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## Assessment of antioxidant effect of biochanin: A on isoprenaline-induced cardiac fibrosis in mice

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#### Abstract

Biochanin-A, an isoflavonoid abundantly existed in red clover and peanuts, has been reported to possess anti-inflammatory and anti-proliferative effects in different conditions. However, protective antioxidant effects on isoprenaline (ISP)-induced cardiac fibrosis in mice model have not been reported. Thus, the present study was undertaken to assess the antioxidant effect of biochanin-A against ISP-induced cardiac fibrosis in mice. Cardiac fibrosis was induced by administration of ISP @ 20 mg/kg body weight via subcutaneous (S/C) route for 14 days. Biochanin-A @ 30 mg/kg (orally) was co-administered with ISP (S/C) in treatment group for 14 days. Serum samples from different groups were assessed for catalase, superoxide dismutase (SOD), reduced glutathione and MDA activity. Serum level of catalase, SOD, reduced glutathione and MDA levels were altered following ISP-induced cardiac fibrosis which have been improved by biochanin-A-co-administration. In conclusion, biochanin-A may have potential to check progression of ISP-induced myocardial fibrosis due to its anti-oxidative effects.

**Keywords:** Biochanin-A, isoprenaline, cardiac fibrosis, antitoxin

#### 1. Introduction

Myocardial injury leads to fibrotic alterations and impaired heart architecture which are the reason for cardiac dysfunction (Whittaker *et al.*, 1989) [14]. Oxidative stress plays a key role in the pathogenesis of cardiac injury and responsible for the cardiac dysfunction and further, promotes cardiac fibrosis (Ning *et al.*, 2017) [9]. Low levels of reactive oxygen species (ROS) are required for the normal cellular functions, however, excess production of ROS during oxidative stress induces fibrosis by enhancing the inflammatory response as well as modulating collagen synthesis (Wattanapityakul & Bauer, 2001) [13]. Myocardial fibrosis occurs due to oxidative injury which is characterized by the deposition of extracellular matrix and this extracellular matrix contains collagen and non-collagen contents secreted from activated my fibroblast (Wippt *et al.*, 2008). It was reported previously that prolong administration of isoprenaline produces myocardial oxidative stress and enhances synthesis of pro-inflammatory cytokines and ultimately leads to cardiac hypertrophy, necrosis and fibrosis (Davel *et al.*, 2014) [4]. In addition, enhanced concentrations of Ca<sup>2+</sup> ions inside the cell, activation of adenylate cyclase enzyme and depletion of ATP level have been associated with ISP-induced myocardial structural damage (Filho *et al.*, 2011) [7].

Medicinal plants are believed to possess antioxidant activity and play an important role in the management of various diseases including cardiovascular diseases (Goyal *et al.*, 2015) [5]. Previously, it has been shown that inhibition of oxidative stress through plant derived products could be an important strategy to attenuate isoprenaline-induced fibrotic response (Zhao *et al.*, 2017) [17]. Biochanin-A, an isoflavones present in the red clover, cabbage and alfalfa, has been reported to have antioxidant and anti-inflammatory effects (Liu *et al.*, 2016). Biochanin-A administration enhanced the level of superoxide dismutase and catalase in different conditions through scavenging the ROS (Raheja *et al.*, 2018) [10]. A previous report suggests that biochanin-A showed antioxidant potential to attenuate CCl<sub>4</sub>-induced liver fibrosis (Breikaa *et al.*, 2013) [2] and streptozotocin-induced diabetic cardiomyopathy (Sadri *et al.*, 2017) [11]. However, there is no report available on effect of biochanin-A on oxidative stress in ISP-induced myocardial fibrosis. Therefore, the present study was designed to evaluate the antioxidant effects of biochanin-A in ISP-induced cardiac fibrosis in mice model.

## 2. Material & Methods

### 2.1 Animals

Healthy adult male Albino mice (25-30 g) were procured from the Laboratory Animal Resource (LAR) Section, ICAR-Indian Veterinary Research Institute, Izatnagar, U.P. Animals were kept for an acclimatization period of seven days before the conduction of experiments.

They were housed in polypropylene cages at a constant room temperature and light cycle (12:12 h dark-light) with free access to feed and water. All protocols and surgical procedures employed were in accordance with the Institutional Animal Ethics Committee, ICAR-Indian Veterinary Research Institute, Izatnagar.

