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## **Antioxidant potential of arjunolic acid in an *in-vitro* system of precision cut goat liver slices subjected to oxidative stress**

Lavanya Yaidikar and Santh Rani Thakur

Division of Pharmacology, Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati-517502, Andhra Pradesh, India

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### **ABSTRACT**

Oxidative stress upshots from imbalance between antioxidant defense system and reactive oxygen species. It often leads to various pathological diseases like cancer, ischemia, inflammation, atherosclerosis and other neurodegenerative disorders. Precision cut goat liver slices are used as an *in-vitro* model for the evaluation of antioxidant activity by crafting *in-vivo* tissue environment and helps in curtailing the use of intact animals. Plant derived products have been engrossed for the therapy of oxidative stress associated pathological diseases. One such phytochemical is Arjunolic acid, a terpenoid saponin from *Terminalia arjuna* bark. The present study is focused to evaluate the antioxidant potential of Arjunolic acid in an *in-vitro* system of goat liver subjected to oxidative stress. The liver slices treated with hydrogen peroxide (untreated control group) alone showed significant decrease in both enzymatic and non-enzymatic antioxidant levels as compared with normal liver slices. The Arjunolic acid treated liver slices showed significant improvement in both enzymatic and non-enzymatic antioxidant levels as compared with untreated control group. From our results it was observed that Arjunolic acid showed significant antioxidant activity in an *in-vitro* system of goat liver slices subjected to oxidative stress.

**Key Words:** Arjunolic acid, antioxidant activity, precision cut goat liver slices

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### **INTRODUCTION**

Oxidative metabolism is one of the customary metabolic process in the human body and is concomitant with the generation of reactive oxygen species (ROS). The generated ROS are regulated by endogenous antioxidant defense system thereby upholding homeostasis. If any riot in this homeostasis upshots in oxidative stress. During oxidative stress, there is robust production of ROS which overwhelms the antioxidant defense system, attacks lipids, proteins and cause damage to DNA. Such damage by free radicals leads to many pathological diseases such as cancer, inflammation, atherosclerosis including neurodegenerative disorders[1]. Several epidemiological studies conveyed that antioxidant especially from plant origin plays protective role, over and above let down the risk of free radicals associated diseases. Arjunolic acid (AA) is one such active component found in *Terminalia arjuna* bark. AA: 2,3,23-trihydroxyolean-12-en-28-oic acid, a natural pentacyclic triterpenoid saponin.

During the last two decades, ample efforts have been made towards the development and international acceptance of alternative methods to carry out safety experimental studies using laboratory animals. Using intact animals is costly and would cause suffering to animals as well. In order to minimize the use of live animals, in the present study, precision cut goat liver slices (PCGLS) were used as an *in-vitro* model. PCGLS are an appropriate model of *in-vitro* systems for many reasons, including simplicity and effortlessness of preparation, retention of normal organ architecture, ability to obtain multiple slices from each organ [2]. Besides, PCGLS mimics the cellular, structural and functional features of an *in-vivo* tissues. In this study, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is used as oxidant to induce oxidative stress environment to the slices.

Hence in the present study, the precision cut goat liver slices were selected as an *in vitro* model to determine the antioxidant potential of the Arjunolic

acid against hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) induced oxidative stress.

## MATERIALS AND METHODS

**Preparation of AA extract:** Arjunolic acid (AA) was purchased from Organic Changsha herb Inc. China. 2 mg of AA dissolved in 50 µl of dimethyl sulfoxide (DMSO) and was used for the study. The concentration of Arjunolic acid used for antioxidant assay was 100 µg.

**In-vitro model:** Fresh goat liver was obtained from the local slaughterhouse in ice cold phosphate buffer saline (PBS) and maintained at 4 °C till use. Thin slices (1 mm thickness) of the liver were cut using a sterile scalpel and the slices were placed in PBS at a proportion of 0.25 g in 1 ml, in broad, flat bottomed flasks. H<sub>2</sub>O<sub>2</sub> was used as the oxidising agent to induce oxidative stress at a final concentration of 200 mM (4 ml).

