Coulson, 2009).

4.6. Defense against host MAC

Schistosomes have evolved ways to block host complement proteins. Immunocytochemistry techniques have found decay accelerating factor (DAF) protein on the tegument. DAF is found on host cells and protects host cells by blocking formation of MAC. It has also been found that the schistosome genome consists of human CD59 homologs. CD59 inhibits MAC.

Resistance of infection is mainly due to the tegument coats the worm and acts as a physical barrier to host antibodies, complement and Antioxidant Proteins; Host immune defenses are capable of producing superoxide, which has a tremendous detrimental affect on the worm. However, worms are able to produce a number of antioxidant proteins that block the effect of host superoxide. Schistosomes have four superoxide dismutases and levels of these proteins increase as the schistosome develops and matures.

4.7.Symptoms

Many individuals do not experience symptoms. If symptoms do appear, it usually takes four to six weeks from the time of infection. The first symptom of the disease may be a general ill feeling. Within twelve hours of infection, an

individual may complain of a tingling sensation or light rash, commonly referred to as "swimmer's itch", due to irritation at the point of entrance of cercariae. The rash that may develop can mimic scabies and other types of rashes. Other symptoms can occur two to ten weeks later and can include fever, aching, cough, diarrhea, or gland enlargement. These symptoms can also be related to avian schistosomiasis which does not cause any further symptoms in humans.

a. Katayama fever

Katayama fever was described in 1847 in the Katayama district of Japan, and has been known to be caused by *Schistosoma japonicum* since 1904 which is another primary condition develop from infection with these worms, and it can be very difficult to recognize (Jauréguiberry *et.al.*, 2010). Symptoms include fever, lethargy, the eruption of pale temporary bumps associated with severe itching (urticarial) rash, liver and spleen enlargement, and bronchospasm. Katayama fever has been described in people with initial heavy infections caused by *S.mansoni*, *S.haematobium* and especially *S.japonicum*. It is believed to represent a serum sickness-like reaction resulting from circulating immune complexes (Strickland and abdel-warib, 1991) and (Ross *et.al.*, 2007).

b. Intestinal schistosomiasis

Granulomatous reaction, due to lodging of the eggs to the intestinal wall, leads to obstruction of the colon and blood loss. Severe hepatosplenic schistosomiasis (HSS) is a result of the failure of the immunomodulation process. The large bowel is predominantly involved in the milder intestinal form of schistosomiasis, although the entire intestinal wall and liver are affected. Patients typically present with mucoid bloody diarrhea, tenesmus, and abdominal cramping (Ross *et.al.*, 2007).

The infected individual may have what appears to be a potbelly. Eggs can also become lodged in the liver, leading to high blood pressure through the liver, enlarged spleen, the buildup of fluid in the abdomen, and potentially life-threatening dilations or swollen areas in the esophagus or gastrointestinal tract that can tear and bleed profusely (esophageal varices). Rarely, the central nervous system is affected. Individuals with chronic active schistosomiasis may not complain of typical symptoms.

4.8. Diagnosis:

a. Stool Examination

Diagnosis of infection is confirmed by the identification of eggs in stools. Eggs excretion can be detected as early as 5 weeks after initial infection, eggs of *Schistosoma mansoni* are approximately 140 by 60 μm in size and have a lateral spine. The diagnosis by rapid, simple, and inexpensive Kato–Katz thick-smear stool examination, (a semi-quantitative stool examination technique) (Katz *et al.*, 1972), requires 40 to 50 mg of feces and is widely used in field studies and national control programs to determine the burden of eggs in feces. Several population-based studies have demonstrated that mean egg burdens correlate with the mean severity of disease (Ross *et al.*, 1997 and Kardorff *et al.*, 1997).

b. Biopsy and Ultrasonography:

Biopsy of rectal mucosa is more sensitive than fecal egg detection for the diagnosis of schistosomiasis, especially when the crush technique is used. This method involves the examination under low power of biopsy specimens that are crushed between two microscope slides before formalin fixation (Abdel-Hafez and Bolbol, 1992).

A particular useful diagnostic tool is abdominal ultrasonography, which allows accurate measurement of liver and spleen size, grading of hepatic fibrosis and detection of complications of portal hypertension (Hatz *et al.*, 1992).

c. Serological testing:

Other methods which can be used are enzyme linked immunosorbent assay (ELISA) which have been developed for the detection of schistosomal antigens in the sera of actively infected patients. There is a good correlation between antigen level and worm burden, so these assays can also be used to estimate the intensity of infection, they also can be used to monitor the response to therapy, as antigen level in both serum and urine declined rapidly following successful chemotherapy (Deelder *et al.*, 1994; Nibbeling *et al.*, 1998). In addition to circumoval precipitation test (COPT) and alkaline phosphatase immunoassay (APIA) (Turner *et al.*, 2004).

d. Blood Testing:

Blood tests are occasionally useful in supporting the diagnosis or assessing the severity of schistosomal infection. Serologies and polymerase chain reaction (PCR) assay-based testing can confirm a diagnosis (Sandoval *et al.*, 2006 and Lier *et al.*, 2006) Considerations in laboratory testing include the following:

- Complete blood count (CBC) - May reveal peripheral eosinophilia, particularly in acute infection and/or anemia (Jauréguiberry *et al.*, 2010).
- Increased alkaline phosphatase level and gamma-glutamyl transferase (GGT) level - Are observed with hepatic granulomatosis.
- Transaminase levels (ALT, AST) - Generally elevations which are usually caused by coexisting hepatitis.
- Renal function - May be decreased if obstructive nephropathy is severe