Long-Term Consumption Effect of *Eurycoma longifolia* Jack on Histopathological Changes in the Vital Organs in Rats

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**ABSTRACT**

**Introduction:** Presently, the use of *Eurycoma longifolia* Jack (ELJ) (Tongkat Ali) has increased dramatically in Southeast Asia especially Malaysia where it is widely used as an aphrodisiac and anti-malarial agent. Interestingly, its consumption has become popular in daily life as a beverage to enhance energy and stamina especially among males. However, its effect on the safety of vital organs of the body has not been studied adequately. Hence, the main objective of this study was to determine whether or not long-term consumption of ELJ has side effects on the vital organs such as Pancreas, Liver and Kidney in rats. **Materials and methods:** Three different concentrations of aqueous extract of ELJ were prepared and dissolved in distilled water. A total of 32 Sprague-Dawley male rats were used and randomly divided into three test groups and control. The test groups were given different doses (low dose 250 mg/kg bw, medium dose 500mg/kg bw and high dose 1000 mg/kg bw) of aqueous extract of ELJ, respectively. Control group was given distilled water alone. Doses were given orally and daily for 5 weeks. After 5 weeks, animals were sacrificed; whole liver tissues were obtained, fixed in 10 percent formaldehyde overnight for histological examination. **Result:** Histological observations showed mild to moderate degrees of hemorrhage, hepatocytes degeneration and severe fatty changes in liver tissue of the test groups treated with ELJ as compared to control. **Conclusion:** In conclusion, the long-term daily consumption of ELJ in large quantity as beverage may cause fatty changes, hemorrhage and hepatocytes degeneration in the liver tissue. The Pancreatic tissues on the three groups treated with different concentration doses do not show any fatty changes or inflammation in the treated groups.

**INTRODUCTION**

Medicinal plants have been used since the time immemorial for medical purposes with respect to benefit mankind. Different kinds of plant extracts or derivatives are used in folklore healing systems such as Japanese (Kampo) medicine, Indian (Ayurveda) or Chinese herbal medicine, and are now widely practiced in fully developed countries including United States of America where their use is often integrated into conventional medicine to get more benefits. The therapeutic efficacy of several plant based medicines has been established, however, for many others this is not the case, often because the research has not been done properly. *Eurycoma longifolia* Jack (ELJ) (also known as Tongkat Ali or Pasak Bumi) is one of the plants of such category. Even though toxicity and safety evaluation studies have been carried out, still a major gap exists in providing scientific base for commercial utilization and clearance of the Tongkat Ali’s products with regard to consumer’s safety. Tongkat Ali is well known among various ethnic groups in Malaysia for treating various diseases and enhancing health and as such, it is sometimes referred to as Malaysian ginseng [1]. ELJ belongs to Simaroubaceae family and is a tropical herbal plant found in several parts of Southeast Asia including Malaysia. It is an evergreen, slow growing herbal plant and reaches a maximum height of 15-18 m and fruits after approximately 2-3 years of cultivation. However, it is generally believed that for complete maturation of the plant, it might take up to 25 years. But, for commercial usages, most of the times, roots are harvested after four years of cultivation. The fruits are green in color, 2-3 cm long and turn to dark red after ripening. The leaves are pinnate, spirally arranged, long (10-15 inches) with 10-30 leaflets. The flowers are produced in large panicles.
and the plant is dioecious with female and male flowers borne on different trees [2]. The plant parts are rich in various bioactive compounds (eurycomanone, eurycomaoside, eurycolactone, eurycomalactone, and pasakkumin-B) among which the quassinoids and alkaloids form a major portion. Certain quassinoids, isolated from ELJ have been reported to display a variety of biological activities, including antitumor, anti-viral, anti-amoebic and anti-inflammatory activities [1, 2]. It has been claimed to improve men’s strength and power during sexual activities [3]. A study conducted on rats showed that the oral administration of ELJ extract containing eurycomanone as a major compound was found to increase sperm quality and testosterone level. [4].

The water extracts of ELJ have been reported to have a better market value as beverage and capsules [5]. In our previous study we found that water extract of ELJ caused no damage to hepatocytes in rats when given alone orally at dose 750 mg/kg bw for 3 days [6]. Recently, a study conducted on rats reported that different fractions of ELJ (chloroform, methanol, water) could enhance the libido in sexually experienced male rats [7]; and initiation of sexual performance of inexperienced male rats [8,9]. The long term consumption of ELJ as beverage among men and women for its vitality during copulation, and traditional uses in the management of various chronic diseases incited us to evaluate its effect on the safety of the vital organs such as Pancreas, Liver and Kidney. The root extract has been correlated with sleep disturbances, hot-headiness with facial flushing, pressure in the testicles in men, and hyper aggressiveness. However, there is a lack of scientific evidence for such deleterious effects [10]. Owing to the lack of published data on the efficacy of long term consumption of ELJ on the vital organs such as; Pancreas, Liver and Kidney; we were prompted to evaluate its effect on this vital organs of the body. In the present study we aimed to determine if long term consumption of ELJ as beverage could have any deleterious effect on the vital organs of the body or not.