### 2.2 Experimental Design

Mice were randomly divided into four groups and each group had minimum six mice. Control mice (Group-I) were received normal saline as vehicle for 14 days via subcutaneous route (S/C). ISP and biochanin-A were administered (Group-II and Group-III) @ 20 mg.kg<sup>-1</sup>, S/C and 30 mg.kg<sup>-1</sup> orally for 14 days, respectively. Co-administration of biochanin-A with ISP (S/C) was done with oral route for 14 days in group, IV. Mice were sacrificed on 15<sup>th</sup> day by bleeding from venacava under urethane anesthesia (1.2 g/ kg body weight I/P). Blood samples were collected by cardiac puncture from mice of different groups in serum separator tube (SST) and allowed to clot for two hours at room temperature. Serum was separated by centrifuging at 4000 rpm for 10 min and kept at -80°C for further use. Total Protein concentration was determined in serum by protein estimation kit (GeNei, Cat. No.2601800011730).

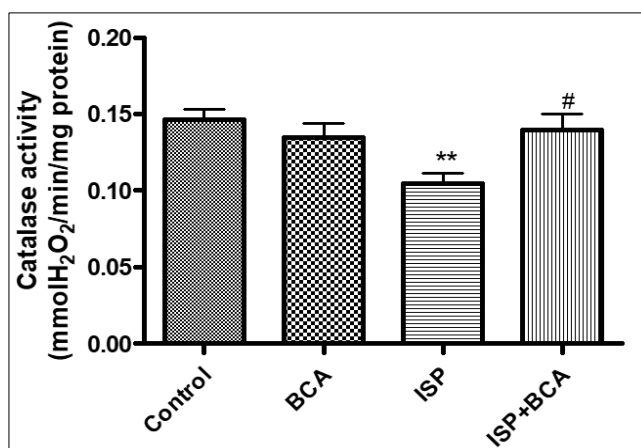
### 2.3 Assessment of antioxidant enzymes and malondialdehyde (MDA) level in serum

Catalase activity was estimated by using method of Aebi (1984) [1]. In brief, serum sample was added to 50 mM phosphate buffer (pH 7.0). 30 mM hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added and a change in absorbance was followed for 30 sec (or 1 min) at 240 nm at 15 sec intervals. The catalase activity was calculated using the mill molar extinction coefficient of H<sub>2</sub>O<sub>2</sub>. Superoxide dismutase (SOD) activity was measured by the method of Mahesh and Balasubramanian (1998) [8]. The reaction mixture, contained 0.65 ml PBS (pH 7.4), MTT (1.25 mM), pyrogallol (100 μM), and serum, was incubated at room temperature for 5 min. The reaction was stopped by adding dimethyl sulfoxide and absorbance was read at 570 nm. Results were presented as SOD units. GSH level was estimated by the method described by Sedlak and Lindsay (1968) [12]. Serum sample, distilled water and 50% trichloroacetic acid was added and incubated at room temperature for 15 min. Then, mixture was centrifuged at 3000 rpm for 15 min. 0.4 ml of supernatant was added to 1 M Tris buffer (pH 8.9) followed by 0.2 ml of 0.01M DTNB (5,5'-dithiobis-2-nitrobenzoic acid). The yellow color was developed and read immediately (within 5 min) at 412 nm. Results were calculated using molar extinction coefficient of Chromophores (13000/M/cm). Lipid peroxidation (malondialdehyde or MDA) was estimated by the method of Buege and Aust (1978) [3]. Serum was treated with TBA-TCA-HCl (Thiobarbituric acid-Trichloroacetic acid-Hydrochloric acid) reagent (TBA 0.37%, 0.25N HCl and 15% TCA) (1:1:1) and placed in water bath for 15 min and cooled. The absorbance of clear supernatant was measured against the reference blank at 535 nm. Concentration was calculated using molar absorptivity of malonaldehyde which is 1.56 × 10<sup>5</sup> M<sup>-1</sup>cm<sup>-1</sup>.

