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Hepatoprotective effect of *Ginkgo biloba* leaf extract on lantadenes-induced hepatotoxicity in guinea pigs

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ABSTRACT

The hepatoprotective effect of freeze-dried methanolic leaf extract of *Ginkgo biloba* was evaluated against lantadenes-induced hepatic damage in guinea pigs. The reversed-phase HPLC analysis of lantadenes confirmed the presence of 72.82% of lantadene A. UPLC-ESI-MS analysis showed the presence of ginkgolide B, C, bilobalide and traces of ginkgolide A and J in *G. biloba* extract. The concentration of ginkgolide B in the sample was found as 0.29%. The elevated levels of serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase due to lantadenes were significantly restored towards normal values by *G. biloba* extract in a dose-dependent manner. The effects of lantadenes and *G. biloba* extract on lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase were assayed in liver homogenates to evaluate the antioxidant activity. *G. biloba* extract in a dose-dependent manner produced significant decrease in lantadenes-induced increased levels of LPO. The lantadene-induced decreased levels of SOD, GSH and catalase were elevated by *G. biloba* extract. The findings of biochemical and antioxidant enzyme levels were supported by gross and histopathological observations. Moreover, liver sections of *G. biloba* group also showed a marked decrease in apoptosis in comparison to lantadenes group. This study suggested that *G. biloba* could be used as a promising hepatoprotectant against lantadenes-induced hepatic damage. Future studies are needed to elucidate the precise mechanism of hepatoprotection for practical application.

1. Introduction

Lantana camara Linn. (Locally known as *bara phulnoo* in Himachal Pradesh), an ornamental shrub, belongs to the family Verbenaceae. The branches of the plant possess curved prickles, grows to a height of 2–3 m, and its spreaded branches cover an area of about 1 m^2 (Sharma et al., 1981b). The most prevalent and most noxious variety of *L. camara* is red flower variety (*L. camara* var. *aculeata*), which causes severe toxicity in grazing animals (Sharma et al., 1991). Mexico and Central America are considered as generic epicentre of *L. camara* (Spies, 1984) from where it was introduced to the rest of world via Europe (Stirton, 1977). It is the principal weed in 12 countries and has been found in nearly 50 countries (Ghisalberti, 2000). In India, *L. camara* was introduced in the nineteenth century and has spread all over the country (Sharma et al., 1981a). This plant is spread widely over Kangra valley in Himachal Pradesh (Sharma and Makkar, 1981), Western

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ghats in South India, Kumaun hills, Garhwal in Uttarakhand, Allahabad, Kukrail forests of Lucknow in Uttar Pradesh, Maharashtra, Tamil Nadu, and in many other parts of the country (Sharma et al., 2007). In a survey of the Kangra valley of Himachal Pradesh in India, Sharma and Makkar (1981) observed lantana poisoning as the main cause for loss of livestock.

L. camara toxicity caused by lantadenes is characterized by intrahepatic cholestasis, associated liver damage and photosensitization. Both ruminants including cattle, sheep, buffalo, goats, and non-ruminants like horses, guinea pigs, rabbits, female rats are susceptible to lantana toxicity. Guinea pigs, from non-ruminant group, exhibit the most typical symptoms comparable to experimental or field cases of lantana toxicosis in ruminants (Sharma et al., 1992). The toxic compounds of *L. camara* are lantadenes, which are pentacyclic triterpenoids in nature (Sharma et al., 1988; Pass, 1991). The major constituents of the leaves of the red flower variety are lantadene A (LA), lantadene B (LB), lantadene C (LC) and lantadene D (LD) (Sharma et al., 1991). Reduced lantadene A (RLA) and reduced lantadene B (RLB) are the minor constituents (Sharma et al., 1991). In ruminants, *L. camara* toxicity primarily attacks liver and kidneys and also causes photosensitization. Inappetance and constipation within 24 h of dosing followed by ictericity and photosensitization are typical signs of lantana toxicity (Sharma et al., 1992).

