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SHORT COMMUNICATION

Hepatotoxic Effect of *Aralia mandshurica* Dried Root Extract in Pigs

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The hepatotoxic effect of a dried root extract of *Aralia mandshurica* over a period of 60 days (0.16 g/kg, 1.5 g/kg and 3 g/kg) was studied in Landrace pigs of both sexes. The toxic effect of *Aralia mandshurica* was evaluated by measuring serum alanine amino transferase (ALT), gamma glutamil transpeptidase (gGT) and serum alkaline phosphatase (SAP). Blood samples were obtained by venopuncture on days 0, 7, 30, and 60 after the administration of *Aralia mandshurica* and the body weight was registered weekly. At the end of the experiment the liver was examined histologically. The levels of ALT and gGT were increased significantly with all the concentrations of *Aralia mandshurica* at day 60. A subclinical hepatitis characterized by the presence of lymphocytes and polymorphonuclears in the portal and periportal region was observed. A hepatobiliary toxic effect of *Aralia mandshurica* dried root extract after chronic administration in pigs is concluded.

Keywords: Aralia mandshurica; hepatotoxicity; transaminases; pigs; subchronic toxicity

INTRODUCTION

Herbal medicines are largely advertised to be devoid of adverse toxic effects in contrast to conventional drugs. However, recently for some herbal medicines after prolonged use, a potentially toxic effect has been described (MacGregor *et al.*, 1989). *Aralia mandshurica* (Rupr. et Maxim) (Araliaceae) is a medicinal plant classified as an 'adaptogen' (Brekhman and Dardymov, 1969), reputed to be completely innocuous and capable of increasing the nonspecific resistance of organisms against the adverse influences of various origins (Brekhman and Dardymov, 1969).

In an earlier work we described the subchronic toxic effect of *Aralia m.* (Rupr. et Maxim) (Araliaceae) root dried extract in rats (Burgos *et al.*, 1994). At day 60 of administration of *Aralia m.* (3.9 g/kg p.o.), a decrease in body weight was observed, this effect being more pronounced at day 90 (Burgos *et al.*, 1994). Also, at day 90, (3.9 g/kg) *Aralia m.* provoked an increase in the serum level of SAP, AST and in the liver weight. A possible toxic effect of *Aralia m.* on the billary canaliculi and/or an enzymatic induction mechanism of action was proposed. However, no histopathological findings were observed in the rat liver (Burgos *et al.*, 1994). The above findings reveal an unspecific toxic effect of *Aralia m.* in rats leaving the question open as to whether *Aralia m.* could exert a long term toxic specific effect on the liver or if in another animal model it is possible to observe similar effects. In this study we investigated the possible subchronic toxic effect of *Aralia m.* on the liver in pigs.

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MATERIAL AND METHODS

Materials. The dried root extract of *Aralia m.* was obtained from the Swedish Herbal Institute. The procedure of extraction has been described previously (Burgos *et al.*, 1994).

Kits for ALT, gGT, SAP, and other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Animals. 24 Landrace pigs of both sexes were utilized, weighing 9.0 ± 0.8 kg. All pigs were clinically healthy. The animals were identified by ear tattoo and were separated at random into four groups, each consisting of three females and three males separately. An acclimatization period of 7 days was allowed before experimentation. The animals were kept under controlled temperature (22° ± 5°C) and humidity (relative 50%) conditions. Water was given *ad libitum*. Food composition was 25% of protein, 2%–3% crude fibre, 2900 kcal/kg.

Experimental design. All pigs were weighed weekly. While one group of pigs was administered food only, the other three groups were given *Aralia m.* extract mixed in the following percentages 0.5%, 3% and 6% in the food. This last concentration was considered as the maximum possible dose to administer, as is described elsewhere (Poole and Leslie, 1989). Doses higher than 6% of *Aralia m.* decrease the consumption of food in pigs. Concentrations of 0.5%, 3% and 6% are equivalent to 0.16 g/kg, 1.5 g/kg and 3 g/kg, respectively. The amount of food was given according to the nutritional requirements and adjusted weekly according to body weight. On days 0, 7, 30, and 60 of administration of *Aralia m.* blood samples were obtained by cava puncture

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with a vacutainer tube. On day 60, the pigs were killed by electronarcosis.

Biochemical analysis. After obtaining blood samples, these were centrifuged at 2000 rpm for 5 min. The serum (1.5 mL) was stored at −25°C. gGT, ALT and SAP were measured utilizing the enzymatic kinetic method of Sigma Chemical Co. Diagnosis (USA).