The treatment groups are:

Group 1: Untreated control containing the liver slices alone

Group 2: Positive control in which the liver slices were treated with H<sub>2</sub>O<sub>2</sub>

Group 3: Treatment control in which the liver slices were treated with AA 100 µg in presence of oxidant H<sub>2</sub>O<sub>2</sub>.

Group 4: Negative Control in which in which the liver slices were treated with AA 100 µg in absence of oxidant H<sub>2</sub>O<sub>2</sub>.

The liver slices were treated with H<sub>2</sub>O<sub>2</sub> both in the presence and the absence of the AA and incubated at room temperature for 1 h with mild shaking. After incubation, the mixture was homogenized followed by centrifugation and the supernatant was used for the analysis.

**Analysis of enzymatic antioxidant activity:** The superoxide dismutase (SOD) activity estimated by the method of Misra and Fridovich [3]. Catalase activity (CAT) was estimated by the method of Aebi [4]. The glutathione peroxidase (GPx) activity was assayed using the method proposed by Jagetia *et al.*, [5]. Glutathione reductase (GR) activity was assayed as per the method of Calberg and Mannervick [6].

**Analysis of the levels of non-enzymatic antioxidants:** Ascorbic acid (Vitamin C) levels were estimated based on the method of Roe and Keuther [7]. The reduced glutathione (GSH) level was estimated by the method of Ellman [8].

**Statistical analysis:** Data were expressed as Mean ± standard error of mean (SEM; n=3). Statistical significance was determined by one way analysis

of variance (ANOVA) with P < 0.05 considered to be significant using Graphpad Prism (Version 5.0).

## RESULTS AND DISCUSSION

**Effect of AA on enzymatic antioxidants:** In our study H<sub>2</sub>O<sub>2</sub> is used as oxidizing agent to induce oxidative stress environment in the goat liver. H<sub>2</sub>O<sub>2</sub> is a stable ROS, upon hemolytic reaction it readily converts to hydroxyl radical and superoxide radical, which are powerful oxidants can lead to degradation of haeme proteins, inactivation of enzymes, oxidation of lipids and DNA damage [9]. Overproduction of these radicals overwhelms the antioxidant defense mechanism results in oxidative stress. In our study, enzymatic antioxidant levels were assessed in liver slices subjected to oxidative stress in the presence and in the absence of AA. We observed that the positive control group (liver slices treated with H<sub>2</sub>O<sub>2</sub>) showed decreased levels of SOD, CAT, GPx, GR significantly (P < 0.001) as compared with untreated control group (Table 1) indicating the development of oxidative stress in positive control group. No significant change in these enzymatic antioxidant levels were observed in negative control group (liver slices treated with AA 100 µg in absence of H<sub>2</sub>O<sub>2</sub>) as compared with untreated control group. The treatment control group showed significant (P < 0.001) improvement in these enzymatic antioxidant levels as compared with positive control group. SOD, CAT, GPx are three primary enzymes which involved in direct elimination of reactive oxygen species (hydroxyl radical, superoxide radical, hydrogen peroxide) whereas GR acts as secondary enzyme, which help in the conversion of glutathione disulphide GSSG to GSH thereby maintain GSH levels. The treatment control group significantly restored the antioxidant enzyme levels by eliminating the reactive radicals and maintains the homeostasis between antioxidant defense system and reactive oxygen species.

**Effect of AA on non-enzymatic antioxidants:** Apart from enzymatic antioxidants, non-enzymatic antioxidant also found to play a major role in rendering oxidative stress to maintain homeostasis. Non enzymatic antioxidants like vitamin C, GSH were assessed in goat liver slices subjected to oxidative stress. We observed that the positive control group (liver slices treated with H<sub>2</sub>O<sub>2</sub>) showed significantly (P < 0.001) decreased levels of vitamin C and GSH as compared with untreated control group (Table 2) indicating the development of oxidative stress in positive control group. No significant change in these non-enzymatic antioxidant levels were observed in negative control group (liver slices treated with AA 100 µg

in absence of H<sub>2</sub>O<sub>2</sub>) as compared with untreated control group.