A specific treatment for *L. camara* toxicity is still lacking. The conventionally used treatment includes rumenotomy to remove toxic rumen contents (McSweeney and Pass, 1982). Administration of activated charcoal in the early phase of intoxication successfully treats the affected animals provided this treatment is started before the onset of absorption of toxins (Pass and Stewart, 1984). A number of treatments have been attempted, after the onset of clinical symptoms but are not very effective (Sharma et al., 1981a). *Ginkgo biloba* is the oldest living fossil tree on the earth, more than 200 million years old (Michal, 1986). Ginkgo is a pretty, ornamental tree with unique, beautifully-shaped bilobed leaves, belonging to the family Ginkgoaceae. In north India, it is grown in the Nambalbal area of Pampore, Jammu and Kashmir state and also in the Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology (CSIR-IHBT), Palampur, Himachal Pradesh state. It is used by medical professionals to aid the treatment of problems typically associated with ageing, such as poor circulation, mental confusion and memory loss (Gertz and Kiefer, 2004). The active constituents of *G. biloba* leaf are diterpene lactones namely Ginkgolides A, B, C, M and J and bilobalide, flavone glycosides (kaempferol, quercitin and isorhamnetin), biflavones (ginkgetin, isoginkgetin, bilobetin) and organic acids such as 4- hydroxybenzoic acid and shikimic acid (Kleijnen and Knipschild, 1992). Among these, the ginkgolides are structurally unique in the way that they are found only in this particular plant and they co-occur with bilobalide (Nakanishi et al., 1971; Weinges et al., 1987). The present experiment was designed to evaluate the hepatoprotective activity of *G. biloba* leaf extract by studying its effects on liver function tests, various enzymatic and non-enzymatic antioxidant parameters, gross pathology, histopathological examination and apoptotic reaction during lantadenes-induced hepatotoxicity in guinea pigs.

2. Materials and methods

2.1. Chemicals

Reduced glutathione, NADH disodium salt, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), and trichloroacetic acid were procured from Sisco Research Laboratories, Mumbai, India. Chloroform, sodium pyrophosphate, Triton X-100, hydrogen peroxide and methanol were procured from Merck Specialities Pvt. Ltd, Mumbai, India. Silymarin, thiobarbituric acid and p-nitrophenylphosphate was procured from Sigma–Aldrich Chemicals Pvt. Ltd, New Delhi, India. DeadEnd™ colourimetric TUNEL (TdT-mediated dUTP Nick-End Labeling) system was procured from RFCL Ltd, Promega, India. Solvents used for TLC, extraction, and oxidation stress estimation were of analytical grade. All the solvents used for HPLC and sample preparation were of HPLC or above grade. Before use, the solvents were filtered through 0.45 μ membrane filter, followed by sonication for 15 min.

2.2. Experimental animals

Twenty guinea pigs of around 45 days old, weighing 200–250 g and of either sex were procured from Laboratory Animal Resources Section, Indian Veterinary Research Institute (IVRI), Izatnagar. The animals were maintained in the Laboratory Animal Housing Facility of IVRI Regional Station, Palampur in polypropylene cages under standard conditions of humidity (55 ± 10% RH) and temperature (23 ± 2 °C). All sanitary and hygienic measures were observed as per the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines. The animals were provided *ad libitum* access to standard laboratory animal diet added with vitamin C (Limcee, 1000 mg/kg feed) and clean water during the period of the experiment. The experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee.

2.3. Collection of L. camara leaves and purification of lantadenes

L. camara leaves were collected during the month of September–October from an area adjoining Palampur town located at an altitude of 1200 m above mean sea level. The sample was oven dried at 55 °C and ground to a fine powder of 1 mm particle size using a grinder. Powdered leaves of *L. camara* were extracted as per the protocol described by Sharma et al. (1999) with some modifications so as to obtain lantadenes. Lantana leaf powder (100 g) was extracted with 500 ml methanol with intermittent shaking and was filtered through two layers of muslin cloth. The residue was extracted once again with 200 ml methanol. The methanolic extracts were pooled and decolorized with 15 g of activated charcoal. The methanol was removed *in vacuo* at 60 °C in rotary evaporator (Heidolph Laborota 4000). The residue so obtained was extracted with 100 ml chloroform. Chloroform was removed *in vacuo* at 40 °C in rotary evaporator. Dried