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Nutritional Supplementation with *Ailanthus altissima* and *Ziziphus spina christi* to Compensate for Some Metabolic Disorders in *Schistosoma mansoni* Infected Mice

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Abstract: Different protein fractions having variable molecular weights were electrophoretically separated and quantitated in serum of control and *S. mansoni* infected mice with and without oral administration of chloroform extract of *Alilanthus altissima* stem bark and alcoholic extract of *Ziziphus spina christi* roots. Also total protein, alkaline phosphatase, cholesterol, bilirubin and nitric oxide levels were measured in sera of the same mice groups. The results obtained showed that administration of both extracts to normal mice increased total protein, albumin and most protein fractions. Also both extracts reduced alkaline phosphatase (ALP), cholesterol and nitric oxide (NO) while bilirubin was elevated. Infection with *S. mansoni* caused a decline in total protein and all protein fractions and an elevation in all other biochemical parameters with respect to control. However, administration of either extracts to infected mice upregulated the levels of the different parameters. It could be concluded that *A. altissima* and *Z. spina christi* extract supplements can compensate for the depletions in different proteins resulting by schistosomal infection and can also decrease liver disorders presumably due to their high nutritional value and their antioxidant and hepatoprotective constituents.

Key words: Protein, *A. altissima*, *Z. spina*, schistosomiasis

INTRODUCTION

Infection with schistosomiasis results in serious health problems such as hepatosplenic complications and can lead to liver cirrhosis (Fahim *et al.*, 2000). Following infection schistosome larvae travel through blood vessels to the lungs, abstract serum proteins and camouflage themselves and secrete β -macroglobulin which helps to confuse the macrophages later. In addition, adult worms ingest whole erythrocytes, using the globins component of hemoglobin as a nutrient source for their metabolism and the cysteine proteases associated with their gut digest host proteins to absorbable nutrients (Brindley *et al.*, 1997).

In this respect, host blood provides a rich source for the production of energy, amino acids and fatty acids for the synthesis of parasite molecules and for egg production (John *et al.*, 2004).

Serum protein profiling pattern is a useful tool for diagnosis of liver diseases such as cirrhosis or infections. Thousands of individual serum proteins which vary in their physiological functions enable the discovery of disease biomarkers (O'Connell *et al.*, 2005). The levels of total protein shows how well the liver is making proteins that the body needs to fight

infection and perform other metabolic functions. Sometimes when there is a problem with the liver such as in schistosoma infection it cannot make proteins as well, so the protein levels are lower. In addition, total cholesterol, alkaline phosphatase and total bilirubin are marker parameters for liver damage during schistosomal infection (El-Sokkary *et al.*, 2002). Also nitric oxide is a major effective molecule produced by macrophages against helminth parasites (Zhou *et al.*, 2005).

In order to compensate for the depletion in proteins due to malnutrition and host protein consumption by the parasite during schistosomiasis and also to reduce liver disorders, supplementation with natural extracts rich in protein and other effective components can be of high nutritional value and of significant medicinal importance.

So, in the present study, both the chloroform extract of *Ailanthus altissima* stem bark and the ethanol extract of *Ziziphus spina christi* root were investigated against different disorders induced by *S. mansoni* infection. The study is conducted on the recent approach of development of new drugs from natural products for treatment of human diseases especially in developing countries which still rely on traditional medicinal plants

for their primary health care (WHO, 2002). *A. altissima* and *Z. spina christi* plant extracts were selected owing to their previously reported wide range of biological activities. *A. altissima* possesses antituberculosic activity (Buzina *et al.*, 2001), antiplasmodial activity (Okunade *et al.*, 2003) and antitumor activity (Tamura *et al.*, 2003). On the other hand, *Z. spina christi* shows antidiabetic activity (Glombitza *et al.*, 2002), antimicrobial activity (Shahat *et al.*, 2001) and antidiarrheal activity (Adzu *et al.*, 2003). Thus it was of interest to study the hepatoprotective effect of these extracts in mice infected with *S. mansoni* and developing certain liver disorders. In a related and recent study, Ali and Hamed (2006) deduced that both extracts under study caused reduction in worm burden, ova count and granuloma size as well as improvement in the histopathological picture of the liver, kidney and spleen of mice infected with *S. mansoni*. This confirms that the two medicinal plants reduce inflammatory and fibrotic reactions of parasite toxins. Also El-Rigal *et al.* (2006) studied the antioxidant and hepatic marker enzyme levels in *S. mansoni* infected mice and treated with both extracts under study and showed that they possessed a pronounced effect in improving liver damage caused by *S. mansoni* infection.