Vitamin C is a water-soluble antioxidant, neutralizes the reactive oxygen species. GSH is a ROS scavenger and redox buffer and is an important constituent of intracellular protective mechanisms against various noxious stimuli including oxidative stress. It has been proposed that antioxidants, which maintain the concentration of reduced GSH, may restore the cellular defense mechanisms and thus protect against the oxidative tissue damage [10]. In our study, we observed that the treatment control group showed significant ( $P < 0.001$ ) improvement in Vitamin C and GSH levels as compared with positive control group, which combats the oxidative stress by scavenging the reactive species, keeping the cellular redox state in balance.

## CONCLUSION

In our present study, precision cut goat liver slices are used as alternative in-vitro model for evaluating antioxidant potential of Arjunolic acid against hydrogen peroxide induced stress in-vitro. This in-vitro model parodists the in-vivo system and aids in minimizing the use of live animals. Enzymatic and non-enzymatic antioxidant levels were analysed in the goat liver slices subjected to oxidative stress in the presence and absence of Arjunolic acid. From our results, it was observed that H<sub>2</sub>O<sub>2</sub> exposed liver slices showed significant decrease in antioxidant levels which was reverted significantly with the administration of Arjunolic acid and confirmed that Arjunolic acid can improve the antioxidant status in an oxidatively stressed tissue, which strengthens the antioxidant potential of Arjunolic acid.

**Table 1: Effect of AA on enzymatic antioxidants in goat liver slices exposed *in-vitro* to H<sub>2</sub>O<sub>2</sub>**

Groups	SOD (Units/g tissue)	CAT (Units/g tissue)	GPx (Units/g tissue)	GR (Units/g tissue)
Liver slices + vehicle	15.44± 1.22	76.06±0.88	30.12±1.45	2.53±1.55
Liver slices + H <sub>2</sub> O <sub>2</sub>	10.42±0.98 <sup>***</sup>	31.09±1.02 <sup>***</sup>	18.88±0.66 <sup>***</sup>	1.19±1.31 <sup>***</sup>
Liver slices + H <sub>2</sub> O <sub>2</sub> + AA	13.61±1.09 <sup>*+</sup>	69.09±1.11 <sup>+++</sup>	26.41±1.07 <sup>+++</sup>	2.36±0.77 <sup>+++</sup>
Liver slices + AA	15.69±1.32 <sup>+++</sup>	77.33±1.41 <sup>+++</sup>	29.71±1.44 <sup>+++</sup>	2.47±0.79 <sup>+++</sup>

Values are expressed as mean ± SEM (n=3); enzyme activity was expressed as units/g liver tissue. Analysed by one way ANOVA followed by Dunnett 't' test; \*( $P < 0.05$ ), \*\*\* ( $P < 0.001$ ) vs untreated control group; +++ ( $P < 0.001$ ) vs H<sub>2</sub>O<sub>2</sub> control group;

**Table 2: Effect of AA on non-enzymatic antioxidants in goat liver slices exposed *in-vitro* to H<sub>2</sub>O<sub>2</sub>**

Groups	Vitamin C(mg/g tissue)	GSH(nmol/g tissue)
Liver slices + vehicle	0.53±1.09	2.93±0.25
Liver slices + H <sub>2</sub> O <sub>2</sub>	0.17±1.54 <sup>***</sup>	1.27±1.07 <sup>***</sup>
Liver slices + H <sub>2</sub> O <sub>2</sub> + AA	0.48±0.89 <sup>+++</sup>	2.81±1.06 <sup>+++</sup>
Liver slices + AA	0.52±1.09 <sup>+++</sup>	2.91±1.11 <sup>+++</sup>

Values are expressed as mean ± SEM (n=3); enzyme activity was expressed as units/g liver tissue. Analysed by one way ANOVA followed by Dunnett 't' test; \*( $P < 0.05$ ), \*\*\* ( $P < 0.001$ ) vs untreated control group; +++ ( $P < 0.001$ ) vs H<sub>2</sub>O<sub>2</sub> control group

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