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# Investigating on the antioxidant effects of soybean extract on histopathological changes of liver in Gelofen treated mice

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**Abstract**
**Introduction:** Gelofen (Ibuprofen) is a non-steroidal, non-inflammatory drug that is used to relieve moderate to severe pains. However, the drug has proven side effects such as production of free radicals and interfacing with cellular events. So finding a proper antioxidant is necessary to reduce the side effects of this drug. It was determined that Gelofen by disorder in prostaglandins function and production of free radicals can probably damage liver tissues. Use of soybean extract as a proper antioxidant is recommended in this article to reduce Gelofen induced-complications. For the present experiment, 56 rats were randomly divided into 7 equal groups that received different dose of Gelofen and soybean. After anesthesia histological studies were performed on their livers. The results show that liver tissues in the Gelofen groups had extensive degenerative changes, centrilobular necrosis, lymphocyte accumulation and bleeding. And these changes improved in the soybean groups.

**Keywords:** Soybean, Gelofen, Histopathology, Liver, Rat.

## 1. Introduction
In addition to main active ingredients, medicinal plants have substances with therapeutic effects. These substances can intensify therapeutic effects, and in many cases they can prevent from toxicity and complications [1]. Today, finding a new formulation especially of plant origin is one of the therapeutic strategies of diseases such as hepatic cancer. Soybean which is used to treat numerous diseases is an annual plant belonging to *Legume* family. The plant is grown due to its seeds that are rich in proteins [2]. Soybean and its supplements are a rich source of phytoestrogens. Soybean phytoestrogens are isoflavones types, which include genistein and daidzein [3, 4]. Various studies show that soybean isoflavones can reduce prostate tumors' growth [5] and can also reduce menopause symptoms including flare-ups and bone loss [6].
On the other hand, Gelofen is one of the most prescribed drugs by generic name of ibuprofen. It is a non-steroidal, anti-inflammatory drug (NSAID) and is non-opioid, analgesic, and anti-fever [7] and it is practically insoluble in water [8]. Ibuprofen as a non-steroidal, anti-inflammatory drug with analgesic, anti-fever and anti-inflammation effects was introduced in 1969 by Boots UK Company and in 1974 through Upjohn Company United States to pharmaceutical markets to treat rheumatoid. The effectiveness of the drug in rheumatoid arthritis and osteoarthritis has been approved by FDA [9] and it is practically insoluble in water [10]. Anti-inflammatory activity of NSAIDs mainly occurs through inhibition of prostaglandins synthesis. NSAIDs block arachidonic acid conversion to Endoperoxides interfaces, through inhibition of cyclooxygenase enzymatic activity and then of course prostaglandins will not be synthesized. Various NSAIDs may also have other impact mechanisms including inhibition of chemotaxis, negative regulation of interleukin-1 production and interfering with calcium mediated-performed intracellular events [11]. Given to the above, as well as possible side effects of Gelofen as a widely used drug on histological changes of liver, finding a proper anti-oxidant is vital and necessary to reduce the drug's side effects. So, the study is performed by the purpose of investigation on anti-oxidant effects of soybean plant on histological changes of liver in rats treated with Gelofen.

## 2. Materials and Methods
The study was performed from June 2014 to October 2014. All ethical use of laboratory animals has been respected in this study according to Shiraz University Animal Welfare Committee guidelines. The used animals in the research were a number of 56 adult female

Wistar rats weighing approximately 185-195 g and age of 2-3 months. The rats were prepared from the Animal Breeding Center of Yasouj University of Medical Science. The study was performed during a period of 21 days. Rats were exposed to 12 hours of light and 12 hours of dark, during the 21-day period of experiment. They received urban water and fed from mouse foods. Based on the weight range, rats were randomly divided into 7 groups of 8 each, as follows:

1- The control group: they were kept in normal condition without receiving of any drugs.
2- The blank group: they only received distilled water as a drug solvent and extract by injection.
3- The experimental group 1: Gelofen (400 mg/kg) was intraperitoneally injected to them.
4- The experimental group 2: they orally received a dose of 80 mg/kg of soybean extract, only.
5- The experimental group 3: they inraperitoneally received Gelofen at a dose of 400 mg/kg associated with soybean extract at a dose of 3 g/kg.
6- The experimental group 4: they inraperitoneally received Gelofen at a dose of 400 mg/kg associated with orally soybean extract at a dose of 6 g/kg.
7- The experimental group 5: they inraperitoneally received Gelofen at a dose of 400 mg/kg associated with orally soybean extract at a dose of 15 g/kg.

