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Abstract

Background: The therapeutic safety of herbal medicine is a major concern for consumers and users. After studying the effects of linseed on hair growth in rabbits, the turn is to evaluate its safety by the observation of some clinical, biological and anatomo-pathological aspects.

Materials and Methods: A study was conducted during a period of three months on two groups of rabbits (control and test). Test group daily received feed supplemented with 3g of ground linseed while the control animals received the same feed without any additives. Weekly, rabbits were weighed and monthly blood samples were taken. By the end of the trial, liver and kidneys biopsies were analyzed for histological and cytological lesions.

Results: There was no significant improvement in weight gain in the test group rabbits, in which biochemical parameters had differentially evolved with a decrease in their Glycemia and cholesterolemia. There were also no modifications in their serum hepatic and renal marker enzymes and their liver and kidneys exhibited noticeably normal histology without any anatomically detectable anomalies.

Conclusion: These findings confirm that prolonged linseed ingestion in rabbits is safe.

Key words: *Linum usitatissimum*, rabbit, ingestion, safety.

Introduction

Nowadays, phytotherapy is worldwide used and it is more regarded as an alternative to modern medicine in developed countries (De Smet, 2002). According to the World Health Organization, the worldwide consumption of herbal medicines is enormous and about 80% of populations in developing countries practice medical herbalism, based on the use of plants or their extracts (Bagnis et al., 2004).

As for any product, there are particular health concerns for consumers in relation to the quality and safety of herbal products and supplements. This is why the consumer should be cautioned about the resultant risks of phytotherapeutic ingredients use (Stewart et al., 1998). To avoid or at least reduce these worrisome risks, it is primordial to improve control systems and evaluation procedures of natural products' therapeutic effects, their toxicity and their interactions with prescription drugs (Skalli et al., 2007).

Linseed (*Linum usitatissimum*) is a rich source of fibres, omega 3 fatty acids and phyto-estrogen mucilage. Year after year, this plant is gaining more importance because of the therapeutic effects of its components. It exhibits antioxidant, antineoplastic, antidiabetic, antiviral, antibacterial, antifungal, anti-inflammatory and anti-atherosclerosis properties (Prasad 1997, 2000; Chen et al., 2002).

Additionally, flaxseed is active against angina pectoris and hypercholesterolemia. It is very useful in treating constipation and removing secretions within the respiratory tract. Daily flaxseed oil intake protects gastric and urinary tracts membranes, heals scars, protects inflamed skin and improves its elasticity. It nourishes and regulates also hair follicles cycle (Halligudi, 2012; Beroual et al., 2013). After linseed oil extraction, resultant flaxseed meal is used as a protein supplement in livestock feeds (Maddock et al., 2005).

The present work aims to evaluate the toxicity of ingested flaxseed in rabbits and to contribute to quality and safety assessment of foods of animal origin.

Materials and Methods

Vegetal Material

Linseed and linseed oil were purchased from a local herbalist. Specimens of the two products are deposited at the laboratory of Pharmacology-Toxicology-Institute of Veterinary Sciences. University Frères-Mentouri. Constantine. Algeria.

Animals and Husbandry

The experiment has been carried out on 16 white New Zealand male rabbits, weighing approximately 2.5 Kg and aged six months. Animals were kept in individual standard cages in one room under the same environmental conditions (temperature, relative humidity and hygiene practices). Daily, they were individually fed 180g of the same feed during an acclimatization period of seven days.

Experimental Design

The trial was conducted during three months. Animals were equally divided into two groups (control and test). The control (CTRL) group did not receive any feed supplement, while the tested (LSI for linseed ingestion) group daily received the same feed but supplemented with 3 g of crashed linseed per rabbit.

Weekly, rabbits were weighed at the same day and the same hour before feed distribution. To evaluate the effects of linseed ingestion on the metabolic profile, blood samples were taken (on the 1st day, the 1st month, the 2nd and the 3rd) before feed distribution. Blood was

collected on heparinized tubes from the marginal ear vein using vacutainer system[®]. Plasma was obtained by blood centrifugation at 3000 rpm for 5 min and then kept at -18°C until used for analysis to dose the following parameters: glycemia (Gly), creatinemia (Crea), uraemia (Ure), albuminemia (Alb), bilirubinaemia (Bil), total protein (TP), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), cholesterolemia (Chol) and triglycerides (Tri) using an automatic biochemistry analyser (Architect ci8200).

By the end of the trial, rabbits of both groups were sacrificed and biopsies of their livers and kidneys were taken and conserved in 10% neutral formalin buffer solution. Histology slides were prepared for anatomo-pathological analysis and description.

