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Effect of Zingiber Extract on Histopathologic Changes in Mice Kidneys

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Abstract: The use of botanical medicine is ancient and plant chemicals are still the backbone of the pharmacopoeia because >50% of drugs used in western pharmacopoeia are isolated from herbs or derived from modification of chemicals first found in plants. *Zingiber officinale* Roscoe. (Ginger) is a spice that has been used from 2,000 years ago as a medicine in several Asian countries. Recent studies showed that it has various pharmacological effects. So, in this study the effect of Zingiber on mice renal system has investigated. Ginger ethanol extract was administered every 48 h for a period of 20 days, Intraperitoneally (IP) to male mice. The liver, kidney and bladder were collected as tissue samples from executed animals for pathological examination. On pathological examination, there were no morphological changes under light microscope in the liver. A histological examination of kidneys shows that after treatment with the low and middle doses (10, 20 mg/kg/48 h) the ginger produced little damaging effects on the kidney, it appears that ginger has no adverse effects in renal function but the extract in higher doses (40 mg/kg/48 h) may have some negative effects on the renal function. Undergo observable chances the renal toxicity was significantly enhanced there were interstitial inflammation. Formation of hyaline cast, regeneration of renal tubules, glomerulosclerosis, hypertrophy of glomeruli and basement membrane thickening. This indicated that with regards to the results ginger in high doses induce histological changes in the kidneys indicating the need to identify a safe dose range for ginger.

Key words: Zingiber, kidney, mice, pathology, histology, Iran

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is a plant from Zingiberaceae family is commonly part of food diet in some parts of the world. From common ginger rhizome a powder that called ginger spice is made which its hot and fragrant taste were used for spicing the food from old times (Baliga *et al*., 2003). Its natural growth is in eastern parts of India, specially in Gingi and Zingi, Malaber, Srilanka and China. Its cultivation is common in South Asia, Japan, North Africa, Mexico until islands and specially Jamaica. This plant hasn't any normal growth in Iran (Frondoza *et al*., 2004). The Greek physician Galen used ginger as a purificant of body. He used ginger to treat conditions caused by imbalances in body (Thomson *et al*., 2002). In classic medicine ginger used as moisture absorption around the head, throat and stomach and by eating or using ginger as eye liner cure the eye darkness from moisture (Momeni *et al*., 2000; Blomhoff, 2004). In recent researches found that ginger because of its various active component including shogaols and gingerols which are responsible for its strong scent. Ginger in also a plant which contain the most antioxidant (Jagetia *et al*., 2003; Lako *et al*., 2004; Liu *et al*., 2003) and used as medicine and food spice. Until now many studies have been done on it and its therapeutic effects for curing different illnesses have been studied. More studies showed that zingiber extract have anti-inflammatory effects (Tan and Vanitha, 2004), Antibacterial effect (Jagetia *et al*., 2003), antifungal (Rinn *et al*., 2004; Smith, 2002), immunomidulatory and antimicrobial effects. *In vitro* studies showed that zingiber is novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite (Lako *et al*., 2004) and could inhibit and/or scaving radicals of rat body (Rinn *et al*., 2004). With respect of all this and by considering that renal system is one of body main systems which is considered in most routine toxicity determination experiment protocols. More over, kidneys almost receive 25% of cardiac output which increases chemical substances distribution to kidneys as well.

The kidneys are routinely exposed to high concentrations of medications or their metabolites because their intrinsic function is to metabolize, concentrate and excrete compounds. Therefore, it is not surprising that as with prescribed medications, many dietary supplements have been associated with nephrotoxicity either as a direct toxic effect or secondary to liver dysfunction, rhabdomyolysis or nephrolithiasis.

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It is clear that although, many dietary supplements may not be harmful, some have been associated with renal dysfunction and others have the potential to do so (Thomson *et al*., 2002). The renal effects of various herbs can be harmful or beneficial. Harmful effects include: polyuria causing dehydration, acute renal failure, chronic renal insufficiency and stone formation. Possible beneficial effects include: diuresis, protection of the kidney from nephrotoxic agents, prevention or amelioration of renal lithiasis and amelioration of kidney failure (Myhre, 2000). Renal system actively involve in drug elimination from body throw renal filtration process, proximal tubule secretion and distal tubule reabsorption. It is well known that most of drugs including: antibiotics, nonsteroid anti-inflammation, radiographic contrast media and some of cancer remedies maybe cause of renal failure. Although, damage maybe reversible, it may cause chronic changes in kidney parenchyma. Likewise unknown usage of herbal medicine may cause a more damage to endanger renal function. Plus direct effects of herbal medicine of kidneys, act of a specific plant may complicate cure of patients. Research has shown that herbal remedy use may be associated with acute renal failure. In addition, the use of herbal remedies may be detrimental for the patient with compromised renal function. Patients with renal insufficiency or renal failure may be at risk for further kidney damage as well as complications related to interactions of herbal remedies with complex renal therapy regimens (Murcia *et al*., 2004). Other potentially nephrotoxic dietary supplements include those that inhibit the enzyme Cyclooxygenase (COX) thereby affecting renal hemodynamic. Dietary supplements that may inhibit COX include *Tanacetum parthenium* (feverfew), *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric). These agents should be used cautiously especially in patients with underlying renal dysfunction (Thomson *et al*., 2002) by reviewing the references, the researchers notice that although, there was abundant researches on zingiber, there isn't any research on renal system so it made us to do the research in this way and study the effects of zingiber on renal function to make a scientific fundamental for safe use of this plant.

