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Effects of Methanolic Extract of *Brassica juncea* Seeds on Biochemical Parameters and Histological Integrity of the Heart and Liver of Albino Rats

Efectos del Extracto Metanólico de las Semillas de *Brassica juncea* sobre los Parámetros Bioquímicos y la Integridad Histológica del Corazón y el Hígado de Ratas Albinas

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SUMMARY: *Brassica juncea* (Indian mustard) seeds are consumed in treatment of high blood pressure, headache and prevention of heart disease. The aim of the present study was to investigate the effects of methanol extract of *Brassica juncea* seeds [BJME] on the heart and liver of adult Albino Wistar rats. A total of 24 albino rats of both sexes were divided into 6 groups [I – VI] of 4 rats per group. Groups I to IV received graded doses of the methanol extract by oral gavage while groups V and VI (controls) received 2 ml/kg body weight of 3 % Tween 80 and water respectively via oral gavage once daily. Treatment lasted for four weeks and the serum levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were estimated. The animals were sacrificed and the heart and liver tissues were excised for further histological processing for light microscopy. There was significant increase in AST and ALT levels following BJME treatment when compared to the controls. ALP activity did not differ significantly among the treatment and control groups. Histopathological changes consistent with toxic injury were observed in the heart and liver tissues of BJME- treated rats. In conclusion, the results of this study show that sub-acute administration of methanol seed extract of *Brassica juncea* can exert cardiotoxic and hepatotoxic effects in rats.

KEY WORDS: *Brassica juncea***; Seeds; histopathology; Heart; Liver; Serum biochemistry.**

INTRODUCTION

Brassica juncea L. commonly known as Indian mustard is a species of Brassicaceae family (Cruciferea) and is well known for its medicinal and nutritive properties (Manohar *et al*., 2009). The plant parts are used traditionally as expectorants, stimulants, diuretics, rubefacient, food flavoring, forage, emetic and as topical treatment for inflammatory conditions such as arthritis and rheumatism (Farrell, 1999; Anuradha *et al*., 2012).

Mustard seeds are a very good source of phosphorus, manganese, dietary fiber, magnesium, selenium, iron, calcium, protein, niacin, zinc and omega-3 fatty acids (Kumar *et al*., 2011). Pharmacological activities of isolated constituents from *B. juncea* including antioxidant, anxiolytic, antitumor, hypoglycemic, antidiabetic, antifungicidal, antimicrobial, goitrogenic and allergenicity activities have been documented (Kumar *et al*.). An acute toxicity study established an oral median lethal dose of 5 g/kg body weight in rats (Duy & Trang, 2016).

In Nigeria, the use of mustard seeds is gaining more popularity as it is believed to proffer 'spiritual protection'. There is thus a need to investigate on the associated risk with its use on some visceral organs due to paucity of scientific literature on sub-acute toxicity studies. The present research, therefore, sought to investigate the effect of prolonged administration of *Brassica juncea* seeds on the heart and liver of albino rats.

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MATERIAL AND METHOD

Plant Material Collection and Preparation. Mustard seeds (*B. juncea*) samples of about 1800 g were purchased from Ogbete Main Market in Enugu, Enugu State, Nigeria. They were ground using gasoline powered grinding machine. Methanol (2.5 L) was added to the ground seed powder which was homogenized by stirring and left for 72 h. The homogenized mixture was then sieved using a muslin cloth and filtered with Whatmann No.1 filter paper. The filtrate obtained was placed inside an oven at 40 ºC for the methanol to evaporate. The residue obtained was weighed and kept in the refrigerator at 4±2 ºC until needed. Prior to administration, reconstitution of the extract was performed by dissolving 50 g of *B. juncea* methanol extract [BJME] in 3 % Tween 80 and made up to 240 ml with the solvent.

Experimental animals. Twenty-four albino rats weighing between 200 g and 225 g were used for the study. The rats were obtained from the Animal House of the College of Veterinary Medicine, University of Nigeria Nsukka and were housed at the College of Medicine Animal House, University of Nigeria, Enugu Campus in well ventilated steel cages to acclimatize for two weeks. The animals were housed under standard laboratory conditions (12h:12h dark-light cycle, room temperature of 23±1 ºC and 55±3 % humidity) and fed with Top feed® and tap water ad libitum.