MATERIALS AND METHODS

Animals: Forty-eight male albino mice weight range (20-25 g) were caged with a free supply of food and water. After acclimatization, they were randomly assigned into six groups of eight mice each and were treated as follows:

The 1st group was left uninfected untreated and served as control. The 2nd and 3rd groups were treated after three months from the start of the experiment with a total dose of 1/4 LD₅₀ of both the chloroform extract of *A. altissima* stem bark (500 mg kg⁻¹ bw, using serial concentrations ranging from 100-4000 mg kg⁻¹ bw) and 70% ethanolic root bark extract of *Z. spina christi* (560 mg kg⁻¹ bw, according to Adzu *et al.*, 2003), respectively five times weekly for one month.

The 4th group was infected with cercariae of the Egyptian strain *S. mansoni* by the tail immersion method and were kept to develop liver granuloma for four months. The 5th group and 6th groups were infected with *S. mansoni*, left for three months, then treated with the previous test extracts, respectively five times weekly for one month.

Mice of all groups were sacrificed after four months. Appropriate anaesthetic and sacrifice procedures were followed ensuring that animals did not suffer at any stage of the experiments. Anaesthetic procedures are complied according to legal ethical guidelines approved by the Ethical Committee of the Federal Legislation and

National Institute of Health Guidelines in USA. An overdose of ether was given gradually to mice and then the abdomen was opened by a midline incision and livers were separated. The livers and sera of all groups were subjected to the different biochemical assays.

Extraction of plant material: One kilogram of *A. altissima* stem bark or *Z. spina christi* root bark was extracted with chloroform and 70% alcohol, respectively in a continuous extraction Soxhlet apparatus till exhaustion and the extracts were concentrated under reduced pressure. The extracts were phytochemically screened and the results showed the presence of alkaloids, sterols and/or triterpenes in both extracts while saponins were also present in *Z. spina christi* extract.

Biochemical assays

Estimation of total protein: Protein was estimated by the method of Bradford (1976). Bradford solution was added to of 5% homogenate and the developed blue color was measured after 5 min at 595 nm in spectrometer (Nova spec, LKB, Sweden) against blank containing water instead of the homogenate. The amount of protein was calculated from a standard curve using serial concentrations of bovine serum albumin (1-10 µg).

Estimation of protein fractions: Protein fractions were estimated by SDS-one dimensional electrophoresis (Fig. 1). Separated on gradient polyacrylamide gel (4-30%), the method of determination and staining of protein is carried out according to De Moreno *et al.* (1985). Drying of gel plate is determined using the method of Jaung *et al.* (1984). The individual standard protein

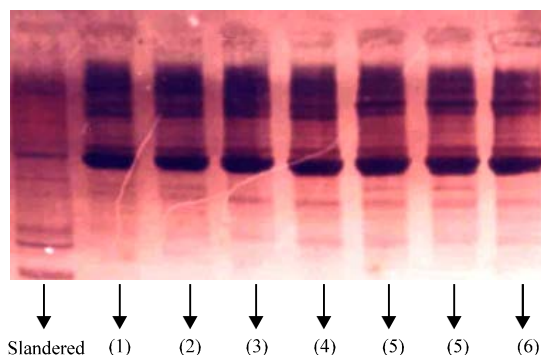


Fig. 1: Electrophoretic profile of plasma proteins on SDS-polyacrylamide gel electrophoresis SDS-PAGE (one-dimensional electrophoresis) 1: Control, 2: Control+ethanolic extract, 3: Control+chloroform extract, 4: Infected, 5: Infected+ethanolic extract, 6: Infected+chloroform extract

fractions designed for molecular weight determination on SDS-PAGE were, α_2 -macroglobulin (180 kDa), β -galactosidase (116 kDa), phosphorylase b (97.4 kDa), serum albumin (66.0 kDa), fumarase (48.5 kDa), carbonic anhydrase (29 kDa), β -lactoglobulin 18.4 (kDa), α -lactalbumin (14.2 kDa) and aprotinin (6.5 kDa). The electrophoretic results of protein fractions are related to the concentration of total protein and expressed as mg protein mL⁻¹ (Laemmli, 1970). The electrophoretic separation bands were measured by Helena France Scanner at 600 nm using the standard protein marker as internal reference.