MATERIALS AND METHODS

The study was conducted after an approval from the Institutional Animal Care committee, No IIUM/IACUC Approval/2014/(3) (16). 500 gm powdered aqueous extract of Eurycoma longifolia Jack (ELJ) was purchased from MKI (M) Sdn. Bhd. No 469700V, which was thawed in distilled water. A total of thirty-two Sprague-Dawley rats (male) were obtained from the animal house (breeding-lab), University Putra Malaysia (UPM), Malaysia. The rats were randomly divided into three test and one control groups (n=8). The test groups were given ELJ aqueous extract at 250 mg/kg bw, 500 mg/kg bw and 1000 mg/kg bw orally by gastric lavage respectively. Control group was given distilled water only. Doses were given daily for 5 weeks using an appropriate needle. Animals were allowed free access to food and water daily for 5 weeks. After five weeks, animals were sacrificed, entire liver tissues were collected. The specimens then underwent automated tissue processing for 24 hours using a tissue processor (Leica TP-1020, Germany). Sections were imbedded in paraffin and cut into 5μm slices. Each section was then stained with haematoxylin and eosin (H&E). The slides were examined by two different pathologist observers and both reading were blinded observation.

Results:

Clinical observations:

There was no evidence of infection in any of the animals before and during the experiment. All animals gained weight during the period of the experiment. The animals tolerated the oral dose from low to high (250, 500, 1000 mg/kg bw) quite well. During the entire period of study, no mortality among rats was observed. All rats appeared normal, active and healthy.

Histological examination of the liver tissue:

The histological appearance of the Pancreatic, hepatocytes and kidney tissues in all groups are shown in figures 1-3. The Pancreatic tissues treated orally with different concentrated doses (250mg, 500mg & 1000mg/kg bw) of ELJ water extract appeared as normal as the control (Figure 1A-1D- respectively).

There was a clear evidence of mild inflammation alone in the liver tissues in the test group with low dose (250 mg/kg bw) of ELJ water extract. No fatty changes were observed in this group (Figure 2A). The mild fatty changes and inflammation were seen in the hepatocytes of the group treated with medium dose (500 mg/kg bw) of ELJ water extract, (figure 2B). The high dose (1000 mg/kg bw) of ELJ water extract, treated group showed hepatocytes with moderate hemorrhage & severe fatty changes (Figure 2C). The liver tissue appeared normal and healthy in the control group was received distilled water alone for 5 weeks (figure 1D). All doses were administrated orally and daily for 5 weeks.

The histological appearance of Kidney tissues in all groups is shown in figures 3A-3D. There was no clear evidence of hemorrhage and fatty changes in the kidney tissues observed at low and medium doses (250 mg & 500 mg/kg bw-respectively) of ELJ water extract (figure 3A). The high dose (1000mg/kg bw) ELJ water extract treated group showed moderate hemorrhage and degree of degeneration of glomeruli (figure 3C), compared to control group (figure 3D). All test groups in this experiment were compared with control group.
Fig. 1: Microscopic analysis by H & E (1A, 1B, 1C and 1D) staining of rat pancreatic tissue 5-week post oral administration of ELJ water extract. The rats in the three test groups were administrated with low, medium and high doses (250 mg & 500 mg & 1000 mg / kg bw) as shown in figure 1 respectively exhibited normal pancreatic tissues in all the three treated groups compared to control group (1D). Distilled water gavages group (control) showing normal structure of liver tissue. Original magnification × 20. CT-connective tissue, BV-blood vessel, IL-islet of Langerhans.

Fig. 2: Microscopic analysis by H & E (1A, 1B, 1C and 1D) staining of rat liver tissue 5-week post oral administration of ELJ water extract. The rats were administrated with low and medium doses (250 mg & 500 mg / kg bw) exhibited mild fatty changes and haemorrhage and mono nuclear infiltration (1A & 1B). High doses (1000 mg/kg, bw) treated groups showing severe degeneration of hepatocytes and fatty changes, hepatocytes haemorrhage. (1C), compared to control group (1D). Distilled water gavages group (control) showing normal structure of liver tissue. Original magnification × 20. Inflammation, CV-central vein, PA-portal area, FC-fatty changes and BiD- Bile duct.

