Toxic Effects of *Grewia mollis* Stem Bark in Experimental Rats

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Abstract: *Grewia mollis* stem bark used locally in Nigeria as food additive was mixed with the normal diet at 0, 1, 5 and 10% and fed male Wister rats over a four week period. No deaths or remarkable changes in general appearance or behaviour were observed in treated animals. Significant ($p < 0.05$) increases in serum transaminases activities, accompanied by decreased food intake were observed in rats fed the stem bark at 10% dietary level. Treatments had no effect on serum alkaline phosphatase activity, urea, creatinine, triglycerides, cholesterol, glucose concentrations and body and organ weights determined. These findings suggest that dietary exposure of rats to *Grewia mollis* stem bark at high concentrations may cause some adverse effects, especially liver injury.

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1.0 Introduction

Plant materials have been used as alternatives to chemical food additives to enhance the quality of food products. Recently, there has been a general increased interest in the use of natural additives presumably due to their relatively higher availability, affordability, and the perceived lower risk compared to synthetic additives. Consequently, various plant preparations have now been introduced as additives in food. Although generally regarded as safe, plant materials differ in their chemical composition and toxicity resulting from consumption of some plant products have been reported (Burkhard *et al.*,1999;Haller and Benowitz,2000; Nortier *et al.*,2000;Ernst 2002), indicating the need for their continual safety assessment.

Grewia mollis (Tiliaceae) is a shrub or tree widely distributed in northern Nigeria and some African countries. Various parts of the plant are used in food and medicine. In Nigeria, the stem bark powder or mucilage is used as a thickener in local cakes made from beans or corn flour commonly called "Kosai" and "Punkasau" in Hausa (Nigeria), respectively . The dried stem bark is ground and the powder mixed with beans or corn flour thereby enhancing the texture of the food product. Some findings demonstrated that the mucilage obtained from the stem bark can serve as a good binder in paracetamol formulations (Martins *et al.,* 2008 and Muazu *et al.,* 2009). In addition, the mucilaginous property of the bark is used traditionally by the Yoruba people of Nigeria at child birth. Phytochemical studies on the leaves and stem bark of *Grewia mollis* indicated the presence of tannins, saponins, flavonoids, glycosides, phenols, steroids and the absence of alkaloids (Onwuliri *et al.,* 2006). Although *Grewia mollis* stem bark powder or mucilage is widely used in Nigeria, few studies have been reported on the safety of the stem bark to consumers as a food additive. The purpose of this study was to determine the potential toxic effects of *Grewia mollis* stem bark powder in male Wister rats after dietary exposure for four weeks.

2.0 Materials and Methods

2.1 Plant material and preparation of the stem bark powder.

Fresh samples of the stem bark of *Grewia mollis* were collected along Ganye-Sugu road, Adamawa state, Nigeria. The specimens were identified at Forestry Department, Federal University of Technology Yola, Adamawa state, Nigeria. The hard outer part of the stem bark was removed by peeling manually and the remaining inner soft tissue cut into small pieces and dried to constant weight in an oven at about 50^0C. The dried samples were pulverized using pestle and mortar, and sieved to obtain a fine powder that was stored in brown glass containers at room temperature until use in the experimental diet.

2.2 Experimental diet

A pelleted standard diet purchased from Grand cereals Ltd., Jos, Nigeria, was used in the entire study. The diet was made to powder using pestle and mortar to obtain a good homogeneity when admixed with *Grewia mollis* stem bark powder. Diets

containing the stem bark were prepared on a daily basis.

2.3 Animals

Male Wister rats weighing 170±10g were purchased from the animal unit of the National Veterinary Research Institute (NVRI) Vom, Plateau state, Nigeria. The animals were housed in plastic cages, kept at room temperature, allowed free access to water and fed standard diet with or without the stem bark powder *ad libitum*. The animals were allowed to acclimatize for two weeks prior to the commencement of the treatment.

2.4 Experimental design

Rats were randomized and divided into four groups of six animals each and fed diet containing 0, 1, 5 or 10% (w/w) *Grewia mollis* stem bark daily for four weeks. Rats were fasted overnight at the completion of the treatment period. Blood samples were collected from rats by cardiac puncture under diethylether anaesthesia and used for the determination of serum biochemical parameters. Clinical signs and general appearance of animals were observed daily for signs of toxicity. Body weights and food intake were also monitored. The animal weights were measured immediately prior to the commencement of the study at weekly intervals and at the end of the study. Final mean body weights and body weight gain were calculated.

The animal diet was weighed and given to animals daily. Food intake was calculated by subtracting the mass of the feed remaining from the known mass provided to the rats and the food consumption per g/rat/day determined. Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities and serum urea, creatinine, triglycerides, cholesterol and glucose concentrations were assayed using commercial kits (Randox laboratories Co.Antrium, UK) following the manufacturer's instructions. The kidney and liver of all animals were removed immediately after sacrifice and weighed. The relative organ weights for each group were calculated.

2.5 Statistical analysis

All results are expressed as mean ± SEM. The data were analyzed by ANOVA and Dunnett's test (Dunnett, 1955). The level of significance was set at $p<0.05$.