Statistical Analysis

Body and organs weight measurements were expressed as mean ± SD and the biochemical analyses were presented as median ± IQR. To assess the differences between animals of the two groups, Student *t* test was applied for parametric variables and Mann-Whitney *U* test for non-parametric ones using the *GraphPad Instat prism 6.04* (GraphPad Software, Inc., San Diego, CA, USA, 2014). The statistical significance was set at a *P* value <5%.

Results and Discussion

Body Weight and Clinical Observations

Animals of both groups remained clinically normal throughout the whole study period. Weekly weight measurements showed that diet supplementation with flaxseed didn't block rabbits' growth and in contrast, we recorded a very trivial improvement of the LSI group weight with less heavy carcasses than the CTRL one, but not in a significant way (Tables 1).

Table 1: Body, carcass and organs weight of rabbits used in the experiment

| | Body (g) | | Carcass (g) | Liver (g) | Liver/Carcass | Kidney/Carcass |
|------------|--------------|--------------|--------------|--------------|---------------|----------------|
| | Beginning | End | | | | |
| LSI group | 2593 ± 62.77 | 3124 ± 163.3 | 1814 ± 81.10 | 98.25 ± 6.15 | 0.051 | 0.01 |
| CTRL group | 2550 ± 202.1 | 3096 ± 173.5 | 1960 ± 82.18 | 87.06 ± 6.22 | 0.045 | 0.009 |
| <i>p</i> | 0.827 | 0.911 | 0.136 | 0.467 | 0.084 | 0.065 |

These results are in concordance with those reported by Brant et al. (2012), who found flaxseed to prevent excess of body weight gain in pregnant rats because of its beneficial effects on lipids and glucose profiles. This same observation had also been made by Daleprane et al. (2010) in weaned rats, for the reason that flaxseed fibres and long-chain N-3 polyunsaturated fatty acid content could reduce both appetite sensation and energy intake (Kristensen et al., 2013). According to Bielanski and Kowalska (2008), the addition of linseed oil to rabbit diets has a favourable effect on the composition of the lipid fraction of its meat, with a significant decrease in total saturated fatty acids and cholesterol, as well as an increase in polyunsaturated fatty acids contents.

Blood Parameters

Regarding biochemical data, all blood parameters analyzed in our study (Figure1) were within the physiological range as described in the literature (RAR, 2015). Furthermore, mean concentrations of some of these parameters seem to be less important in the LSI group than the CTRL one but the difference reached significance only for creatinemia (Table 2). In the LSI animals, 9% and 22% decreases were recorded in glycaemia and cholesterolemia respectively.

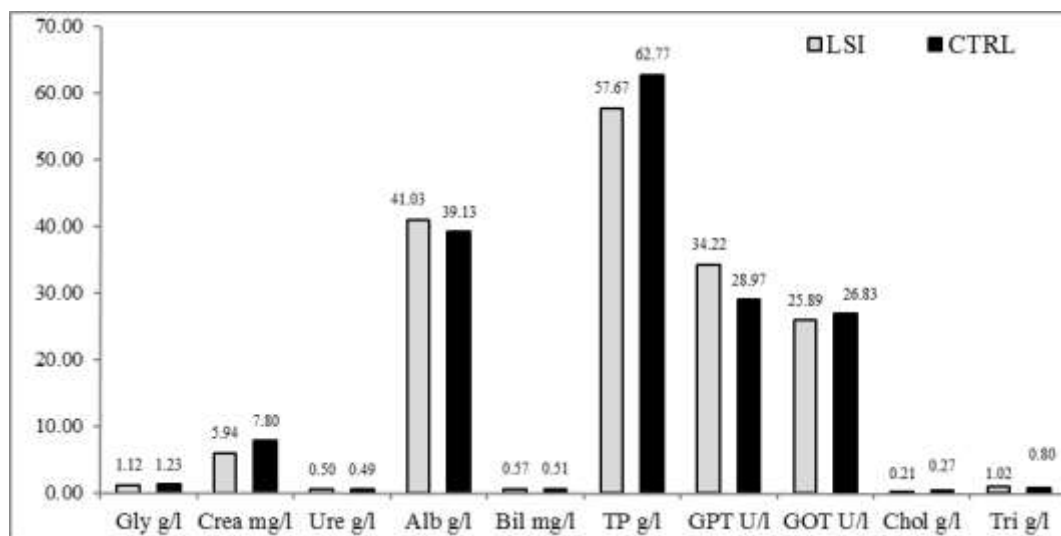


Figure1: Mean concentrations of blood parameters in the LSI group and the CTRL one all along the experiment.

This is due to flaxseed richness with soluble fibres (about one-third of the fibres in flaxseed) which may help to lower blood lipids and cholesterol and to regulate levels of blood sugar (Institute of Medicine, 2002). The hypoglycemia can be related to a drop in the carbohydrate

absorption from the gut and the hypocholesterolemia may be attributed to a reduced absorption of cholesterol and/or bile acid reabsorption (Cintra et al., 2006; Pellizzon et al., 2007).

