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Full Length Research Article

THE CHEMOPREVENTIVE PROPERTIES OF ISOTHIOCYANATE ISOLATED FROM THE SEEDS OF *MORINGA OLEIFERA* LAM

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ABSTRACT
Isothiocyanates (ITCs) are a group of plant phytochemicals believed to have numerous therapeutic properties. In the current study, Glucomoringin Isothiocyanate (GMG-ITC) was isolated and purified from the seeds of *Moringa oleifera* Lam. and used to treat Diethylnitrosamine-Induced Liver Cancer in rats. We observed that GMG-ITC treatment attenuated liver damage and significantly prevented the release of liver enzymes into blood plasma. In addition, the treatment significantly increased total antioxidant capacity of liver cancer induced rats that could have decreased the level of alanine amino transferase (ALT) and aspartate amino transferase (AST) in the blood. Thus, we postulate that pure isothiocyanate from the seeds of *M. oleifera* has potential anti-Liver Cancer activity.

Keywords: Isothiocyanate, Liver Cancer, Alanine Transferase

INTRODUCTION

Primary Liver Cancer is the second leading cause of Cancer related deaths and accounts for over 750,000 annual cases globally (Villanueva, 2019). The three major forms of liver cancer include hepatocellular carcinoma (HCC), cholangiocarcinoma and hepatoblastoma, out of which hepatocellular carcinoma is the most common and accounts for over 80% of all liver cancer cases worldwide (Ferlay *et al*., 2010; Nordenstedt *et al*., 2010). The disease is a public health malady that affects quality of life.

In spite of the availability of clinically used drugs such as sorafenib and regorafenib for management of the disease, there is still no cure for liver cancer (Liovet *et al*., 2008; Kudo *et al*., 2018). Thus, identification of alternative remedies that could effectively and completely destroy the liver tumors is fundamental.

Isothiocyanate (ITC) are hydrolysis products of a group of Sulphur containing phytochemicals known as glucosinolates that are found in numerous cruciferous vegetables including but not limited to *Moringa oleifera* Lam., broccoli and sporous (Fahey *et al*., 2001; Amaglo *et al*., 2010; Leone *et al*., 2015). In recent years, GMG-ITCs have majorly been extracted from *Moringa oleifera*, a tree that is native to Africa and Asia and is the most predominant species of the family, *Moringaceae*. The *M. oleifera* tree has obvious significance in African folklore and tradition because of its numerous medicinal properties (Leone *et al*., 2015). Isothiocyanates from the seeds of *M. oleifera* has been used for the treatment of hypertension, bacterial infections and gastric disorders (Anwar *et al*., 2015; Karim *et al*., 2016). More recently, the anti-prostate cancer effect of GMG-ITCs was reported (Jaafaru *et al.,* 2018; Krishna *et al*., 2018).

In the current study, we isolated the ITCs from the seeds of *M. oleifera* and investigated its chemopreventive properties in rodents. To the best of our knowledge, this is the first study to investigate the anti-liver cancer properties of GMG-ITC.

MATERIALS AND METHOD

The seeds of *Moringa oleifera* Lam. were collected from Zaria, Kaduna State and identified by a resident botanist with the Kaduna State University. The plant material was assigned the voucher specimen no, 1121.

Extraction of Glucomoringin

The Glucomoringin-isothiocyanate (GMG-ITC) rich fraction was obtained using a modified protocol of Jaafaru *et al*. (2018). About 10 g of the dried *M. oleifera* seed was ground to fine powder and soaked in 200 mL of 50% methanol (Analytical grade). The solution was vortexed for 15 s, incubated at room temperature for 1 hour and then sonicated at 30^{o}C for 35 mins. The mixture was centrifuged for 30 mins at 5000 rpm to collect supernatant that was loaded on to a column parked with DAEA Sephadex (A-25). About 60 mL of a mixture of 50% methanol, acetic acid and water in the ratio, 1:1:3 was used to flush the column. Thereafter, the GMG-ITC fraction was eluted with 50 mL 5% ethanol in 0.5 M K_2SO_4. The GMG-ITC fraction was dried and purified further using cold ethanol, and dried again on a rotary evaporator at 40^{o}C.

High Performance Liquid Chromatography (HPLC)

The purity of the extracted GMG-ITC was confirmed using High Performance Liquid Chromatography (HPLC) employing sinigrin as standard.

In vivo assay

Experimental animals

About 25 albino rats were purchased and housed in cages in the animal house of the Department of Biochemistry, KASU. The animals had free access to food and water with a 12-h light-dark cycle and were kept to acclimatize for at least 2 weeks before the commencement of the experiment. The animals were grouped into five (5); I: (Negative control), II: (Positive control), III: (100 mg/kg body weight treatment with GMG-ITC), IV: (200 mg/kg body weight treatment with GMG-ITC) and V (300 mg/kg body weight treatment with GMG-ITC). Animals were handled based on the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No 85-23).

