PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 13 (10): 584-588, 2014
ISSN 1680-5194

Effect of Blood Leaf (*Iresine herbstii*) Leaf Extract and Powder on the Biochemical Profile of Adult Male Albino Wistar Rats

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Abstract: This study assessed effect of *Iresine herbstii* leaf extract and powder on biochemical profile of adult male albino Wistar rats. Twenty adult male albino Wistar rats divided into four groups of five rats each were used for the study. All the animals received rat-chow and water *ad-libitum*. Group A was the control. Groups B, C and D received in addition, fresh leaf extract, shade-dried leaf extract and shade-dried leaf powder, respectively. Blood samples were obtained from the rats for lipid profile, liver and kidney function tests. Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) for windows version 18. $p<0.05$ was accepted as level of significance. Serum AST was significantly ($p<0.05$) increased in rats fed fresh leaf extract. There was a significant ($p<0.05$) reduction in serum ALT of rats fed shade-dried leaf extract and powder. However, serum ALT of rats fed fresh leaf extract was significantly ($p<0.05$) increased. Serum ACP was significantly ($p<0.05$) increased in the three treatment groups. Serum creatinine was significantly ($p<0.05$) increased in rats fed fresh leaf extract. Rats fed shade-dried leaf powder showed a significant ($p<0.05$) increase in serum urea after treatment. Serum urea of rats fed fresh leaf extract was slightly reduced. There was a significant ($p<0.05$) increase in total cholesterol of rats fed fresh leaf extract whereas rats fed shade-dried leaf powder showed a significant ($p<0.05$) decrease in total cholesterol. Serum LDL-C of three treatment groups were increased. However, this was only significant ($p<0.05$) in rats fed shade-dried leaf extract.

Key words: *Iresine herbstii,* extracts, liver function test, kidney function test, lipid profile

INTRODUCTION

Focus on plant research has increased in recent times all over the world (Amagloh and Benang, 2009). Several studies have established that some vegetables species are potentially toxic to humans. Plant chemical compounds, toxic to humans and livestock are produced as part of the plant's defense mechanism against being eaten by pests, herbivores and to gain advantages over competing plants (Dowling and McKenzie, 1993). The natural toxins of plants may also have adverse effects on the health of people who eat the plant. Plant poisons are highly active substances that may cause acute effects when ingested in high concentrations and chronic effects when accumulated over time (Kofi-Tsekpo, 1977). In many cases of poisoning resulting from consumption of endogenous toxicants such as those in toxic vegetables, death or prolonged and serious disabilities are reported (Orech *et al*., 2005).

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the endogenous and exogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism (Ramachandra *et al*., 2007). Hepatic damage is associated with distortion of these metabolic functions (Wolf, 1999). Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (Ilango and Chitra, 2009). The kidney is an important organ having not only excretory function but also other functions such as production of the substances that activates a living body, enzymatic reaction, immunization, etc (Subir *et al*., 2008). Plasma concentrations of creatinine and urea could be used as indicators of nephrotoxicity (Pagana, 1998; Brenner and Floyd, 1999; Burtis and Edward, 1999; Wallach, 2000; Henry, 2001). Low clearance of creatinine or/and urea indicates a diminished impaired ability of the kidneys to filter these waste products from the blood and excrete them in urine (Saka *et al*., 2010). As their clearance values decrease, their blood levels increase. Hence, an abnormally elevated blood creatinine is diagnostic of impaired renal function (Pagana, 1998; Brenner and Floyd, 1999; Burtis and Edward, 1999; Wallach, 2000; Henry, 2001). Excess cholesterol that circulates in the blood can stick to the walls of the arteries leading to a predisposition to CVDs. High LDL-C is also a risk factor for heart disease.