Table 2: Plasma chemistry values in rabbits of the LSI and the control groups during all the trial

| Blood parameters | CTRL (n=8) (Median ± IQR) | LSI (n=8) (Median ± IQR) | U | P |
|------------------|------------------------------|-----------------------------|-------|---------|
| Gly (g/L) | 1.150 (1.015 – 1.250) | 1.190 (0.920 – 1.400) | 67.50 | 0.667 |
| Crea (mg/L) | 23.00 (18.50 – 23.00) | 10.50 (3.50 – 17.00) | 18.50 | 0.0006* |
| Ure (g/L) | 0.300 (0.240 – 0.347) | 0.330 (0.300 – 0.410) | 53 | 0.231 |
| Alb (g/L) | 35.00 (32.00 – 50.75) | 36.00 (29.00 – 56.00) | 74.50 | 0.948 |
| Bil (mg /L) | 0.400 (0.200 – 1.00) | 0.500 (0.100 – 1.00) | 72.50 | 0.873 |
| TP (g/L) | 63.00 (15.00 – 65.00) | 60.00 (53.00 – 62.00) | 78.50 | 0.271 |
| GPT (U/L) | 28.00 (19.25 – 42.00) | 29.00 (22.00 – 35.00) | 76 | 0.999 |
| GOT (U/L) | 20.00 (11.25 – 41.75) | 20.00 (16.00 – 27.00) | 71.50 | 0.825 |
| Chol (g/L) | 0.230 (0.162 – 0.310) | 0.270 (0.170 – 0.410) | 65 | 0.575 |
| Tri (g/L) | 0.675 (0.492 – 1.255) | 0,960 (0.460 – 1.160) | 70 | 0.766 |

*Significant at P<0.01 (two-tailed test)

An increase in triglyceridemia was recorded in the LSI group. This corroborates well with previous studies (in rats and rabbits) and confirms that some non-lipid components of linseed (Lignan and soluble fibres) may increase triglyceride levels through enhanced fat absorption and delayed clearance of either chylomicron or very low density lipoproteins (Prasad et al., 1998; Babu et al., 2000).

A drop in serum proteins level was observed in the LSI group when compared with normal control, but still within the physiological range. This is usually the result of decreased protein synthesis in the liver or increased protein loss through the kidney. It may also be due to malabsorption (Orhue et al., 2005).

It is clear that the measured values for enzymes in our study are within the normal physiological values, which confirms the findings of previous works on the positive pharmacological effects of linseed (Hermier et al., 2004; Weill and Mairesse, 2010). The recorded serum amounts of hepatic marker transaminases didn't differ from physiological standards in rabbits fixed by the RAR (2015) and accordingly it removes the idea of liver dysfunction or overloading. It is known that the leakage of these intracellular hepatic enzymes into the blood stream is an indicator of hepatic cells damage frequently related to the susceptibility of their walls to oxidative stress (Das et al., 2010). Our findings are consolidated by previous studies in which flaxseed chutney has demonstrated a hepatoprotective function by decreasing liver marker enzymes GOT, GPT and ALP in the serum (Shakir and Madhusudhan, 2007a). Moreover, flax addition to the diet of rats exposed for a long-term to azoximethane, results in a significant decrease in liver γ -glutamyl transpeptidase profile and liver lipids and micronuclei formation (Shakir and Madhusudhan, 2007b). In mice with cyclophosphamide-induced oxidative stress, linseed oil inhibits the activity of acid phosphatase and oxidized glutathione. It prevents decline in blood reduced glutathione, glutathione peroxidase and alkaline phosphatase (Bhatia et al., 2006).

There were no modifications in bilirubin amounts in the LSI group when compared with the normal control. This brings more confirmation of the liver good functioning. Bilirubin is an intermediate product in heme metabolism in the liver and its estimation in the serum is an important indicator in evaluating the liver function (Mohamed et al., 2009). A high level of bilirubin may derive from its reduced hepatocyte uptake or secretion, its weak conjugation and red blood cells excessive oxidative damage (Nkosi et al., 2005; Dominic et al., 2012).