Induction of hepatocellular carcinogenesis

Hepatocellular carcinoma was induced by intra peritoneal injection of diethylnitrosamine (DEN) three times consecutively within 2

weeks at a dose of 100 mg/kg body weight of rats. The feed and drinks of the rats was supplemented with 0.02% 2% acetylaminofluorene to quicken the process of hepatocarcinogenesis.

Treatment of animals
Animals in groups III, IV and V were treated respectively, with 100, 200 and 400 mg of the GMG-ITC per kg body weight of rats. The positive control group were treated with doxorubicin (Group 4) while the negative control rats were treated with the vehicle, PBS (Group 5). The treatment was done once daily for 3 days consecutively, and the rats were sacrificed and the blood collected for subsequent analysis.

Aspartate amino transferase (AST) assay
The aspartate amino assay of blood sample of experimental rats was assayed using the Mindray AST reagent according to manufacturer's instruction. The blood was spun to collect serum which was used for the assay. The reaction was run and analyzed using the Mindray Chemistry analyzer (BS-230, China). The analysis was performed in triplicates.

Alanine amino transferase (ALT) assay
The alanine amino assay of blood sample of experimental rats was assayed using the *Mindray* ALT reagent according to manufacturer's instruction. The blood was spun to collect serum which was used for the assay. Similarly, the reaction was run and analyzed using the *Mindray Chemistry analyzer* (BS-230, China). The analysis was performed in triplicates.

Antioxidant assay
The total antioxidant assay was done to monitor the effect of the treatment on the antioxidant level in rats. The assay was performed using Total Antioxidant Capacity (T-AOC) Assay Kit following the FRAP's (2018) method. Briefly, 5 µL each of serum of samples and standard were mixed separately with 180 µL of FRAP working solution in 96 well plate and incubated at room temperature for about 15 mins. The absorbance of the samples was read at 593 nm using a microplate reader (Biorad, USA).

Statistical analysis
Data obtained was reported as mean ± standard deviation. The result were analyzed using one way analysis of variance (ANOVA) on the SPSS software (IBM, USA) *version* 22.

RESULTS

HPLC analysis
The HPLC result for the extracted GMG-ITC and the standard is shown in Figures 1(A) and 1(B) respectively. The peak in Figure 1 (A) confirms that the fraction obtained in this study is rich in Glucomoringin-isothiocyanate. Only GMG-ITC was eluted during the analysis at about 150 seconds and intensity of about 27 mAU.

Aspartate amino transferase
Following treatment with the GMG-ITC, the level of AST significantly reduced significantly ($p<0.05$). The lowest decrease was observed after treatment with GMG-ITC at 400 mg/kg and doxorubicin at 20 mg/kg body weight of the rats (Fig 2).

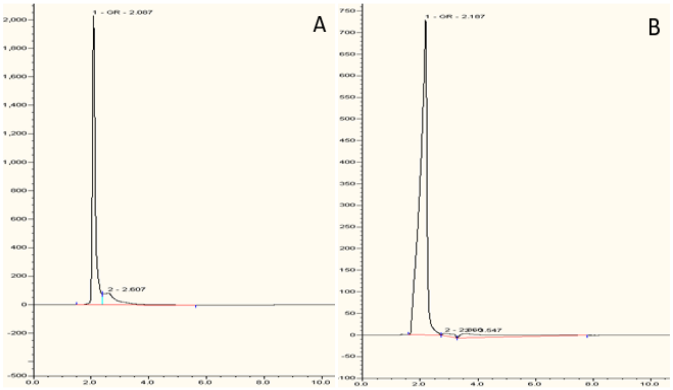

Figure 1: HPLC chromatogram of extracted isothiocyanate (A) and standard sinigrin (B)

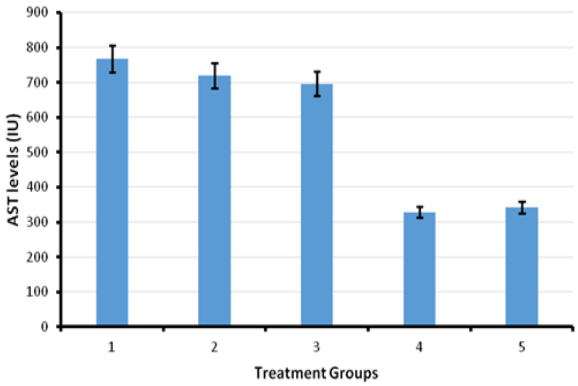

Figure 2: AST level of blood samples after treatment with GMG-ITC and doxorubicin. (Group 1; Negative control, Group 2; 100 mg/kg bw, Group 3; 200 mg/kg bw and Group 4; 400 mg/kg b.w, Group 5; Positive control).