3.0 Results

Clinical signs observed among the rats appeared normal and no deaths were recorded during the four weeks of the experiment. *Table 1,* shows the effects of *Grewia mollis* stem bark administration on some liver marker enzymes in rats. Incorporation of *Grewia mollis* stem bark powder at 10% in the normal diet of rat induced significant ($p< 0.05$) increases in serum transaminases (ALT and AST) activities. There were no significant ($p> 0.05$) differences in serum ALP activity between treated and control animals. The effects of dietary exposure of rats to *Grewia mollis* stem bark on some serum constituents in rats are shown in *Table 2.* No significant ($p> 0.05$) difference in serum urea and creatinine concentrations were observed between control and treated groups. Serum glucose, triglycerides and cholesterol concentrations were also not affected by administration of the stem bark in rats. Mean body weight gain, food intake, absolute and relative organ weight of rats treated with *Grewia mollis* stem bark powder are shown in *Table 3.* Food intake decrease significantly ($p< 0.05$) in animals fed with 10% stem bark powder as compared to control animals. No remarkable changes were observed in mean body weight gain, absolute and relative liver and kidney weights.

Table 1: Effects of *Grewia mollis* stem bark administration on some liver marker enzymes activities in rats.

Dose group (%)	ALT (U/L)	AST (U/L)	ALP (U/L)
0 (control)	74.80±3.20	93.60±2.01	18.58±2.44
1	75.60±2.33	87.80±3.06	21.93±1.84
5	78.76±3.11	96.23±2.67	20.28±2.31
10	94.80±3.21*	107.80±2.75*	19.42±5.61

Tabulated Values are Mean±SEM, n =6

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase

*Significantly different from control group at $p<0.05$

Table 2: Effects of *Grewia mollis* stem bark administration on serum urea, creatinine, triglycerides, cholesterol and glucose concentrations in rats.

Dose group (%)	UREA (mmol/l)	CREATININE (mmol/l)	TRIGLYCERIDES (mmol/l)	CHOLESTEROL (mmol/l)	GLUCOSE (mmol/l)
0 (control)	63.60±3.01	168.56±19.80	5.26±1.98	5.04±1.32	9.56±1.53
1	63.40±4.09	140.75±15.25	4.20±1.91	5.10±1.43	8.71±1.40
5	59.76±5.27	155.08±12.75	3.10±1.34	4.96±1.34	8.98±2.57
10	65.18±3.52	154.23±16.19	4.83±1.77	4.78±1.18	8.72±1.87

Tabulated Values are Mean±SEM, n =6

Table 3: Effects of *Grewia mollis* stem bark administration on body weight gain, food intake, absolute and relative organ weights (g/100g body weight) of rats.

Dose group (%)	Body weight gain (g)	Food intake (g/rat/day)	Absolute organ weight		Relative organ weight	
			Liver	*Kidney*	*Liver*	*Kidney*
0 (control)	44.60±5.20	17.60±2.19	6.58±0.13	1.06±0.02	4.12±0.12	0.67±0.03
1	47.63±4.17	17.73±2.14	6.54±0.16	1.12±0.03	4.20±0.17	0.71±0.02
5	46.41±3.12	15.61±2.56	5.54±0.05	1.06±0.02	3.08±0.05	0.69±0.01
10	39.56±4.23	14.24±2.16*	6.12±0.16	1.12±0.01	4.05±0.18	0.74±0.03

Tabulated Values are Mean±SEM, n =6
*Significantly different from control group at $p<0.05$

4.0 Discussion

The results suggest adverse effects that may involve the liver. Consumption of high concentrations of the *Grewia mollis* stem bark powder resulted in increased serum transaminases (ALT and AST) activities and decreased food intake in rats. Liver cell injury is usually characterised by a rise in plasma transaminases with serum ALT activity more pronounced than AST (Stroev and Makarova, 1989). The elevated levels of ALT and AST activities were considered to reflect the cytosolic release of liver associated enzymes into serum, resulting from the necrotic and degenerative responses of hepatocytes (Lavrijsen, 1992;Kew 2000; Dobbs 2003; Matsumoto, *et al.*, 2006). The absence of significant increases in serum alkaline phosphatase activity may indicate that the stem bark administered to rats had little or no effect on the induction of hepatobilliary distruption (Varley *et al.*, 1980; Somchit *et al.*, 2004).

Since no significant change in serum urea and creatinine concentrations were observed during the course of the study, it was suggested that the administration of *Grewia mollis* stem bark did not interfere with the renal capacity to excrete metabolites. Blood urea and creatinine concentrations are commonly used as indicators of renal injury and are elevated during kidney disease (Imai *et al.*,1981; Narama *et al.*, 1993;Chawla, 1999). Similarly, the non significant changes in serum triglycerides, cholesterol and glucose concentrations suggest normal absorption and metabolism of these substances.

Mean food intake decreased significantly in animals fed with 10% of the stem bark powder. Whether this decrease in mean food intake was due to the toxic effects of the plant or other factors remain to be ascertained. However, incorporation of the *Grewia mollis* stem bark in the fed diet at high concentrations may reduce food palatability and consequently, appetite and food intake in experimental animals. For instance, the presence of some constituents in the stem bark such as tannins (Onwuliri *et al.*, 2006) may cause the observed reduced food intake. Tannins have been shown to reduce feed intake in animals fed tannin diets, which was attributed to the astringent property of tannins and induction of internal malaise in mammals (Hotellier and Delaveau, 1975; Toma *et al.*, 2009). Therefore, the decrease in food intake may not necessarily reflect the severity of the stem bark toxicity. This view is further strengthened by the fact that the body weight gain, absolute and relative liver and kidney weights of the rats were not affected by administration of the stem bark powder.These parameters usually indicate the pathological and

physiological status in man and animals (Ramesh, 2007 and Sellers *et al.*, 2007).

Conclusion

Dietary exposure to *Grewia mollis* stem bark powder at high concentrations (10%) resulted in significant reduction in food intake and elevated serum transaminases activities in rats. It is therefore, reasonable to presume that consumption of the plant material at high concentrations may elicit hepatotoxic effects in rats and possibly in humans.

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