Histological analysis. The liver tissues were fixed in a 10% neutral buffered formalin solution and imbedded later in paraffin. The sections were stained with haematoxylin and eosin.

Statistics. For the biochemical analysis, an ANOVA one-way with randomized block data test was utilized. This allowed the isolation of any effect of sex in the treatments, as is described elsewhere (Steel and Torrie, 1985). A Tukey test of multiple comparisons was used. For the body weight an analysis of covariance and a Scheffe's multiple comparison test was utilized (Steel and Torrie, 1985). The results are expressed as the arithmetic mean and standard error of the mean.

RESULTS

No changes in behaviour, external appearance, consumption of food, or body weight was observed. A significant increase in the serological enzymatic levels of gGT, compared with the control with 0.16 g/kg, 1.5 g/kg and 3 g/kg of *Aralia m.* on day 60 (Fig. 1) was recorded. Significantly higher levels of ALT activity compared with the control were observed with all doses tested (Fig. 2). The concentration of 3 g/kg of *Aralia m.* induced the highest values of ALT in the plasma compared with all other experimental groups. SAP did not show significant differences during the whole experiment (data not shown). The animals treated with *Aralia m.* showed some histological changes in the hepatocytes. The cytoplasm showed irregular eosinophilic clumping, usually focal, involving either part or the entire cytoplasm. The latter resulted in dark eosinophilic cells without nuclear change. Portal tract changes varied from minor inflammatory infiltration, predominantly lymphocytes and polymorphonuclears indicative of a toxic hepatitis (Popper and Franklin, 1948). The portal inflammation involved small or large areas of the tract exhibiting different intensity. The periportal inflammation with destruction of the periportal area indicates a focal necrosis and in some cases included portal tract inflammations (Fig. 4).

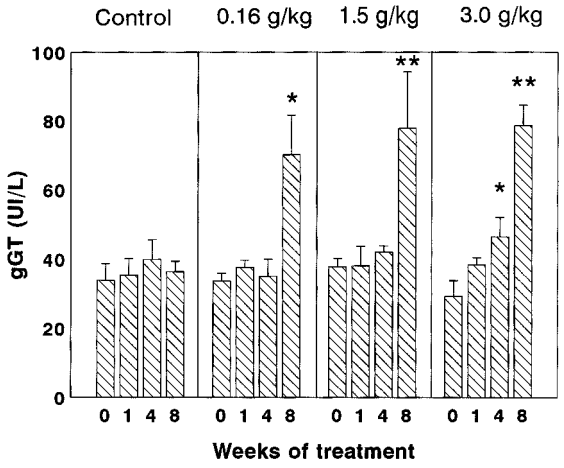

Figure 1. Mean serum concentration of gGT (UI/L) in pigs administered different doses of *Aralia mandshurica* for 60 days. Each value represents the mean±SEM. *$p<0.05$, **$p<0.01$ compared with day 0 of respective group.

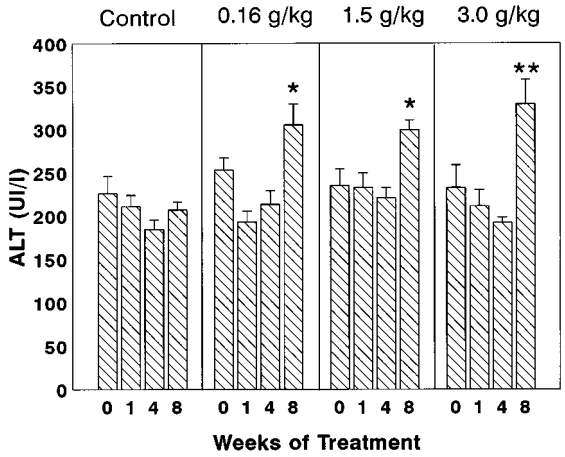

Figure 2. Mean serum concentration of ALT (UI/L) in pigs administered different doses of *Aralia mandshurica* for 60 days. Each value represents the mean±SEM. *$p<0.05$, **$p<0.01$ compared with day 0 of respective group.