Serum bilirubin was determined according to the method of Henry (1974) by reaction with diazotized sulfonilic acid, in the presence of caffeine, with the final production of an azopigment that is measured at 478 nm.

Serum alkaline phosphatase was determined according to the method of Belfield and Goldberg (1971). The method described is based on measuring the liberated phenol at 510 nm in the presence of amino-4-antipyrine and potassium ferricyanide.

Serum total cholesterol was estimated by using Randox diagnostic kit. The method is based on the enzymatic hydrolysis and oxidation of cholesterol. The resulting H₂O₂ reacts with 4-aminoantipyrine in the presence of peroxidase forming quinoneimine which is measured at 500 nm.

Nitric oxide, measured as nitrite, is determined by the method of Montgomery and Dymock (1961). Sulphanilamide is diazotised with the formed nitrous acid and the product is coupled with N-(1-naphthyl) ethylene diamine to form a reddish purple azodye which is read at 540 nm.

Statistical analysis: The statistical significance of the results was determined by analysis of variance (ANOVA) accompanied with post-hoc (SPSS computer program).

RESULTS

Data shown reveal the effect of *Ailanthus altissima* and *Ziziphus spina christi* extracts on total protein, on the high molecular weight protein fractions (180, 116, 97.4 and 66 kDa), (Table 1) and on the low molecular weight protein fractions (Table 2) in control mice, *Schistosoma mansoni* infected mice and treated mice groups with or without infection. It is shown that infection caused reduction in total protein and the different fractions, while treatment with both extracts induced an elevation in these proteins. Figure 2 and 3 reveal the percentage changes between controls, infected and treated groups for the total protein and the different protein fractions, respectively.

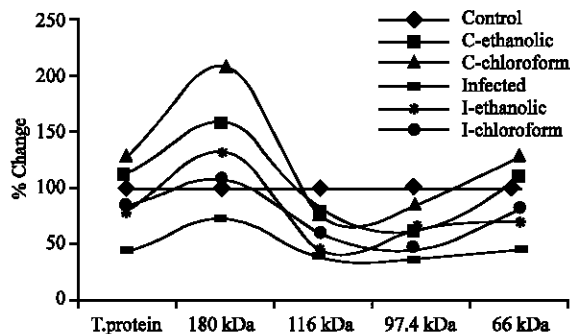


Fig. 2: The percentage change of total protein and the high M.wt.protein fractions (180, 116, 97.4 and 66 kDa) in different mice groups compared to control

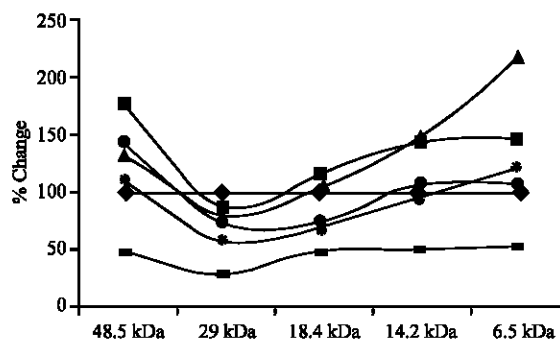


Fig. 3: The percentage change of low M.wt. proteins (48.5, 29, 18.4, 14.2 and 6.5 kDa) in different mice groups compared to control

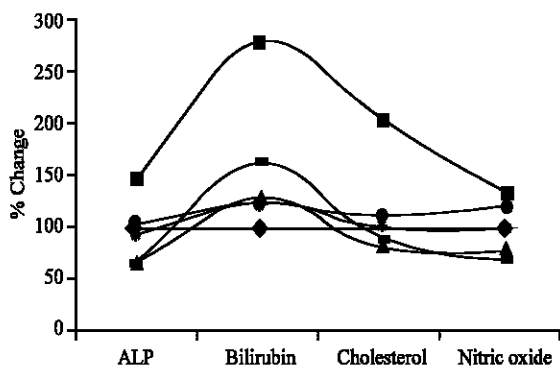


Fig. 4: The percentage change of ALP, bilirubin, cholesterol and nitric oxide in different groups of mice compared to control

Table 3 shows that treatment with both extracts decreased the levels of ALP, cholesterol and nitric oxide while bilirubin increased with respect to normal control while infection caused elevation in all these parameters. On the other hand, treatment with both extracts reduced

