



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Radio Protective Activity of the Plant *Aquilaria Malaccensis* Leaves

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ABSTRACT

The present study was designed to evaluate the radio protective potency of the ethanolic extract of *Aquilaria malaccensis* (Agar wood) leaves by assessing the endogenous oxidant level, haematological parameters and the histopathology of liver. Extract was given orally at a dose of 200 mg/kg, and 400 mg/kg orally for 15 days prior to exposure of Gamma radiation (2.5 Gy). The irradiated mice were kept under observation for 15 days for any sign of radiation sickness, morbidity and mortality which showed no changes. The haematological parameters showed a significant decrease in the irradiated mice and increase in the drug treated mice when compared with the control group. Radiation induced mice showed increases in the level of endogenous enzymes, when compared to normal animals. Treatment with extract caused reversal of these changes towards the normal. Histopathological changes in the liver section of treated animals showed improved cellular architecture when compared to the normal group. The present study indicates that supplementation with *Aquilaria malaccensis* has significant antioxidant activity and act as probable radio protector against gamma radiation induced oxidative damage. The finding of the present study provides the evidence that, alcoholic extract of *Aquilaria malaccensis* may be beneficial as a potential supplement in the radiotherapy to protect normal cells from the destructive effects of radiation.

Keywords: *Aquilaria malaccensis*, Gamma-radiation, Radioprotection

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Received 01 November 2018, Accepted 23 November 2018

Please cite this article as: Singh MN *et al.*, Development of Naringenin Nanocrystals for Enhanced Solubility and Bioavailability. American Journal of PharmTech Research 2018.

INTRODUCTION

Protection of biological systems from ionizing radiation is of paramount importance in planned as well as unplanned accidental exposures to radiation and the development of novel and effective agents to combat radiation damages using nontoxic radio protectors is of considerable interest in defense, nuclear industry, space travels, and health care, particularly in radio diagnostics and therapy¹. It has been recognized that radiation-induced effects remain a significant risk.

Because radiation-induced cellular damage is attributed primarily to the harmful effects of free radicals, molecules with radical scavenging properties are particularly promising as radioprotectors². However, most of them produce serious side effects, and some are considered to be toxic at the doses required for radioprotection². So, the development of radio protective agents has been the subject of intense research in view of their potential for the use within a radiation environment, such as space exploration, radiotherapy, nuclear power plants reactor accidents and even nuclear warfare³. Up to 80 percent of cancer patients need radiotherapy either for curative or palliative purpose. In order to obtain better tumor control with a higher dose, the normal tissues should be protected and this could be achieved by various techniques as well as radio protective agents. Thus the role of radio protective compounds is very important in clinical radiotherapy.

Many synthetic as well as natural compounds have been investigated the recent past for their efficacy to protect the biological systems against the deleterious effects of radiations. They include sulfhydryl compounds, antioxidants, immunomodulators, and other agents. The most pragmatic approach to select the possible candidate to evaluate radio protective effect is to look into the available properties of the substance. Whether a substance has anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, free radical scavenging or anti-stress properties, if so, it may act as a potential radio protector and could be the right candidate for evaluation of its radio protective activity. So the search for alternative sources, including plants, has been ongoing for several decades. Several plants have been used to treat free radical-mediated ailments and, therefore, it is logical to expect that such plants may also render some protection against radiation induced damage.

One such plant, *Aquilaria malaccensis* (Agar wood) belongs to the family Thymelaeaceae, locally known as Eagle wood distributed in India, Burma, Malaysia, Philippines and Indonesia. Agar wood leaves are reported to possess antidiabetic, anti-inflammatory, antioxidant, antibacterial, antidepressant and antiviral activities^{4,5,6}. The extract of the leaves of agar wood is reported to contain more than a dozen chemical constituents belongs to flavonoids, alkaloids, terpenoids and glycosides class of secondary metabolites that can be extracted⁵. They have a wide use in medicines

like general pain reducer, kidney and rheumatic diseases, as a venom repellent and free radical scavenging properties. Further the agar wood is reported to possess anticancer activity⁷. Moreover, the presence of glycoside moieties like saponins, anthraquinones, steroidal glycosides and flavonoids could inhibit tumor growth and act as potential radical scavengers⁸. The leaves of the plant are also used by tribal healers of India to ameliorate many diseases. The presence of these arrays of chemical constituents and radical scavenging potentials might possess radio protective effect. However, there is no scientific data are available to justify the radio protective effect of *Aquilaria malaccensis*. Keeping the above information in view, in the present study the ethanolic extract of the leaves of *Aquilaria malaccensis* was evaluated for its radio protective activity.