Discussion:

It is well established that *E. longifolia* Jack (ELJ) extract is a traditional medicine that has been used for decades in Southeast Asian nations in the management of many disorders. Several studies on ELJ have been conducted on small animals with respect to find out its various biological effects. However, its efficacy on the safety of body organs is not adequately studied. Currently, the levels of safety for the use of herbal drugs have become the center of attention. Various herbal drugs in the market are prescribed for various infirmities without including any toxicity profile. Such prescriptions may pose serious or fatal problems for the patients who are
dependent on such traditional medications. The major drawback of employing folklore herbal remedies is the lack of ample supporting scientific evidences on the levels of safety, quality and toxicity related to such herbal drugs. To our knowledge, at present, there are no available data in the literature on the safety and on the side effects or any deleterious effect of long term use of the products prepared from the ELJ plant. Furthermore, the plant extracts used for commercial preparations might not fulfill the standard criteria regarding the concentration of the active principles, as there might be wide variations with regard to age, growth conditions, environmental effect and plant source, [9].

Fig. 3: Microscopic analysis by H & E (3A, 3B, 3C and 1D) staining of rat liver tissue 5-week post oral administration of ELJ water extract. The rats were administrated with low and medium doses (250 mg & 500 mg / kg bw) exhibited normal structure of the kidney tissue (3A & 3B). High doses (1000 mg/kg, bw) treated groups shown moderate haemorrhage and degree of degeneration of glomeruli (3C), compared to control group (1D). Distilled water gavages group (control) showing normal structure of kidney tissue. Original magnification × 20. Bc- Bowman’s capsule, Glom- Glomerulus, Dt - Distal tube, Ct-Collecting tube.

Our previous study has indicated that ELJ (300mg & 750 mg/kg bw) water extract has moderate hepatic protection against hepatotoxicity induced by carbon tetrachloride, [6]”, furthermore, another study which was carried out on rat pancreas treated with oral dose of ELJ water extract did not show any signs of deleterious effects on the pancreatic tissues when examined by light microscope, [14]”, ELJ is popularly consumed by people of Asian origin in the form of beverage especially for its energy enhancement and aphrodisiac effects. A hepatoprotective, kidney activity and its safe use as daily beverage if any would be an added advantage to its use. Traditionally, ELJ is used for its aphrodisiac, anti-fever effects as well as a general tonic, [10]”. It possesses anti-malarial activity,[11]”, as well as exhibits potent anti-ulcer activity,[12,13]”. The study of long-term consumption of ELJ as daily beverage and its potential efficacy on the safety of some vital organs such as liver kidney and pancreas is not fully studied yet. Therefore, the current study was an attempt to find out the effect of long-term use of ELJ water extract on hepatocytes, kidney and pancreatic tissues in rats.

In the current study, we targeted rat’s liver, kidney and pancreas and the time of dose of ELJ intake was increased to 10 times than in our previous study work, [6]”. The result showed mild inflammation only with low dose, but moderate hepatocytes degeneration, fatty changes and hemorrhage with medium dose. Whereas, the severe fatty changes, hepatocytes degeneration and hemorrhage were clearly observed in liver tissue of the group that received high dose. Interestingly the pancreatic tissue in the three different concentration treated groups of ELJ did not show any degree of hemorrhage, fatty changes or degeneration. Changes were examined under light microscope and compared to control (distilled water alone). On the other hand, the histological examination of rat’s Kidney tissue after 5 weeks of the low and medium doses of ELJ water extract intake did not show any signs of fatty changes degeneration in kidney tissue when compared to control (distilled water alone). However, kidney tissue treated with high dose shown moderate hemorrhage and degree of degeneration of glomeruli, compared to control group. Distilled water gavages groups (control) showing normal structure of kidney tissue as well. The result of the clinical observation showed rats treated with low and medium doses were active before and after ELJ water extracts administration and gained body weight during the study period that reflects the health status of rats, safety of oral intake of ELJ and absence of toxicity of ELJ. However, the
rats treated with high dose of ELJ water extract were sluggish two to three hours after ELJ intake. During the experiment, all the rats were permitted adequate access to food and water and there was no hypodipsia or loss of weight. From our finding we strongly suggest that ELJ does not induce any anorexia and hypodipsia or loss of weight. During the entire period of experiments, no mortality among rats was observed. All rats appeared physically normal.

**Conclusion:**

In this study we found that use of *Eurycoma longifolia* Jack (ELJ) as beverage or capsules at low doses did not appear to cause any toxic effect on the liver, pancreas and kidney in rats’ model.

The long-term daily consumption of ELJ when taken in large quantity either as beverage or capsules may cause fatty changes, haemorrhage and hepatocytes degeneration in vital organs in rat model.

Further studies are recommended to prove the effect of long-term consumption in different animal models

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**REFERENCES**