Table 1: Effect of *Ailanthus altissima* and *Ziziphus spina christi* extracts on total protein and protein fractions (180, 116, 97.4 and 66 kDa) in control and *Schistosoma mansoni* infected mice

	Control	Control-ethanolic	Control-Chloroform	Infected	Infected-ethanol	Infected-chloroform	
Parameters	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	ANOVA
Total protein	65.9±4.8 (2,3,4,5,6)	74.7±10.09 (1,3,4,5,6)	83.7±8.14 (1,2,4,5,6)	28.3±3.1 (1,2,3,5,6)	52.8±5.08 (1,2,3,4)	55.3±5.12 (1,2,3,4)	< 0.0001
180 kDa	1.52±0.37 (2,3,4,5)	2.41±0.17 (1,3,4,5,6)	3.16±0.39 (1,2,4,5,6)	1.11±0.25 (1,2,3,5,6)	2.00±0.29 (1,2,3,4,6)	1.64±0.18 (2,3,4,5)	< 0.0001
116 kDa	3.25±0.21 (2,3,4,5,6)	2.49±0.16 (1,4,5,6)	2.34±0.39 (1,4,5,6)	1.18±0.28 (1,2,3,6)	1.37±0.14 (1,2,3,6)	1.84±0.13 (1,2,3,4,5)	< 0.0001
97.4 kDa	3.49±0.21 (2,3,4,5,6)	2.19±0.27 (1,3,4,6)	3.04±0.41 (1,2,4,5,6)	1.29±0.09 (1,2,3,5)	2.23±0.34 (1,3,4,6)	1.58±0.134 (1,2,3,5)	< 0.0001
66 kDa	9.17±0.29 (2,3,4,5,6)	10.17±0.33 (1,3,4,5,6)	11.86±0.46 (1,2,4,5,6)	4.19±0.13 (1,2,3,5,6)	6.45±0.28 (1,2,3,4,6)	7.5±0.39 (1,2,3,4,5)	< 0.0001

Data are means±SD of six mice in each group, Total protein expressed as mg protein mL⁻¹ serum. Protein fractions are expressed as mg 0.5 mL⁻¹ serum, p is level of significance, where p<0.05 is significant, Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (SPSS Computer Programme)

Table 2: Effect of *Ailanthus altissima* and *Ziziphus spina christi* extracts on protein fractions (48.5, 29, 18.4, 14.2 and 6.5 kDa) in control and *Schistosoma mansoni* infected mice

	Control	Control-chloroform	Control-ethanol	Infected	Infected-chloroform	Infected-ethanol	
Parameters	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	ANOVA
48.5 kDa	2.41±0.33 (2,3,4,5,6)	4.27±0.17 (1,3,4,5,6)	3.25±0.23 (1,2,4,5)	1.17±0.11 (1,2,3,5,6)	2.67±0.14 (1,2,3,4,6)	3.41±0.22 (1,2,4,5)	< 0.0001
29 kDa	3.71±0.74 (2,3,4,5,6)	3.24±0.19 (1,4,5,6)	2.99±0.3 (1,4,5)	1.04±0.12 (1,2,3,5,6)	2.15±0.11 (1,2,3,4,6)	2.73±0.19 (1,2,4,5)	< 0.0001
18.4 kDa	3.41±0.53 (4,5,6)	3.63±0.21 (4,5,6)	3.59±0.34 (4,5,6)	1.72±0.08 (1,2,3,5,6)	2.37±0.11 (1,2,3,4)	2.54±0.2 (1,2,3,4)	< 0.0001
14.2 kDa	2.71±0.26 (2,3,4)	3.89±0.17 (1,4,5,6)	3.98±0.43 (1,4,5,6)	1.36±0.17 (1,2,3,5,6)	2.60±0.15 (2,3,4,6)	2.92±0.3 (2,3,4,5)	< 0.0001
6.5 kDa	3.29±0.85 (2,3,4)	4.87±0.66 (1,3,4,6)	7.16±1.15 (1,2,4,5,6)	1.77±0.59 (1,2,3,5,6)	4.01±0.42 (3,4)	3.52±0.65 (2,3,4)	< 0.0001

Data are means±SD of six mice in each group, Values of protein fractions are expressed as mg/0.5 mL serum, p is level of significance, where p≤0.05 is significant, Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (SPSS Computer Programme)