Iresine herbstii could be found in homes and are known as blood boosters/tonic by local users. Sometimes, the infusion or extract of the fresh leaves are drunk as quick sources of nutrients and medicines among local communities. Hence, the leaves are consumed without

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fear of toxicity. No work has addressed the toxicology of *Iresine herbstii* leaf extract and powder to some biochemical parameters of the body system leaving the area un-highlighted. This study, therefore, investigated the effects of *Iresine herbstii* leaf extract and powder on the liver, kidney and lipid profile of adult male albino Wistar rats.

MATERIALS AND METHODS

Source and identification of *Iresine herbstii* leaves: The leaves of *Iresine herbstii* were harvested from its plants from a farmland at the back of the Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka, Nigeria. It was botanically identified at the Department of Plant Science and Biotechnology, of the same University.

Sample preparation: Fresh leaves of *Iresine herbstii* were harvested from the plants. The fresh leaf extract was obtained by weighing out 300 g of the leaves and washing it in clean water. The washed leaves were squeezed with hand in 200 mL of water, sieved and filtered using a 0.1 mm mesh size filter. The shade-dried leaf extract was obtained by washing 30 g of the fresh leaves in clean water and shade-drying it for four days. The shade-dried leaves were squeezed with the hand in 200 mL of water, sieved and filtered using a 0.1 mm mesh size filter. The shade-dried leaf powder was prepared by washing the fresh leaves in clean water, shade-drying for four days and grinding into fine powder.

Animal study: Twenty adult male albino rats (190-250 g) were used for the study. The rats were divided into four groups (Groups A, B, C and D) of five rats each in a way that the difference in weight between rats in a group was not more than 5 g. They were allotted into individual metabolic cages. The animals were acclimatized for five days during which they were fed soybean and maize flour blend and water *ad-libitum* and even during the experimental period:

1: Group A (control) = no treatment
2: Group B = fresh leaf extract
3: Group C = shade-dried leaf extract
4: Group D = shade-dried leaf powder

Biochemical analysis: Five milliliters of blood samples were collected after acclimatization and at the end of the study from the medial canthus of the rats. The blood samples collected were used for liver and kidney function tests and lipid profile determination.

Liver function test: Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using QCA test kit. Serum alkaline phosphatase (ALP) was determined using the King and Kind calorimetric method. Serum acid phosphatase (ACP) was determined using King's method for *in-vitro* determination.

Kidney function test: Serum urea and creatinine test were determined using QCA test kit.

Lipid profile determination: Total cholesterol and low density lipoprotein cholesterol (LDL-C) were determined using Chod-pap method.

Statistical analysis: Data obtained from this study was analyzed using the computer program, Statistical Package for Social Sciences (SPSS) for windows version 16. Main analysis included means and standard error of mean. One way analysis of Variance (ANOVA) and Duncan's Studentized New Multiple Range Test was used to separate and compare means within groups of rats. Level of significance was $p<0.05$.

RESULTS

Table 1 shows the effect of *Iresine herbstii* leaf extracts and powder on liver function. Rats fed the shade-dried leaf extract showed a significant ($p<0.05$) increase in serum ALP. Serum AST was significantly ($p<0.05$) increased in the rats fed the fresh leaf extract, however serum AST significantly ($p<0.05$) decreased in the rats fed the shade-dried leaf powder. There was a significant ($p<0.05$) reduction in the serum ALT of rats fed the shade-dried leaf extract and powder however, the serum ALT of rats fed the fresh leaf extract was significantly ($p<0.05$) increased. Serum ACP was significantly ($p<0.05$) increased in the three treatment groups.

Table 2 shows the effect of *Iresine herbstii* leaf extracts and powder on kidney function. Serum creatinine was significantly ($p<0.05$) increased in the rats fed the fresh leaf extract however, serum creatinine was significantly ($p<0.05$) decreased in the rats fed the shade-dried leaf powder. Rats fed the shade-dried leaf powder showed a significant ($p<0.05$) increase in serum urea after treatment. Serum urea of the rats fed the fresh leaf extract was slightly reduced whereas serum urea of rats fed the shade-dried leaf extract was increased though it was not significant.