Experimental design and conduct. The animals were divided into 6 groups [I - VI] of 4 rats each. Groups I - IV served as the treatment groups and received 100, 200, 300 and 400 mg/kg body weight respectively while Groups V and VI (controls) received 2 ml/kg body weight of the vehicle (3 % Tween 80) and water respectively. The treatments were given orally via orogastric tube, once daily for four weeks. All animals were handled in accordance to Institutional approved guidelines for the care and use of animals for scientific research by the University of Nigeria.

Biochemical analysis. At the end of the treatment, blood samples were obtained through retro-orbital puncture of the median canthus of the eye into plain dry bottles. Sera obtained were used for the estimation of serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline Phosphatase levels using standard methods (Cheesbrough, 2006).

Histological processing. The rats were sacrificed under mild chloroform anaesthesia and the heart and liver tissues were excised, fixed with 10 % formal saline for 48 h prior to histological processing using paraffin embedding technique for light microscopy. Sections of 3-5 microns were obtained stained with Haematoxylin and Eosin (H-E) staining technique as described by Baker & Silverton (2014). The sections were examined using an OlympusTM binocular microscope with in-built lighting system.

Statistical analysis. Data was analyzed using the SPSS Inc. software version 20. All data were expressed as the mean value ± SEM. The level of significance was determined by the students' t test while the main effects on treatment groups were determined by the "one-way analysis of variance" (ANOVA). $P < 0.05$ was considered significant.

RESULTS

Effect of methanol extract of *B. juncea* seeds on serum enzyme levels. The mean serum levels of AST, ALT and ALP activities are represented in Table I. Significant differences in levels of AST and ALT were observed among the groups ($p = 0.003$ and 0.021 respectively). Elevated levels of AST and ALT were observed in all BJME – treated groups with most groups showing significant differences when compared with the controls ($p<0.05$). ALP activity did not differ among the groups ($p = 0.622$). Lower doses of BJME appeared to have slightly elevated the ALP levels,

Table I. Effects of Brassica juncea crude methanol seed extract on serum enzyme levels [AST, ALT and ALP] when compared with the controls.

Groups	Treatment	Parameters		
		Aspartate Transaminase (iu/l)	Alanine transaminases (iu/l)	Alkaline phosphatase (iu/l)
I	100 mg/kg *BJME*	38.00±5.83*	89.75±14.10 *	81.68±21.70
II	200 mg/kg *BJME*	35.50±2.99*	51.25±11.32	79.43±20.33
III	300 mg/kg *BJME*	41.50±2.99$*$	82.25±13.55$*$	63.05±14.93
IV	400 mg/kg *BJME*	40.25±2.39$*$	73.75±17.23*	45.90±16.22
V	2ml/kg Tween 80	28.25±3.71	41.50±2.33	57.25±5.48
VI	Water	20.50±0.65	38.75±0.85	64.75±11.83
	F-ratio	5.504	3.525	0.712
	Sig.	0.003	0.021	0.622

Data expressed in mean ± SEM; * and $ - p<0.05 when compared to the groups V and VI respectively