Table 3: Effect of *Ailanthus altissima* and *Ziziphus spina christi* extracts on alkaline phosphatase (ALP), bilirubin, cholesterol and nitric oxide in control and *Schistosoma mansoni* infected mice

	Control	Control-ethanol	Control-chloroform	Infected	Infected-ethanol	Infected-chloroform	
Parameters	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	ANOVA
ALP	0.656±0.081 (2,3)	0.437±0.081 (1,4,5,6)	0.432±0.113 (1,4,5,6)	0.962±0.195 (2,3)	0.603±0.179 (2,3)	0.673±0.147 (2,3)	< 0.0007
Bilirubin	5.689±2.31 (2,4)	9.294±1.886 (1,4)	7.34±1.68 (4)	15.90±1.97 (1,2,3,5,6)	7.11±2.86 (4)	6.94±2.103 (4)	< 0.0001
Cholesterol	9.168±1.46 (2,4)	8.173±1.004 (1,4,5,6)	7.343±1.101 (4,6)	18.703±1.487 (1,2,3,5)	9.088±0.752 (2,4)	10.078±1.656 (2,3)	< 0.0001
Nitric oxide	1.103±0.220 (3,4)	0.760±0.049 (4,6)	0.867±0.053 (1,4,5,6)	1.482±0.203 (1,2,3,5,6)	1.087±0.105 (3,4)	1.323±0.375 (2,3,4)	< 0.0001

Data are means±SD of six mice in each group, ALP expressed as mol min⁻¹ mg⁻¹ protein, Bilirubin as μmol L⁻¹ mg⁻¹ protein, cholesterol as μmol μg dL⁻¹ mg⁻¹ protein, nitric oxide as mol mg⁻¹ protein, p is level of significance, where p<0.05 is significant, Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (SPSS Computer Programme)

the levels of these parameters with respect to the infected group, values being ameliorated to nearly the normal values. Figure 4 show the percentage changes in ALP, cholesterol, bilirubin and nitric oxide between different groups.

DISCUSSION

Serum potentially carries an archive of important information whose determination could serve as drug target and biomarkers for disease

diagnosis. The analysis of serum however is analytically challenging due to the high dynamic concentration range of constituent proteins and peptides, with wide range of biological activities (Merrell *et al.*, 2004). However, serum protein electrophoresis continues to be a useful tool for identifying serum protein disorders (O'Connell *et al.*, 2005).

It should be pointed out that the different proteins separated cover a wide range of protein molecules, with varying and physiological functions.

In the present study total protein, albumin and the different molecular weight proteins separated electrophoretically were significantly decreased by schistosomal infection. This reduction reflects an impaired protein synthesis in the liver associated with malnutrition and protein malabsorption during schistosomiasis which agrees with the findings of (Schieg, 1996) that serum albumin level is an excellent gauge of hepatic protein synthetic ability and that it is highly reduced with total protein in chronic schistosomiasis.

Also schistosome infection has been found to induce inflammatory reactions in the host leading to changes not only in immunoglobulin, but also in acute phase proteins including α_2 -macroglobulin, which inhibits cathepsin-like cysteine and aspartic proteases, the enzymes that break host protein by parasite gut during infections (Arinola and Salimonu, 1998). This breakdown in protein macromolecules by schistosomes confirms the reduction in the various protein fractions separated (Charles, 1997; John *et al.*, 2004).

Concerning other metabolic parameters, the obtained data revealed remarkable elevation in ALP, cholesterol, NO and bilirubin in *S. mansoni* infected mice. In agreement with these data, El-Sokkary *et al.* (2002) reported increased NO, ALP and cholesterol associated with *S. mansoni* infection as evident biomarkers of liver damage. Marzouki and Amin (1997) found significant increase in serum total cholesterol (14-20 week post infection with *S. mansoni*) and attributed this to metabolites released by the parasite which affect host hepatic tissue while Soliman *et al.* (2002) confirmed the elevation in serum ALP to be associated with *S. mansoni* infection.