Table 3 shows the effect of *Iresine herbstii* leaf extracts and powder on lipid profile. There was a significant ($p<0.05$) increase in the total cholesterol of rats fed the fresh leaf extract whereas rats fed the shade-dried leaf powder showed a significant ($p<0.05$) decrease in total cholesterol. Rats fed the shade-dried leaf extract showed an increased total cholesterol though this was not significant ($p<0.05$). Serum LDL-C of three treatment groups were increased, however, this was only significant ($p<0.05$) in the rats fed the shade-dried leaf extract.

Table 1: Effect of *Iresine herbstii* leaf extracts and powder on liver function test

	Group A	Group B	Group C	Group D
ALP (IU/L)				
Before treatment	27.25±0.20[a]	26.55±0.09[a]	27.69±1.00[a]	20.72±0.86[a]
After treatment	30.99±0.58[a]	30.12±0.29[a]	34.56±1.48[b]	27.79±1.67[a]
AST (IU/L)				
Before treatment	14.67±1.20[a]	17.33±3.18[a]	22.00±1.53[a]	21.00±2.00[a]
After treatment	21.33±0.88[b]	23.00±1.00[b]	22.00±1.00[a]	14.33±0.33[b]
ALT (IU/L)				
Before treatment	13.61±0.57[a]	13.33±0.88[a]	15.33±0.33[a]	14.67±0.88[a]
After treatment	13.67±0.33[a]	16.00±0.57[b]	12.03±0.73[b]	4.93±0.03[b]
ACP (IU/L)				
Before treatment	11.67±1.67[a]	4.00±1.53[a]	4.67±0.33[a]	7.32±0.88[a]
After treatment	5.67±0.33[b]	30.00±0.00[b]	22.67±4.30[b]	17.00±3.05[b]

Group A (control) = no treatment; Group B = rats fed the fresh leaf extract; Group C = rats fed the shade-dried leaf extract; Group D = rats fed the shade-dried leaf powder

Table 2: Effect of *Iresine herbstii* leaf extracts and powder on kidney function test

	Group A	Group B	Group C	Group D
Creatinine (µmol/L)				
Before treatment	78.90±9.33[a]	51.81±3.80[a]	88.00±7.17[a]	62.63±0.07[a]
After treatment	82.30±4.57[a]	81.50±4.27[b]	74.81±5.79[a]	21.33±3.27[b]
Urea (mmol/L)				
Before treatment	5.17±0.05[a]	5.50±0.04[a]	4.93±0.05[a]	6.30±0.05[a]
After treatment	5.00±0.19[a]	5.47±1.18[a]	4.10±0.10[a]	9.10±1.20[b]

Group A (control) = no treatment; Group B = rats fed the fresh leaf extract; Group C = rats fed the shade-dried leaf extract; Group D = rats fed the shade-dried leaf powder

Table 3: Effect of *Iresine herbstii* leaf extracts and powder on lipid profile

	Group A	Group B	Group C	Group D
Total cholesterol (mmol/L)				
Before treatment	2.63±0.08[a]	1.73±0.12[a]	2.59±0.11[a]	3.11±0.12[a]
After treatment	4.20±0.20[b]	3.08±0.04[b]	2.75±0.08[a]	2.17±0.09[b]
LDL-C (mmol/L)				
Before treatment	1.49±0.07[a]	1.17±0.03[a]	1.23±0.09[a]	1.16±0.09[a]
After treatment	2.52±0.09[b]	1.50±0.10[a]	1.56±0.06[b]	1.30±0.03[a]

Group A (control) = no treatment; Group B = rats fed the fresh leaf extract; Group C = rats fed the shade-dried leaf extract; Group D = rats fed the shade-dried leaf powder

DISCUSSION

Increased serum ALP levels in rats fed fresh and shade-dried *Iresine herbstii* leaf extract and shade-dried *Iresine herbstii* leaf powder is indicative of tissue damage which is often used in the diagnosis of liver and bone marrow disease and hepatitis (Nelson and Cox, 2000; Anderson, 1968). This increase was however significant ($p<0.05$) in the rats fed the shade-dried *Iresine herbstii* leaf extract. ALP occurs in the liver next to the bile ducts and in the bone (Amadi *et al*., 2013). It leaks into the bloodstream in a manner similar to AST and ALP (Friday, 2004; Mathew, 2000).