### 2.1. Preparation Method of Different Doses of Gelofen

Gelatin capsules of gelofen were prepared as a dose of 400 mg. Then, the capsules layers were cut with a sterile blade and their liquid gels extracted. Each capsule was then diluted with 2 cc of distilled water and a dose-dependent-drug was intraperitoneally injected to each rat by insulin syringe, given to every kilograms of rats' body weight.

### 2.2. Preparation Method Extraction of Soybean

The required soybeans were bought primarily from Sobhan Company. The beans were powdered and then percolation method was used for extraction. Thus, the dried powder was poured in cylindrical portion of percolator device, which was filled up to two-third with alcohol (80%) and the rest with water. The valves of device were closed, when the first solution had been exited from the last valve and after passing of 24 hours, they opened and the extract collected. Then it was dried at a temperature of 40-30 ˚C under a microbe-free environment [12].

After a period of 21 days and performing of medical treatments, the animals were unconscious with ether and their hepatic tissues were removed for Histological studies. Hematoxylin and Eosin staining was used for microscopic investigations (cellular necrosis, lipid changes, lymphatic invasion, swelling of hepatocytes, cloudy swelling or ballooning degeneration of cells, and granularity of cytoplasm) and finally, the samples were photographed by a microscope.

## 3. Results

Histological evaluations show that hepatocytes in the control group, which did not receive any substance, had maintained their natural shape and any lymphocytic infiltration and tissue damage was not observed. The control group is shown in Figure 1.

Histological results show that any tissue changes did not observe in the blank group received drug solvent compared to the control group (Figure 2). Histological evaluations show that lymphocytic infiltration and accumulation of mononuclear cells was observed in the Gelofen groups, (Figure 3). Hydropic swelling of hepatocytes cells and cellular necrosis, which show the destructive effects of Gelofen, were extremely observed in the Gelofen groups (Figure 3).

No change was observed in hepatic tissues in soybean groups at the dose of 15 g/kg and hepatocytes were normal (Figure 4).

Destruction of hepatocytes in the minimal dose-soybean group was less than in the Gelofen group. Liver cells in this group were granular and their cytoplasm was seen darker than the Gelofen group (Figure 5). Sinusoidal hyperemia and lymphocytic infiltration in the soybean group was observed less compared to Gelofen group (Figure 5).

Destruction of hepatocytes in the average-dose-soybean group was less than in the Gelofen group with soybean 3 g/kg. Also granularity of liver cells was observed in this group (Figure 6).

Destruction of hepatocytes in the maximum-dose-soybean group was less than in the Gelofen group with soybean 3 and 6 g/kg. Also granularity of liver cells was observed in this group (Figure 7).