DISCUSSION

The results presented in this paper suggest that the subchronic administration of *Aralia m.* after 60 days induces a subclinical hepatotoxic effect in pigs. The hepatotoxic effect was shown by an increase in the serum level of gGT at day 60 compared with the controls ($p<0.05$) and could represent a specific toxic effect on the hepatobiliary system (Cornelius, 1989; Albilos *et al.*, 1990). This was corroborated by a significant increase of ALT activity at day 60 (Fig. 2). High levels of ALT activity indicate specific hepatocellular lesions (Mcintyre and Rosalki, 1993), with which they were corroborated histologically. The increase of ALT activity (3 g/kg *Aralia m.*) at day 60 was preceded by an increase in the serum level of gGT at day 30 of administration (see Fig. 1). Moreover, the toxic effect is more intense with gGT (2 fold) than with ALT (50%). This observation indicates a higher sensibility of gGT to toxic effect. This could indicate that at these doses the effect of *Aralia m.* is predominantly cholestatic

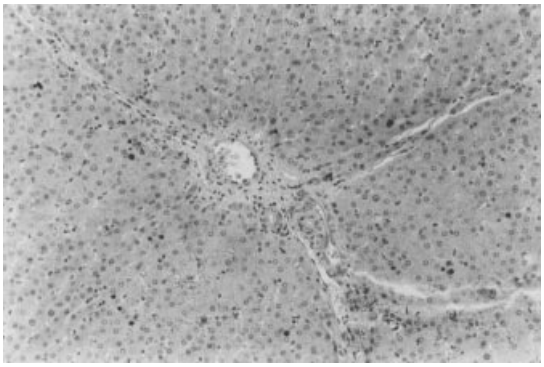

Figure 3. Liver of control animal stained with haematoxylin-eosin (100×).

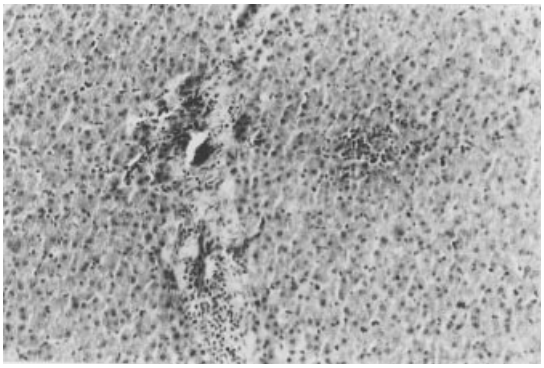

Figure 4. Liver of animal treated with 3.0 g/kg of *Aralia mandshurica* for 60 days and stained with haematoxylin–eosin (100×). Note the periportal inflammation with destruction of the periportal area.

(Cornelius, 1989), and, if this is so, could explain the increase in SAP observed in rats treated with *Aralia m.*. In these animals the increase of SAP preceded the increase in AST (Burgos *et al.*, 1994).

Several lesions were recorded around the portal duct. Microscopically, the main findings were infiltrative lymphocytes and polymorphonuclear cells in the periportal and portal zone (see Fig. 4). The lesions induced by *Aralia* are similar to other drugs which damage the intrahepatic bile duct (Lefkowitch, 1993; Berg and Klein, 1993). Indeed, the hepatic lesions indicate a different sensitivity of piglets to *Aralia m.* as compared with the rat model (Burgos *et al.*, 1994). The absence of clinical manifestations could indicate that the hepatic lesions are subclinical as is the case of other drugs (Kaplowitz *et al.*, 1986; Scheuer and Bianchi, 1974) or that a larger administration period is required in order to manifest the toxic effect of *Aralia m.* (Burgos *et al.*, 1994).

The extract contains mainly saponin structures which are steroidal (Lutomsky and Nguyen, 1977; Samochoviec, 1983; Voskanyan *et al.*, 1983; Wojcicki *et al.*, 1977). It is possible that these steroids will be responsible for the hepatotoxic effect. In support of this suggestion, it is known that oestrogen or derivatives can induce hepatotoxicity (Berr *et al.*, 1984), moreover this effect is characterized by no alterations in SAP activity (Heikel and Lathe, 1970; Schreiber and Simon, 1983) and a wide range of histopathological lesions (Berg and Klein, 1993), similar to those observed with *Aralia m.* adminstration.

The use of other herbal medicines such as germander, valerian, asafetida, hops, skullcap, and gentian may induce hepatitis after a prolonged period of intake (MacGregor *et al.*, 1989), as well as ingestion of senna fruit extracts (Beuers *et al.*, 1991), chinese herbs (Davies *et al.*, 1991), mistletoe (Harvey and Collin-Jones, 1981) and chaparral leaf (Katz and Saibil, 1990). This fact is of importance if we consider that *Aralia m.* is a medicinal plant of tonic action, indexed in the Russian Pharmacopea as innocuous for humans. It can be concluded that *Aralia m.* dried root extract induced a subclinic hepatotoxicity, after 60 days of administration, characterized by an increase in gGT and ALT, associated with portal and periportal inflammation.

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