MATERIALS AND METHODS

Experimental animals: In order to do these laboratory adult mice (white mice Souris) with scientific name *Mus musculus* Var. albinos has been used. Experimental mouse belongs to rodent's animals from albino variety from normal mouse (Huang *et al*., 2003). These mice bought from pharmacology and medicine animal nest and kept in Isfahan Payam e Noor University animal nest. Mice have sexual dimorphism in their kidneys; this difference is not only in their size but also seen in their cellular (Baliga *et al*., 2003) and sub cellular structure (Murcia *et al*., 2004), gene expression and enzyme activity so, in this experiment it has been used male mice. Mice weighted 28.49 g mean which had kept for 2 weeks for new environment adaptation with normal light period in temperature with 30.11°C mean.

Table 1: Mice treatment

Groups	Treatments
Control	No injection has done
Placebo	Injection of placebo which contains physiologic sera+ ethylic alcohol
Group one	Injection of zingiber extract with 40 mg/kg/48 h
Group two	Injection of zingiber extract with 20 mg/kg/48 h
Group three	Injection of zingiber extract with 10 mg/kg/48 h

Making herbal extract: Zingiber rhizomes have been prepared under expert's supervision from grocery. With use of food processor zingiber rhizomes have been powdered and mixed with 90% ethylic alcohol by 1 part in 3. Container closed with par film and covered with aluminum foil to avoid potentially light effect on extract components. Before use, extracts kept in refrigerator. Since, for each 10 g of mice body weight 0.2 mL of provided concentration were injected to mice in whole experiment period, 40, 20, 10 mg/kg/48 h doses were provided for treatment groups.

Groups: In this research 30 mice grouped in 5 their was 4-8 mice in each (Table 1). Since, the mice were male the researchers tried to put them in cages so they have less possible contact and fight less and live peacefully together in all experiment period. In research with mice the researchers tried to respect moral ethics as possible.

Treatment: After grouping and ending the adaptation period to temperature, moisture and new nest environment, mice were weighted and marked for individual identification. Treatment performed for 20 every other days.

Injection and sampling: Between, 11-14 at noon, at first every mouse was weighted and upon its weight for every 10 g of body weight 0.2 mL injection solution was taken. After that abdomen were antisepticised by alchoholated cotton and by insulin syringe intraperitoneal (almost with 10 degree angle) injection performed right or left side of abdomen near middle line near of hillum. For blood sampling eye sinus punctuation with use of hematocerite tube were done. After that mouse were decapitated for

sampling of internal organs immediately. Blood biochemical experiments included Creatinine (Cr) and Blood Urea Nitrogen (BUN). For separating serum, experimental tubes were centriphuged with 2000 cycle for 10 min. Thus, according to common measurement methods urea diacetyl from diacetyl mono oxim hydrolysis composed with urea and produced yellow terminal product which by use of spectrophotometer density of produced color were measured by 475 nm wavelength. Also light absorption of orange color produced from combination of creatinine with picric acid in baseic environment measured with use of 500 nm wavelength spectrophotometer (Ficker *et al*., 2003a, b). Collected histological samples routinely prepared and slides were made from them and lamelated. One way ANOVA was used for comparing means of quantitative variables in independent groups and for finding the place of difference between groups if there is Tukey's HSD test were used. If data haven't any normal distribution and variance of studied groups weren't the same Kruskal-Wallis test was used.

RESULTS AND DISCUSSION

Biochemical experiments results: Kidneys function some how wasn't under the effect of herbal extract. In low, medium and high ginger doses (10, 20 and 40 mg/kg/48 h) with comparing by control group showed significant changes ($p<0.01$, 0.1, 0.05) in lowering BUN levels. There weren't any significant changes in creatinine levels in treatment with low and medium zingiber doses. As it shown in Table 2 there was a significant change in BUN on Creatinine ratio in all groups to control group ($p<0.05$). There wasn't any significant change between experimental and placebo group but change difference between BUN between experimental groups and control group were significant.