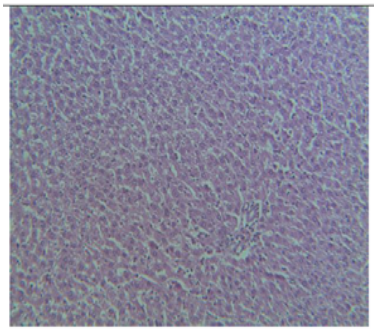

**Fig 1:** Optical photomicrograph of hepatic tissue in control group. Hepatocytes are normal. (H&E staining, × 100)

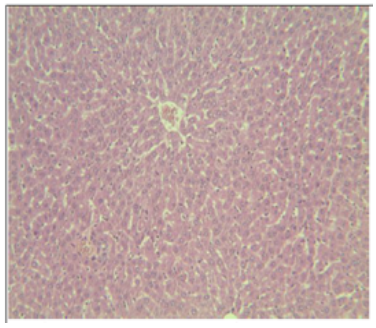

**Fig 2:** Optical photomicrograph of hepatic tissue in blank group. Hepatocytes are normal. (H&E staining, × 100)

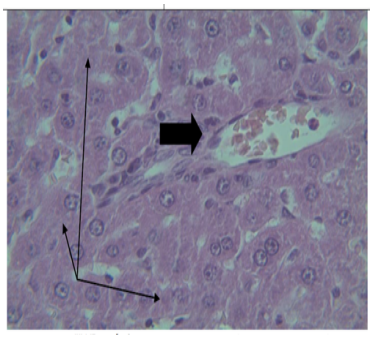

**Fig 3:** optical photomicrograph of hepatic tissue in Gelofen group 400. Hepatocytes are normal. (H&E staining)

Representation of mononuclear and lymphocyte cells accumulation (large arrow); representation of hydropic swelling (narrow arrow); and representation of hepatocytes necrosis (small arrow), × 400

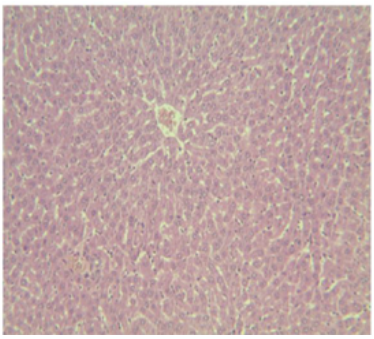

**Fig 4:** optical photomicrograph of hepatic tissue related to group received soybean extract at a dose of 15 g/kg. (H&E staining, × 100)

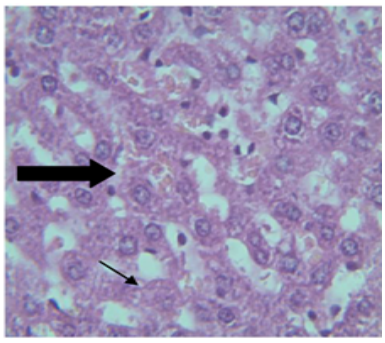

**Fig 5:** optical photomicrograph related to hepatic tissue in experimental group 3, granularity of hepatocytes cytoplasm of hepatic tissue (narrow arrow), and hyperemia and necrosis of hepatocytes cells (large arrow); (H&E staining, × 400)

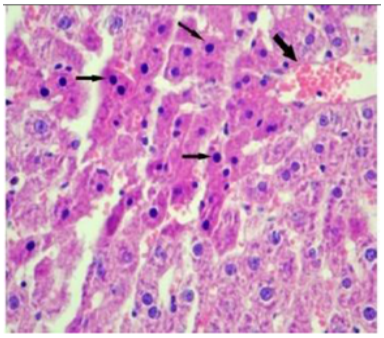

**Fig 6:** optical photomicrograph related to hepatic tissue in experimental group 4; granularity of hepatocytes cytoplasm of hepatic tissue and hyperemia and necrosis of hepatocytes cells (arrow); (H&E staining, × 100)

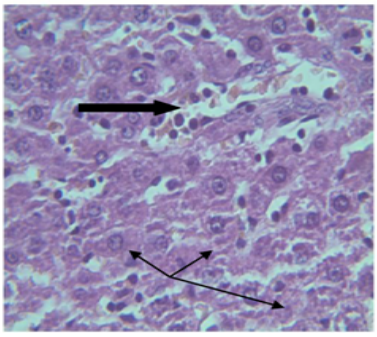

**Fig 7:** optical photomicrograph related to hepatic tissue in experimental group 5; granularity of hepatocytes cytoplasm of hepatic tissue (narrow arrow), and hyperemia in hepatocytes cells (large arrow); (H&E staining, × 400)

## 4. Discussion

Various parameters were studied for investigation of liver histopathologic changes in the experimental groups. Widely degenerative changes, centrilobular necrosis, and bleeding by taking Gelofen were observed in this study in the experimental groups received Gelofen, which represent destructive effects of the drug.