Significant ($p<0.05$) increase in the serum AST and ALT of the rats fed the fresh *Iresine herbstii* leaf extract was observed. Fresh *Iresine herbstii* leaf extract probably caused damage to the liver tissues (hepatotoxic). Balint *et al*. (1997) reported that ALT and AST are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histopathologic changes. According to Kumar *et al*. (2003) increased activities of serum AST and ALT levels could be attributed to the damaged structural integrity of the renal and hepatic cells causing the enzymes which are located in the cytoplasm to be released into circulation, hence the increased serum enzyme activities. Bergmeyer *et al*. (1978) reported that AST and ALT are excellent markers of liver damage caused by exposure to toxic substances. This shows that fresh *Iresine herbstii* leaf extract was toxic to the liver because when the integrity of the hepatocellular membrane is compromised, there is extrusion of the enzymes into the plasma (Moss and Henderson, 1996). Friday (2004) noted that ALT is a more liver specific enzyme for diagnostic use. Most elevations of ALT are caused by liver disease. Rats fed shade-dried *Iresine herbstii* leaf extract and powder had decreased serum AST and ALT showing that the shade-dried *Iresine herbstii* leaf extract and powder were probably not toxic to the liver. Significant ($p<0.05$) changes in the serum ACP levels of the rats fed the fresh and shade-dried *Iresine herbstii* leaf extract and shade-dried *Iresine herbstii* leaf powder was observed. According to Rodwell and Kennelly (2000) an elevated serum ACP may suggest an increased rate of tissue destruction.

Rats fed fresh *Iresine herbstii* leaf extract had a significant ($p<0.05$) increase in their serum creatinine level. This could mean an abnormal functioning kidney since the retention of creatinine in the blood is an evidence of kidney impairment (Amadi *et al*., 2013). Rats fed the shade-dried *Iresine herbstii* leaf powder had a significant ($p<0.05$) decrease in their serum creatinine. However, significant ($p<0.05$) increase in serum urea of rats fed the shade-dried *Iresine herbstii* leaf powder was observed. This shows that the shade-dried *Iresine herbstii* leaf powder may be toxic to the kidney since renal diseases which diminish the glomerular filtration leads to urea retention (Ranjna, 1999).

A significant ($p<0.05$) decrease in the serum total cholesterol of the rats fed shade-dried *Iresine herbstii* leaf powder was observed. This could be attributed to its fibre content. Jenkins *et al*. (1978) reported that the fibre content of vegetables and many fruits has been reported to have beneficial effects on blood cholesterol. Consumption of fruits and vegetables lowers total cholesterol (Dragsted *et al*., 2006). Serum LDL-C was increased in all the groups of rats. This shows that the rats were at risk for cardiovascular disease (CVD) and coronary disease. High plasma concentration of LDL-C is a risk factor for CVD (Ademuyiwa *et al*., 2005; Lichtennstein *et al*., 2006). Epidemiological studies have shown that elevated concentration of LDL-C in the blood is a powerful risk factor for coronary diseases (Law, 1999).

Conclusion: The study showed that the fresh and shade-dried *Iresine herbstii* leaf extract and the shade-dried leaf powder is associated with considerable alterations in enzyme activities and is likely to induce tissue damage and lipid profile abnormalities.

Recommendation: Consumption of the extracts or powder of *Iresine herbstii* for the treatment of various ailments should be with extreme caution as they are likely to induce tissue damage and lipid profile abnormalities.

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