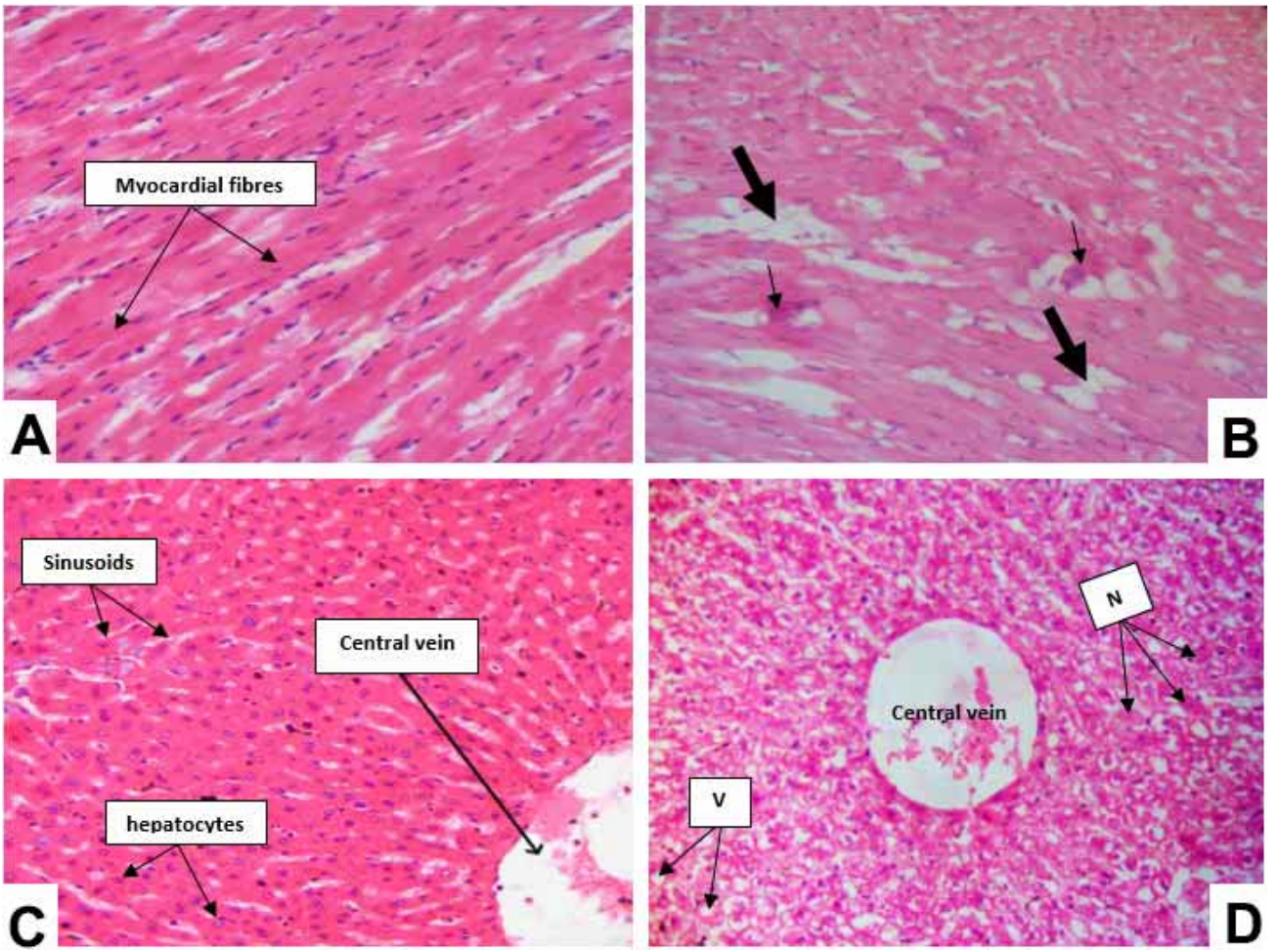

Fig. 1. Light photomicrographs of histological sections from excised organs. A. Heart section from control rat showing normal myocardial fibres; B. Heart section from BJME-treated rat showing distorted histological framework of the myocardium with evidence of vacuolation (thick arrows) and necrosis (thin arrows); C. Liver section from control rat showing normal histoarchitecture of the hepatic tissue with intact central vein, sinusoids flanked by plates of hepatocytes; D. Liver section from BJME-treated rat showing obvious histopathological changes as hepatocytes vacuolation (V) and necrosis (N) [Stain: H&E/Mag. x400].

however, this difference was considered statistically non-significant.

Histological findings. Upon microscopy, histoarchitectural features consistent with normal tissues were observed in the heart and liver control rats (Figs. 1A and 1C, respectively). For BJME- treated rat, the heart and liver sections showed degenerative changes (Figs. 1B and 1D, respectively).