Previous studies indicate that the host response to *S. mansoni* infection involves the production of reactive oxygen species thus, levels of superoxide O_2^- and nitric oxide (NO) production by monocytes significantly increase indicating that these radicals have a role in immunity against such infections, i.e., increase antigenic stimulation and protective immunity (Abou-Shousha *et al.*, 1999; Zhou *et al.*, 2005). In addition, Brunet (2001) reported that NO is an integral component of the host against the invading parasites, the preferential production of pro-inflammatory cytokines predisposes to the inversed synthesis of NO, which mediates host protection through either parasite killing or by limiting parasite growth in schistosomiasis. Thus, NO plays a role in regulation of egg-induced inflammation preventing hepatocyte death and wide spread tissue damage by limiting parasite development (Ascenzi and Gradoni, 2002).

In order to compensate for the depletion in proteins due to malnutrition and protein consumption by the parasite during schistosomiasis, nutritional supplementation by natural extracts rich in protein may be a helpful tool for reducing liver disorders.

In this respect, plants are considered a tremendous source for the discovery of new products of high nutritional and medicinal value for drug development and for protection and recovery from disease disorders (Vanisree *et al.*, 2004).

In the present study, extracts from both *A. altissima* and *Z. spina christi* plants were administered to normal and *S. mansoni* infected mice to elucidate their nutritional and biological effects, since both plants are a good source of protein, of high nutritional value and are of great commercial potential for medicinal purposes (Duke and Ayensu, 1985; Shahat *et al.*, 2001; Nazif, 2002).

It was found that administration of both extracts to normal mice resulted in elevation of total protein content, in the albumin fractions and in the various serum proteins separated. This is directly correlated with the enrichment of both extract with proteins as previously described (Azim *et al.*, 2002). Also, one of the protein fractions, presumably 180 kDa may be correlated with α_2 -macroglobulin which is a protease inhibitor, thus its elevation causes increase in serum proteins.

In addition, both extracts reduced serum ALP and cholesterol relative to the untreated controls which indicates the bioafety of these extracts towards these liver biomarkers. However, mild toxicity of these extracts is indicated by an increase in the level of total serum bilirubin in healthy subjects.

Concerning NO, both extracts under study induced a remarkable decline in its content presumably as a beneficial role of these plant extracts to control the cytotoxic damage to the host own cells in case of exposure to tissue damage, since upregulated production in NO may lead to damages including alterations in neurological functions.

Administration of *A. altissima* and *Z. spina christi* to infected mice caused an increase in both albumin and total protein with respect to infection although levels were not ameliorated to control values. This effect was also reflected on the different separated protein fractions with varying degrees, certain fraction values even exceeding those of controls.

The present results also revealed that treatment of the infected mice with both extracts ameliorated the levels of ALP, cholesterol, bilirubin and NO to almost the normal values with respect to control.

These data demonstrate that the test extracts possess both hepatoprotective and antioxidant effects. Previously, Duke (1992) reported that the chemical constituents present in *A. altissima* which confer its antioxidant and hepatoprotective activities are β -sitosterol, Fisetin, gallic acid, Isoquercitrin, Lignin, Quercetin, Scopoletin and tannin (this also possesses antihelminthic activity). Also, this species has a long history in leek medicine and is used as antispasmodic, antihelminthic and parasiticide. The stem bark of the plant is used to treat dysentery and diarrhea (Ansari and Ali, 1999). However pharmacological research is focusing on the possible use of *A. altissima* for treating cancer, malaria and HIV-1 infections (Chang and Woo, 2003; Okunade *et al.*, 2003). Recently the plant was evaluated for its cytotoxic and antiproliferative activities, the CHCl_3 extract of root reduced cell viability and increased apoptosis in Hela cells (De Feo *et al.*, 2005).

With respect to *Z. spina christi*, almost every part of the plant has been used for medicinal purposes (Adzu *et al.*, 2003). The root bark is rich in alkaloids, triterpenoid and benzylquinoline. Other chemical constituents which confer biological activity are stigmasterol, β -D-glucoside, urosolic acid, octacosanol and flavonoids (Mahran *et al.*, 1996).

Hypoglycemic and antihyperglycemic effects of *Z. spina christi* have been demonstrated (Glombitza *et al.*, 1994; Zakaria *et al.*, 1999). Also *Z. spina christi* extract showed both antioxidant activity and protection against DNA damage (Effraim *et al.*, 1998).

Based on the previous information, both *A. altissima* and *Z. spina christi* can be used as nutritional food supplements especially for compensating protein depletions since their chemical constituents confirm their beneficial use against liver disorders. No significant toxicity changes in the biochemical parameters studied is shown although the use of the extracts should be rationalized to avoid mild adverse side effects.

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