Alanine amino transferase
The treatment of HCC-induced rats significantly reduced ($p<0.05$) the level of ALT in a dose dependent manner (Fig 3). Doxorubicin also significantly reduced the ALT level.

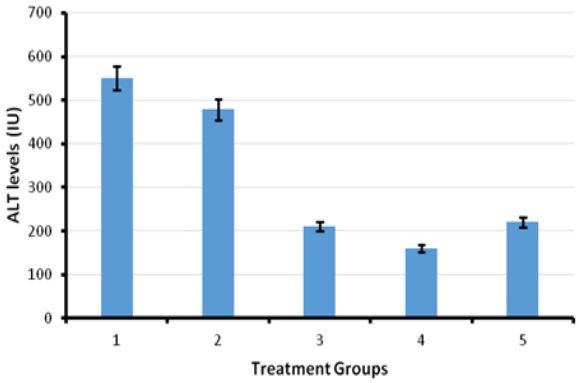

Figure 3: AST level of blood samples after treatment with GMG-ITC and doxorubicin. (Group 1; Negative control, Group 2; 100 mg/kg bw, Group 3; 200 mg/kg bw and Group 4; 400 mg/kg bw, Group 5; Positive control).

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Total Antioxidant assay result

The treatment with GMG-ITC significantly increased ($p<0.05$) the total antioxidant capacity of the rats only at dose of 200 mg/kg body weight. Although insignificant, a decrease in the level of total antioxidant capacity was observed as the treatment dose increased to 400 mg/kg body weight (Fig 4).

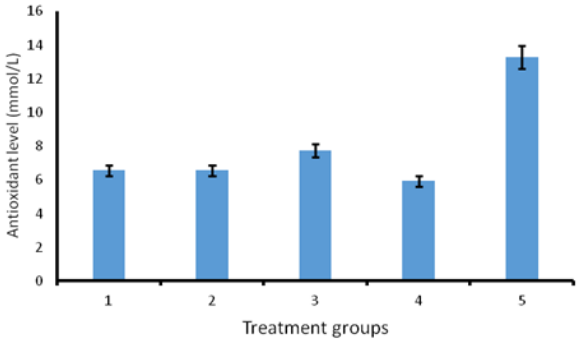

Figure 4: Total antioxidant level of blood samples after treatment with GMG-ITC and doxorubicin. (Group 1; Negative control, Group 2; 100 mg/kg bw, Group 3; 200 mg/kg bw and Group 4; 400 mg/kg bw, Group 5; Positive control).

DISCUSSION

Numerous studies have shown that certain phytochemical in plants have strong anti-cancer effects (Kawasaki *et al*., 2008). Glucomoringin Isothiocyanate (GMG-ITC) for instance, modulate cell signaling, and help to inhibit the proliferation of some tumor cells (Cheung and Kong, 2010). Another study by Wang *et al*. (2014) and Ju *et al*. (2016) showed that GMG-ITC causes apoptosis of cervical and colon cancers respectively. Based on these findings, we decided to investigate the antitumor and chemopreventive properties of GMG-ITC in diethylnitrosamine-induced hepatocellular carcinoma in rats. Our findings show that the extracted ITC inhibited the growth of liver cancer cells. Diethylnitrosamine causes Liver cancer with consequent elevation of alanine amino transferase (ALT) and aspartate amino transferase (AST) levels (Al-Rejaie *et al*., 2009). The severity of damage determines the amount of ALT and AST released into the plasma. In the current study, the amount of ALT and AST increased by over 20 folds following damage caused by the induction of liver cancer in the rats. However, this elevated level of ALT and AST significantly reduced ($p<0.05$) after treating the rats with GMG-ITC isolated from *Moringa oleifera*. By preventing liver damage, the pure GMG-ITC used in this study decreased the amount of ALT and AST released into the blood. The bioactivity of the GMG-ITC increased in a dose dependent manner.

Furthermore, GMG-ITC treatment at a dose of 200 mg/kg body weight of rats caused a significant increase in the total antioxidant capacity of the rats. In a similar study, another strong antioxidant, *Tragopogon porrifolius* was reported to inhibit the proliferation of breast and colon cancer cells (Tenkerian *et al*., 2015). This treatment with GMG-ITC may have triggered the production and activity of the antioxidants as observed in this study. This we presume is one of the mechanisms through which ITC from the seeds of *M. oleifera* contribute to the chemopreventive effects in the liver cancer induced rats.

In conclusion, the findings of this study shows that GMG-ITC isolated from the seeds of *M. oleifera* have potential chemopreventive properties in liver cancer induced rats.

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