DISCUSSION

The present study highlights that consumption of *Brassica juncea* seeds exerts deleterious effects on the heart and liver histology of rats. This report is similar to that documented earlier on the adverse effects of ethanolic extract of *B. juncea* seeds on the microanatomical structure of kidney and brain tissues of rats treated for two weeks (Inyang *et al*., 2014). Since medicinal plant products are used widely in folk medicine, minimal attention has been given to the possible adverse effects these products may have on the body system. Duy & Trang documented that *B. juncea* seeds contain saponins, flavonoids and tannins as the major phyto-constituents. Mustard oil is well known to contain glycerides of erucic acid which is considered harmful to human health (EFSA Panel on Contaminants in the Food Chain *et al*., 2016). The observed effects in this study may be attributed to a singular or combined effect of these phyto-constituents.

Conflicting findings have been associated with the health benefits of mustard oil on the heart. Alpha-linolenic acid and erucic acid are the beneficial and harmful constituents in mustard oil respectively. The cardioprotective

effect of mustard oil have been attributed to alpha-linolenic acid as it's level is thought to outweigh the harmful effect caused by erucic acid (Rastogi *et al*., 2004), however, few researchers disagree with this reasoning. Our study demonstrated histopathological changes (vacuolations and necrosis) on the heart of the treated rats, even at low doses, which are consistent with toxic injury. Vacuolations occur in spaces of tissues occupied by lipids after treatment with clearing agents during histological processing. High consumption of erucic acid is well known to cause cardiac lipidosis which is due to poor b-oxidation of erucic acid in mitochondria thereby contributing to the accumulation and retention of cardiac lipid (EFSA Panel on Contaminants in the Food Chain *et al*.). This may be the plausible mechanism to the effect observed in the heart tissues in this study.

The results of the biochemical analysis correlate with the lesions observed in the liver histology. High serum activities of the ALT, AST and ALP are associated with hepatic damage. ALT is more liver-specific and is increased together with AST in hepatocellular damage. High AST levels are also seen in myocardial injury. Thus the increased levels of AST and ALT as observed in this work suggest that the extract of *B. juncea* possesses cardiotoxic and hepatotoxic properties. However, the mechanism of action for the observed effects was not elucidated in this study.

CONCLUSION. The present study suggests that prolonged consumption of *B. juncea* seed extract is cardiotoxic and hepatotoxic in rats.

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RESUMEN: Las semillas de *Brassica juncea* (mostaza india) se consumen en el tratamiento de la hipertensión arterial, el dolor de cabeza y la prevención de enfermedades del corazón. El objetivo del presente estudio fue investigar los efectos del extracto de metanol de semillas de *Brassica juncea* [*BJME*] en el corazón y el hígado de ratas Albino Wistar adultas. Un total de 24 ratas albinas de ambos sexos se dividieron en 6 grupos [I - VI] de 4 ratas por grupo. Los grupos I a IV recibieron dosis del extracto de metanol por sonda oral progresivamente, mientras que los grupos V y VI (control) recibieron 2 ml / kg de peso corporal de 3 % de 80 y agua, respectivamente, por sonda oral una vez al día. El tratamiento duró cuatro semanas y se estimaronlos niveles séricos de aspartato transaminasa (AST), alanina transaminasa (ALT) y fosfatasa alcalina (ALP). Los animales se sacrificaron y fueron analizados los tejidos del corazón y el hígado, para un procesamiento histológico adicional con microscopía óptica. Hubo un aumento significativo en los niveles de AST y ALT después del tratamiento con *BJME* en comparación con los controles. La actividad de ALP no difirió significativamente entre los grupos de tratamiento y control. Se observaron cambios histopatológicos compatibles con lesiones tóxicas en los tejidos del corazón y el hígado de ratas tratadas con *BJME*. En conclusión, los resultados de este estudio muestran que la administración subaguda de extracto de semilla de metanol de *Brassica juncea* puede ejercer efectos cardiotóxicos y hepatotóxicos en ratas.

PALABRAS CLAVE: Semillas; *Brassica juncea*; **Histopatología; Corazón; Hígado; Bioquímica sérica.**

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