Studies stated that creation of free radicals in cells and their combination with cell membrane and unsaturated fatty acids produce lipids radicals with oxygen molecules. As a result, phospholipids break down in endoplasmic reticulum and cause release of enzymes. These reactions ultimately lead to cell death and cellular necrosis [13, 14].

It is also stated that incidence of degenerative changes and necrosis around central venous, can occur as a result of exposure to toxins [15]. So, the histopathological findings of liver in the present study reflect direct and significant effects of Gelofen toxicity.

Investigations on physiological conditions have found that there is equilibrium between formation of oxidative species and their elimination by antioxidant compounds. Oxidative stress occurs when the equilibrium is disrupted by excessive production of oxygen free radicals and/or weakness of antioxidant defense system [16]. Investigations have found that Gelofen by effecting on cell membrane may cause damage to hepatic cells and impairment in antioxidant system of body [17,18].

Also, studies have found that use of Gelofen causes severe dilation of blood vessels, necrosis of vessels of connective tissues and hepatocytes, and severe congestion of cells [19].

According to the prepared photomicrographs, the present study also showed that hydropic swelling, lymphocytic infiltration, mononuclear cells accumulation, and cellular necrosis were observed in Gelofen groups which more damages seen in taking of higher doses. This is consistent with findings of previous researches. Studies showed that taking Gelofen at a dose of 10 g/kg causes edema, hyperplasia and blood accumulation in body tissues [19]. This agrees with findings of the present research.

Studies have shown that non-steroidal, anti-inflammatory drugs such as Gelofen, probably cause hepatic steatosis and toxicity. What is certain is that Gelofen is mostly used in inflammatory diseases. The presence of excessive plasma within cells is observed in inflammatory responses and this causes increase of lymphocytes and macrophages and as a result, tissue damages. Other studies have shown that Gelofen is mostly removed from body through liver and liver may be damaged or inflamed if the removal action is not done properly. This inflammation that occurs as a result of drug taking is called drug-induced hepatitis [20].

The results of the present study show that tissue damage was observed in soybean group 2. Also, necrosis as well as tissue damage were observed less in experimental group 3, 4, and 4 which simultaneously received the drug and extract compared to experimental 1 which received Gelofen alone. This reflects positive effects of the extract.

Studies indicated that use of medicinal plants in traditional medicine due to their ease of access and low costs has had an important role in health and safety of societies, especially in developing countries including Asian countries [21]. Nowadays, due to side effects induced by taking anti-inflammatory drugs (such as Gelofen), use of natural compounds have been common for treatment of many diseases and inflammations [22].

Several studies have performed on milk [23] and soybean proteins. Several biologically active proteins and peptides are produced during food processing and intestinal digestion [25].

Soybean proteins are introduced as one of the most desirable

herbal proteins that can provide all essential amino acids. Researchers have reported biological benefits of soybean peptides and its antioxidant properties have been widely studied [26, 27]. Bazzoli *et al*. demonstrated that a daily intake of 40 g of soybean protein causes reduce of lipid peroxidation in plasma for 4 weeks. The researchers showed that soybean protein increases antioxidant capacity of plasma; these findings were measured according to assessment of Total Antioxidant Status (TAS). Bazzoli *et al*. reported that consumption of soybean for 4 weeks caused some antioxidant effects. This is consistent with results of Aoki *et al*. study [24, 28]. Also these researchers showed that isolated soybean protein increases anti-oxidative capacity of liver and enzymatic activity of glutathione reductase and glutathione peroxidase [24, 28]. In the present study, according to the photomicrographs of tissues, less tissue damages observed in the experimental groups received soybean extract than the groups received Gelofen, and this may indicate the antioxidant effects of the extract on changes of hepatic tissues. The effect is more seen in higher doses and indicates a dose-dependent manner of the extract. It was found that Gelofen may cause dysfunction in prostaglandins activity, as well as changes in pores of mitochondrial membrane, production of free radicals and abnormality in hepatic tissues. Use of soybean extract as a proper antioxidant is recommended in the present study to reduce Gelofen-induced complications.

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