## 3. Results-5

### 3.1 Effect of biochanin-A on serum catalase activity in ISP- induced cardiac fibrosis

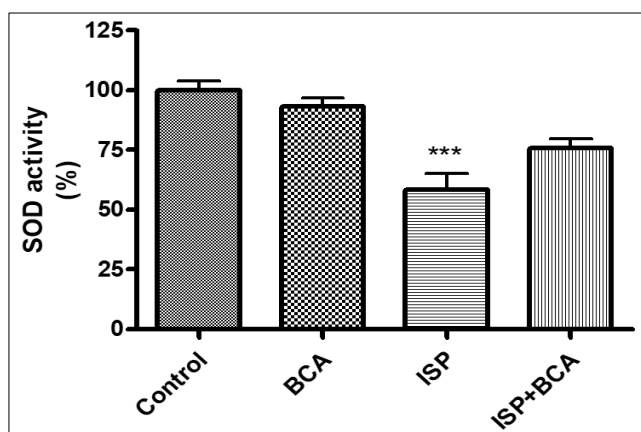
Figure 1 illustrates catalase activity was significantly reduced in ISP-induced cardiac injury group (0.10±0.006 mmolH<sub>2</sub>O<sub>2</sub>/min/mg protein; n=6) in comparison with control (0.15±0.006 mmolH<sub>2</sub>O<sub>2</sub>/min/mg protein; n=6). Biochanin-A had shown significant increment in serum catalase activity in ISP co-administered (0.14±0.009 mmolH<sub>2</sub>O<sub>2</sub>/min/mg protein; n=6) group in comparison with ISP-alone administered injury group. Catalase activity in biochanin-A alone group was 0.13±0.009 mmolH<sub>2</sub>O<sub>2</sub>/min/mg protein; n=6 which was not significantly different from control.



**Fig 1:** Bar diagram showing the effect of biochanin-A on serum catalase activity in ISP-induced cardiac fibrosis in mice. Data are analyzed by one-way ANOVA followed by Tukey's multiple comparison test. \*\**p*<0.01 in comparison with control; #*p*<0.05 in comparison with ISP.

### 3.2 Effect of biochanin-A on serum superoxide dismutase (SOD) activity in ISP-induced cardiac fibrosis

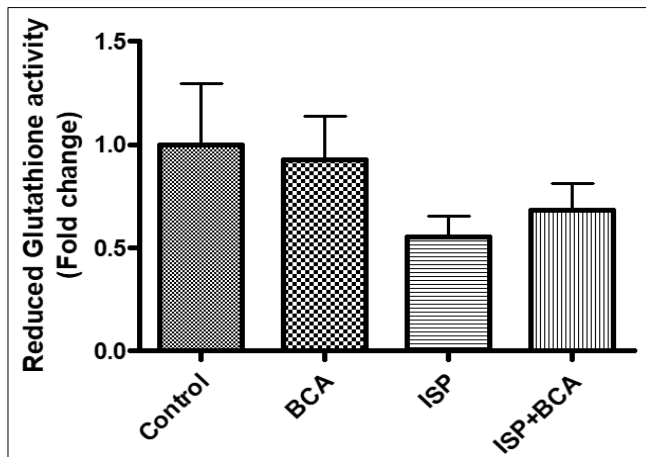
Figure 2 illustrates that SOD activity was reduced significantly in ISP-induced myocardial injury group (58.54±6.71% activity; n=6) in comparison with control (100.0±4.02% activity; n=5). Biochanin-A had increased SOD activity but not at significant level in ISP co-administered (75.68±4.09% activity; n=6) group in comparison with ISP-alone administered injury group (58.54±6.71% activity; n=6). SOD activity in (93.38±3.40% activity; n=5) biochanin-A alone group was not significantly different with control group.



**Fig 2:** Bar diagram showing the effect of biochanin-A on serum SOD activity in ISP-induced cardiac fibrosis in mice. Data are analyzed by one-way ANOVA followed by Tukey's multiple comparison test. \*\*\**p*<0.001 in comparison with control.

### 3.3 Effect of biochanin-A on serum reduced glutathione (GSH) activity in ISP-induced cardiac fibrosis

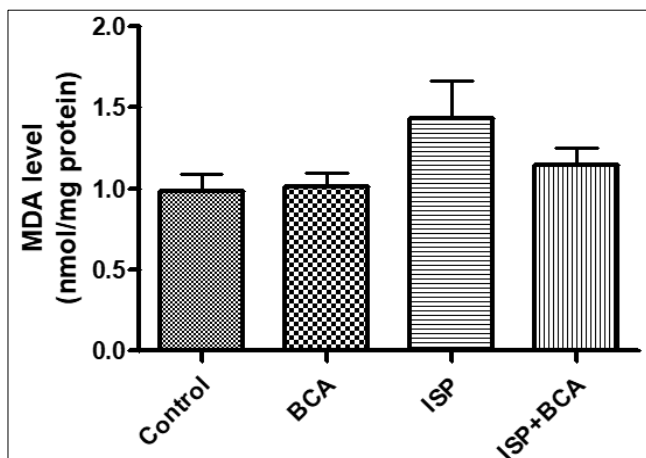
Figure 3 illustrates that reduced glutathione activity was decreased in ISP-induced injury group ( $0.55 \pm 0.10$  fold change;  $n=6$ ) in comparison with control ( $1.00 \pm 0.29$  fold change;  $n=6$ ). Biochanin-A had enhanced GSH activity in ISP co-administered ( $0.68 \pm 0.12$  fold change;  $n=6$ ) group in comparison with ISP-alone administered injury group ( $0.55 \pm 0.10$  fold change;  $n=6$ ). GSH activity in ( $0.92 \pm 0.21$  fold change;  $n=6$ ) biochanin-A alone administered group was almost similar with respect to control group.