MATERIALS AND METHOD

Plant Material and Extraction

The leaves of *A. malaccensis* were collected from the local areas and authenticated by Taxonomist. The collected leaf materials were cleaned and shade dried. The coarse leaf powder was exhaustively extracted with ethanol for 2 days at room temperature. The extraction of grounded leaves was further repeated with ethanol (twice). The entire filtrate was subjected to evaporate under reduced pressure using rotary flash evaporator to give concentrated crude extracts. The dried extract was kept in a glass container until further use.

Experimental Animals

Wistar albino mice (20-25 g) and Wistar albino rats (150-200 g) of either sex maintained under standard room conditions with free access to rodent pellet diet and water were used. The protocol was approved by the Institutional Animal Ethics Committee with the reference number SCP/IAEC/F150/P20/2015.

Preliminary Phytochemical Analysis

Extract was subjected to phytochemical screening for the identification of various phytoconstituents like alkaloids, glycosides, steroids, flavonoids, tannins, etc.

Acute Oral Toxicity Study

The extract was subjected to oral toxicity studies using Wistar albino rats as per OECD guidelines 425⁹. The animals were fasted overnight and the extract administered orally with a starting dose of 2000 mg/kg, to different groups of animals. Animals were observed continuously for first 3h and monitored for 14 days for any mortality and general behavior of animals, signs of discomfort and nervous manifestations.

In-vivo Radio protective Activity

The albino mice were divided in to 4 groups of 6 animals each as normal control, radiation control and irradiated animals treated with extract 200 mg/kg and 400 mg/kg per body weight respectively. All the drugs were administered orally daily for 15 days. The un anaesthetized animals were restrained in ventilated Perspex box and the whole body was exposed to gamma radiation at a distance around 80 cm from the source at the dose rate of 2.5 Gy/min. After the irradiation, the animals were observed for 15 days for any radiation induced lethality and mortality. The radio protective effect of the extract was evaluated by measuring the in-vivo lipid peroxidation and serum endogenous antioxidant enzymes. The haematological assay (RBC, WBC, platelet count, Hb concentration and % PCV) were also evaluated¹⁰. After collection of blood the liver was immediately excised and rinsed in ice cold normal saline, portion of liver was subjected to evaluate for MDA, GSH and catalase activity¹¹. The sections of liver were also examined for the pathological findings of hepatotoxicity.

Statistical Analysis

The data were expressed as mean value \pm SEM and significance was analyzed by one way ANOVA.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

Phytochemical evaluation of *A. malaccensis* leaves extract showed positive result for saponins, alkaloids, flavonoids, terpenoids, tannins, carbohydrate, glycosides, coumarin, emodins, anthraquinones, and resins.

Acute Toxicity Study (LD₅₀)

The oral acute toxicity study was found to be safe up to 2000 mg/kg. There was no mortality and no signs of toxicity observed. Therefore two dose levels i.e., 200 mg/kg and 400 mg/kg per body weight were selected for the present study.

Serum hematological parameters

The haematological parameters of mice against gamma irradiation damages are depicted in the table 1. The WBC, erythrocytes, platelets, packed cell volume and haemoglobin content was decreased significantly in gamma irradiated mice as compared to the control. Pretreatment of animals of group 3 and group 4 with extract caused considerable increase in the hematological parameters.

Serum biochemical parameters

Activities of serum ALT, AST, ALP and LDH were significantly elevated ($p \leq 0.05$) in rats of irradiated group that exposed to whole body irradiation in comparison with control. Serum total cholesterol, triglycerides concentrations were elevated ($p \leq 0.05$) in irradiated animals. Pretreatment

of animals of group 3 and group 4 with extract caused considerable improvement of serum enzymes and lipid profile values dose dependently (Table 2).

Oxidant/antioxidant biomarkers

Hepatic MDA level is significantly increased ($p \leq 0.05$) in irradiated group compared to control. Moreover, hepatic GSH concentration and CAT activity were significantly reduced ($p \leq 0.05$) in comparison to control. All these parameters were relatively improved and shifted toward the normal values in rats of group 3 and group 4 received extract before irradiation (Table 3).

Histopathological findings

There were no histopathological alterations in the liver of control rats and the normal histological structure of the central vein and surrounding hepatocytes were observed (Fig. 1a). In irradiated animals of Group 2 (Fig. 1b), liver showed focal areas of degeneration, congested and dilated sinusoids. However, rats in that receiving irradiation followed extract treatment revealed only slight congestion of sinusoids and showed no abnormalities (Fig. 1c & 1d).