Pathologic experiment results: There weren't any significant changes in liver tissue; herbal extract hasn't any notable change on liver. For studying histological changes in mice kidney's tissue after preparation slide as mentioned before. They were under specialized study by pathology specialist and the changes were ranked. At the end the results were studied as follow: by studying and comparing mean of mice kidney weight to body weight ratio of control group and experimental groups for potential hypertrophy, it have cleared by milligram that in all levels there wasn't any meaningful difference between experimental and control groups (Table 3).

Since, serum BUN and creatinine levels elevation in clinical experiments means renal dysfunction so, the researchers measured serum BUN and creatinine levels to determine amount of kidney damage. BUN levels in control group mice is about 37±5 mg% which in comparing with normal BUN in human blood serum that is between 8-25 mg% (Ficker *et al*., 2003a, b) is noticeably higher. Results showed that ginger treatment in all doses comparing with control group cause meaningful decrease in amount of blood nitrogen urea (Table 2). This result like Huang L. and colleagues studies on *Radix paeoniae* ALBA plant which is from paeoniae (Rinn *et al*., 2004) and so it is like Tirkey studies on *Curcuma longa* L. another member of Zingiberacea family (Akoachere *et al*., 2002) and it is opposite to result of Hsu Hy on *Erycibe obtusifolia* (Lako *et al*., 2004). From these findings the researchers can conclude that zingiber not only hasn't any negative effect on kidney's function but also in some degree it has positive effect on waist exertion. It seems that ginger in end tract of collecting tubules that known to be urea reabsorption tract (Akoachere *et al*., 2002) decreased urea reabsorption in this tract.

Use of plasma creatinine levels is a tool to evaluate kidney's function. With regard that it has reported that Alkaline Pikrate method that is creatinine routine way of measurement shows amount of creatinine higher than real amount of plasma creatinine (Myhre, 2000). Amount of measured creatinine in done experiments is very low. In human creatinine levels are about 0.5-1.3 mg dL^{-1} whereas creatinine mean of control group is 0.35 mg dL^{-1} in mice. With regard to low weight of laboratory mice in same ratio the amount of obtained creatinine from their muscles are very low which amount of low creatinine in mice blood sera confirm it and as you see creatinine levels in different groups by comparing with control group didn't significantly changed. Significant decrease in BUN to creatinine ratio in experimental groups to control group probably because of injected liquid dose volume. Because increase of body water lead to decrease of this ratio. Another possibility for this decrease is hepatic illness. By

Table 2: BUN to creatinine ratio changes

Groups	Mean	SD
Group one (Control)	110.4150	25.3581
Group two (Placebo)	58.0571*	16.1261
Group three (10 mg/kg/48 h)	51.8900*	18.2741
Group four (20 mg/kg/48 h)	55.9717*	7.4491
Group five (40 mg/kg/48 h)	54.3413*	16.4891

*The mean difference is significant at the 0.05 level

Table 3: Descriptive parameters result from study and compare mice kidney's weight to body weight ratio between experimental and control groups

Groups	Mean	SD
Group one (Control)	1.375×10^{-2}	9.5743×10^{-4}
Group two (Placebo)	1.450×10^{-2}	7.0711×10^{-4}
Group three (10 mg/kg/48 h)	1.567×10^{-2}	1.5275×10^{-3}
Group four (20 mg/kg/48 h)	1.650×10^{-2}	2.1213×10^{-3}
Group five (40 mg/kg/48 h)	1.600×10^{-2}	1.4142×10^{-3}

histological studies it has been cleared that there isn't any significant change in liver. So, first possibility is more acceptable. Pathological studies showed that there weren't any macroscopic changes in kidneys, bladder and liver and all of them have normal appearance. In mice kidneys there weren't any hypertrophy, this maybe because short period of treatment and less time for appearing macroscopic symptoms.

Histopathologic changes increases with increase of dose so hyaline casts has seen in kidneys tubules of maximum dose group and its level increases with nephropathy by increase of dose. Meantime the researchers didn't saw any internal medulla atrophy; tubules were firmly beside each other and glumerolar distribution in all doses except major one was normal. This incomplete histology change besides of intraperitoneal injection of ginger maybe due to shortage of length of experiment time or deficiency of injected dose. So, in maximum dose as explained before changes has seen which might be because of increase of blood proteins and precipitation in glomeruls. At end results shows that usage of zingiber with maximum dose (40 mg/kg/48 h) every other day it can effect on kidneys function and until some ranges it make histological and hematologic changes. Probably with dose increase these changes were more significant but have not seen in medium (20 mg/kg/48 h) and low (10 mg/kg/48 h) doses.

CONCLUSION

Many studies have been done on therapeutic effects of zingiber plant that we reviewed some of these studies in previous parts but until now study on renal system haven't been done and findings of this study is first step in this field, the researchers hope it open the way of later studies in this field.

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