**Fig 3:** Bar diagram showing the effect of biochanin-A on serum reduced glutathione activity in ISP-induced cardiac fibrosis in mice. Data are analyzed by one-way ANOVA followed by Tukey's multiple comparison test.

### 3.4 Effect of biochanin-A on serum lipid peroxidation (LPO) in terms of MDA level in ISP-induced cardiac fibrosis

Figure 4 depicts that a marked increment in serum MDA level was observed in ISP-induced cardiac injury group ( $1.43 \pm 0.22$  nmol/mg protein;  $n=6$ ) in comparison with control ( $0.99 \pm 0.10$  nmol/mg protein;  $n=6$ ) mice. However, biochanin-A treatment slightly decreased MDA level in ISP co-administered group ( $1.15 \pm 0.10$  nmol/mg protein;  $n=6$ ) in comparison with ISP-alone administered injury group ( $1.43 \pm 0.22$  nmol/mg protein;  $n=6$ ). MDA level in biochanin-A alone administered group ( $1.00 \pm 0.08$  nmol/mg protein;  $n=6$ ) was not significantly different with control group.



**Fig 4:** Bar diagram showing the effect of biochanin-A on lipid peroxidation in terms of MDA level in ISP-induced cardiac fibrosis. Data are analyzed by one-way ANOVA followed by Tukey's multiple comparison test.

## 4. Discussion

Prolonged administration of ISP increases oxygen and energy consumption and further produces oxidative stress and leads to cardiac toxicity in the target species (Zhao *et al.*, 2017) <sup>[17]</sup>. Oxidative stress has been associated with generation of superoxide anions and finally results in series of inflammatory reactions (Davel *et al.*, 2014) <sup>[4]</sup>. Therefore, it is presumed that inhibition of oxidative stress is an important strategy to prevent cardiac fibrosis. Flavonoids are transferred hydrogen atom from hydroxyl group to free radicals to stabilize them thereby show antioxidant effect (Breikaa *et al.*, 2013) <sup>[2]</sup>. In the present study, SOD and catalase were represented enzymatic part and their activities in serum were significantly decreased in ISP-administered cardiac fibrosis group. However, biochanin-A co-treatment increased the catalase as well as SOD activity. Zhao *et al.* (2017) <sup>[17]</sup> suggested that SOD and catalase are important antioxidant enzymes, and enhancement of their activity can inhibit oxidative stress to delay the progression of cardiac hypertrophy. MDA is one of the end products of lipid peroxidation and GSH is the main endogenous antioxidant in *in-vivo*, and the decreased level of GSH is associated with apparent oxidative damage to cells (Zhang *et al.*, 2016) <sup>[6-16]</sup>. However, enhanced activity of MDA and decreased activity of reduced glutathione in ISP-induced cardiac fibrosis were improved by biochanin-A but not at significant level in our present findings. Thus, it may be speculated that biochanin-A possess antioxidant effect to check the progression of fibrosis. Present investigation is an agreement with the previous study which suggested that biochanin-A had shown activity to scavenge free radicals and thereby reduced oxidative stress (Raheja *et al.*, 2018) <sup>[10]</sup>. Similarly, another study reported that biochanin-A reduces oxidative stress in streptozotocin-induced diabetic cardiomyopathy in rats by decreasing MDA level and increasing the SOD, catalase and reduced glutathione level (Sadri *et al.*, 2017) <sup>[11]</sup>.

## 5. Conclusion

This study revealed that biochanin-A had shown increase in catalase, SOD, reduced glutathione activities and decreased the MDA level in serum which were altered in ISP-induced cardiac fibrosis. Thus, according to our current findings we can suggest that biochanin-A may have potential to check progression of ISP-induced myocardial fibrosis due to its anti-oxidative effects.

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