Table 1: Effect of extract of *Aquilaria malaccensis* on hematological profile

Groups	RBC (millions/cmm)	WBC (/cmm)	Platelet (lakh/cmm)	Hemoglobin (G %)	PCV (%)
Normal	5.5	10000	3.5	14.0	48
Radiation control	4.0	5700	2.5	9.0	29
Radiation+Extract (200mg/kg)	4.5	9000 ^b	3.4 ^a	11.4 ^b	34 ^b
Radiation+Extract (400mg/kg)	5.2 ^b	9500 ^c	3.8 ^b	12.0 ^c	42 ^c

One way ANOVA, n=6, ^aP< 0.05, ^bP< 0.01, ^cP< 0.001 when compared with radiation control group.

Table 2: Effect of extract of *Aquilaria malaccensis* on serum marker enzymes and lipid profile

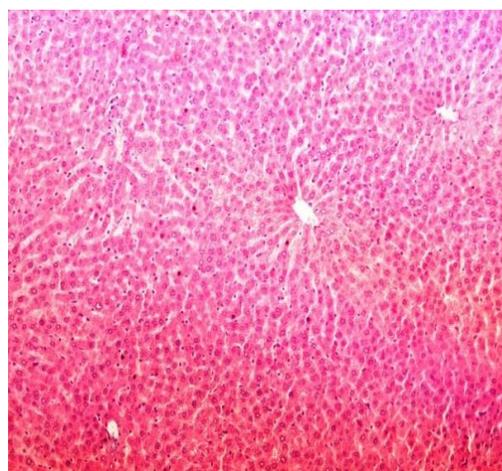
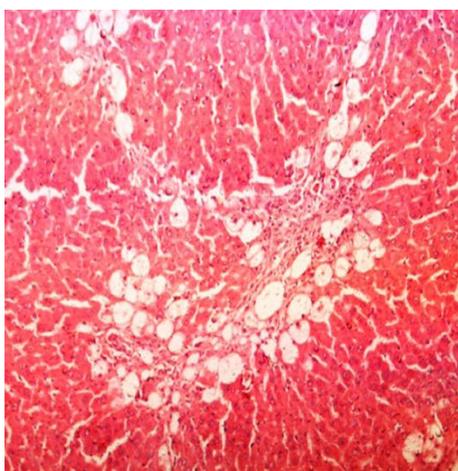
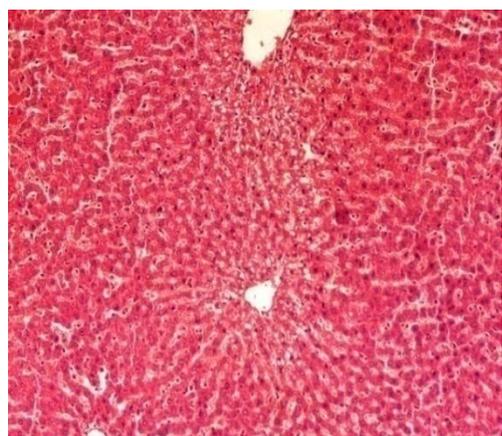
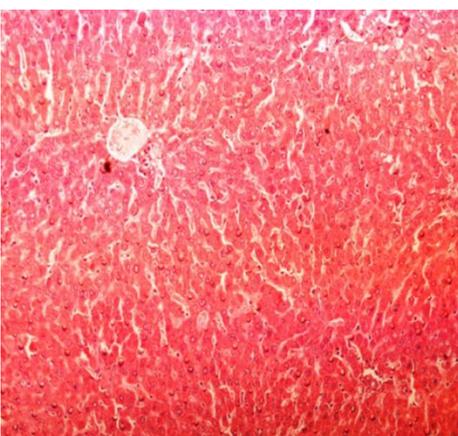
Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	LDH (IU/L)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal	181.09 ± 5.06	63.42 ± 3.44	66.21 ± 2.17	923.68 ± 20.34	66.68 ± 3.97	46.29 ± 1.98
Radiation control	267.72 ± 3.43	194.32 ± 3.56	99.31 ± 4.60	1090.61 ± 28.28	79.22± 3.24	73.69± 3.78
Radiation+Extract (200mg/kg)	261.15 ± 5.42	186.00 ± 5.31	88.15 ± 3.54	1018.32 ± 28.23	74.32± 3.13	62.18± 3.15
Radiation+ Extract (400mg/kg)	228.75 ± 7.47 ^c	114.50 ± 4.95	76.82 ± 2.93	979.28 ± 21.09	69.89 ± 3.13	53.83± 2.85

One way ANOVA, ± SEM, n=6, ^aP< 0.05, ^bP< 0.01, ^cP< 0.001 when compared with radiation control group.