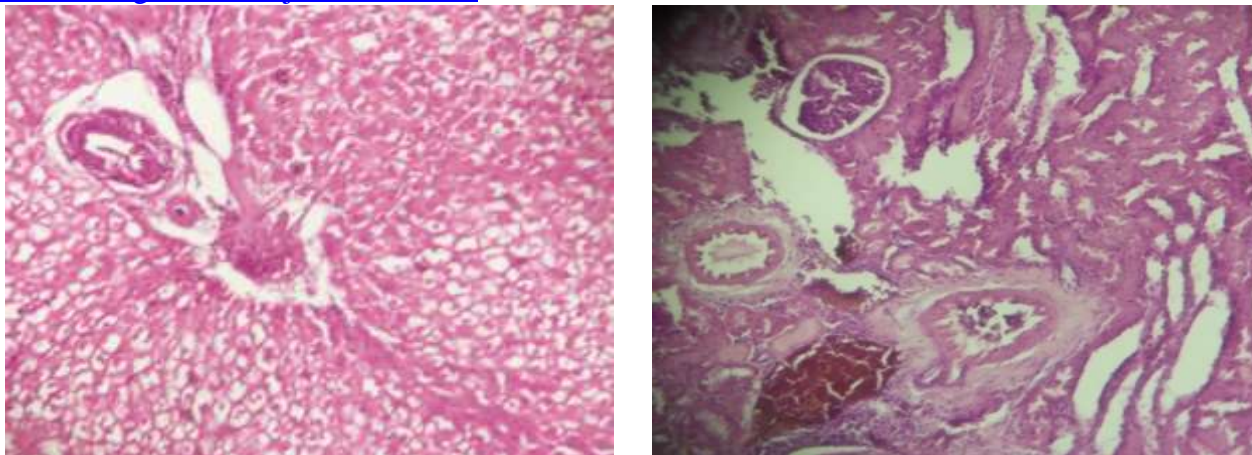
On the other hand, a high urea and creatinine amount is a sign of kidney damage. In the LSI group, these parameters corroborate with standard values reported in the scientific literature for normal rabbits (RAR, 2015) and demonstrate the proper functioning of the kidneys and the good hydration of the animals. In their study on lead acetate induced kidney failure, Abdel-Moneim et al. (2011) reported that the administration of flaxseed oil efficiently decreased the levels of serum creatinine, blood urea nitrogen, uric acid, lipid peroxidation and nitric oxide production.

In contrast with all these previous studies and always in rats, Ahmad et al. (2012) found aqueous methanol extract of flaxseeds to increase serum total proteins, total cholesterol, ALAT and ASAT activity, but without any effect on kidney function.

Antioxidant function of linseed is related to its particular high concentration of lignans (secoisolariciresinol diglycoside) known for their hydrogen-donating antioxidant activity as well as their ability to complex divalent transition metal cations (Prasad, 2000, Touré and Xueming, 2010). According to Barthet et al. (2014), flaxseed antioxidant activities are mainly due to water-soluble system-proteins.

Histological Analyses

The histopathological examination of liver sections of control and test groups showed normal arrangement and architecture of hepatocytes, with clearly visible nuclei, central vein and portal triad. Also, the kidneys' sections demonstrated normal glomeruli and tubules without any architectural derangement of their tissues. There was no necrosis, steatosis, fibrosis or inflammation (Figures 2 and 3).



Figures 2 and 3: Porta hepatis (left) and glomerulus (right) of LSI group rabbits

No differences between liver/carcass and kidney/carcass weight ratios from animals of both groups (Table 1) and no hypertrophy or atrophy have been recorded in these two organs in association to a total absence of any gross lesions on their respective carcasses.

Liver and kidneys are responsible for the metabolism (biotransformation and excretion) of xenobiotics and toxicants (Rose and Hodgson, 2004) which can influence their histological structure and subsequently their weight as well as the weight of the entire body. Changes in the body and organs weights are used as valuable indexes of toxic-related organ damage. In our study, the very slight increase in body weight accompanied with the lack of any change in organs (liver and kidneys) weigh suggest absence of any toxic effect of linseed.

Flaxseed hepatoprotective effect is the consequence of an increase in antioxidant defence mechanisms within the liver, mediated through antioxidant activities of flaxseed phenolic components as well as the neutralization of free radicals responsible of peroxidation of polyunsaturated fatty acids in bio-membranes which results in tissue damage (Kasote et al., 2012).

Linseed kidney protective activity is exerted through three mechanisms: moderation of associated interstitial nephritis, alteration of renal content of polyunsaturated fatty acids and less formation of inflammatory classes of renal prostanoides (Ogbor et al., 1999; Bankovic-Calic et al., 2002). It was concluded from the studies of Ghule et al. (2011, 2012) that renal protective activity of ethanolic extract of flaxseed may result from its action on rennin-angiotensin system and inhibition of renal artery occlusion. This extract restores the levels of renal endogenous antioxidant and membrane-bound enzymes.

Conclusion

In conclusion, the present study clarified that feed supplementation with flaxseed is beneficial to enhance body weight and carcass yield in white male New Zealand rabbits. This plant doesn't affect liver or kidney function parameters and it has no deleterious effects on their histology. However, more studies are needed to assess the effects of greater amounts of these seeds in this same breed of rabbits, in other breeds as well as in other animal species.

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