Table 3: Effect of extract of *Aquilaria malaccensis* on liver tissue lipid peroxidation

Groups	MDA (nmol/g)	GSH (mg/g)	CAT (IU/g)
Normal	39.07 ± 1.48	79.23 ± 2.79	3.78 ± 0.12
Radiation control	68.09 ± 3.74	48.26 ± 2.31	3.51 ± 0.09
Radiation+ Extract (200mg/kg)	58.62 ± 2.56 ^a	56.42 ± 2.98 ^b	3.64 ± 0.08 ^a
Radiation+ Extract (400mg/kg)	50.08 ± 2.48 ^b	68.56 ± 1.82 ^a	3.70 ± 0.08 ^b

One way ANOVA, ± SEM, n=6, ^aP<0.05, ^bP<0.01, ^cP<0.001 when compared with radiation control group.

**A. Normal mice****B. Radiation control mice****C. Extract (200 mg/kg) treated mice****D. Extract (400 mg/kg) treated mice****Figure. 1. Liver histopathology.**

(a) Liver of control rat. (b) Liver of irradiated rat showing focal areas of degeneration, congested and dilated sinusoids. (c) Liver of a rat pretreated with extract (200 mg/kg) before irradiation and (d) Liver of a rat pretreated with extract (400 mg/kg) before irradiation revealing slight congestion of sinusoids with normal architecture.

Human beings constantly struggle against the changing environment condition to maintain optimum health throughout their life, during all the seasons. The human body depends on the continuous hormonal interaction between internal and external factors. When this interaction fails, either due to internal deficiency or hostile environmental factors, the balance is disturbed and leads to disharmony and disease¹².

Ionizing radiation produces its harmful effects through radiolysis which results in releasing of ROS in cells and depletion of cellular antioxidants including glutathione and enzymatic antioxidants. ROS can evoke the inflammatory response by increasing the expression of chemokines, cytokines and endothelial-leukocyte adhesion molecules¹³. The need for radio protective to protect normal tissues during radiotherapy motivated us to study the possible radio protective effect of Agar wood leaves extract in rats.

In the present investigation, the hematological parameters like WBC, erythrocytes, platelets, packed cell volume and haemoglobin content was decreased significantly in gamma irradiated mice, whereas gamma-irradiation of rats increased the activities of serum ALT, AST, ALP and LDH. The elevation of serum transaminases is indicative of hepatocyte injury leading to increase in cell membrane permeability that facilitates the passage of cytoplasmic enzymes to blood. Hepatic ALP is present on the canalicular and luminal domain of the bile duct epithelium and levels rise because of increased synthesis and consequent release into the circulation due to biliary obstruction¹⁴. The increase in serum LDH can be attributed, like transaminases, to enzyme leakage through the damaged membrane of hepatocytes. In addition, it could be due to hypoxia resulting from hepatocyte injury¹⁵.

Our findings also revealed that irradiation of rat induced significant increases in serum cholesterol and triglycerides. The irradiation-induced hyperlipidemia may be attributed to changes in liver lipid metabolism and serum lipoproteins and may be due to indirect effect of radiation through the release of different inflammatory mediators¹⁶.

Results of the present investigation demonstrated that whole body irradiation of rats significantly increased the level of liver MDA, while decreased hepatic glutathione reduced level and catalase activity. This was accompanied with histological alterations including focal areas of degeneration, congestion and dilated sinusoids. Similar findings were previously reported demonstrating that oxidative stress is induced by radiation¹⁷.

The present study demonstrated that leaf extract of *Aquilaria malaccensis* ameliorated the tissue damage induced by whole body irradiation of rats as evidenced by improvement of hematological parameters, liver function and lipid profiles. The antioxidant mechanisms of the body were enhanced

as shown by elevated liver glutathione concentration and catalase activity with reduction of liver malondialdehyde level. In addition, the histopathological observations were in congruence with the biochemical observations in liver and serum.

This protective effect may be attributed to the presence of many constituents in leaf extract of *Aquilaria malaccensis* including saponins, alkaloids, flavonoids, terpenoids, tannins, carbohydrate, glycosides, coumarin, emodins, anthraquinones, and resins.

In conclusion, the leaf extract of *Aquilaria malaccensis* can be effective in reducing radiation-induced oxidative stress, hepatotoxicity, and hyperlipidemia. This makes the substance a potential supplement in the radiotherapy to protect normal cells from the destructive effects of radiation.

ACKNOWLEDGEMENT

The authors are thankful to the Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore for financial assistance to this work.

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