Research Paper

Chemoprevention of colonic aberrant crypt foci by *Gynura procumbens* in rats

Abdrabuh N. Shwtera, Nor Azizan Abdullahb, Mohammed A. Alshawshb, Abdulsamid Alsalahi, Maryam Hajrezaeia, Amel A. Almaqramp, Sameer D. Salemc, Mahmood A. Abdulla

a Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
b Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
c Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

**A R T I C L E   I N F O**

Article history:
Received 3 October 2013
Received in revised form 25 November 2013
Accepted 20 December 2013
Available online 3 January 2014

**Keywords:**
Gynura procumbens
Azoxymethane
Aberrant crypt foci
Antioxidant activity
PCNA
Bcl-2

**A B S T R A C T**

**Ethnopharmacological relevance:** *Gynura procumbens* is commonly used as a traditional medicinal plant in Malaysia for treatment of many diseases. To investigate the chemopreventive properties of *Gynura procumbens* on azoxymethane (AOM)-induced aberrant crypt foci (ACF) in rats.

**Methods:** Five groups of adult male rats were used in this experiment. Normal/control group: the rats were injected subcutaneously with 15 mg/kg of sterile normal saline once a week for two weeks, and orally administered with 10% Tween 20 (5 mL/kg). Carcinogen and treatment groups: the rats were injected subcutaneously each with 15 mg/kg body weight AOM once a week for 2 weeks and were continued to be fed for two months, respectively with 10% Tween 20, 500 and 250 mg/kg body weight plant extracts. Reference group: the rats were injected subcutaneously with 15 mg/kg body weight AOM once a week for 2 weeks, and injected intraperitoneally with fluorouracil 35 mg/kg body weight for five consecutive days.

**Result:** Total ACF detected in methylene blue stained whole mounts of rat colon were 21, 23 and 130 in rats fed with 500, 250 mg/kg body weight treatment and carcinogen groups, respectively. Treatment with high and low doses of the plant extract led to 83.6% and 82.2% decrease in the total crypts in the groups fed 500 mg/kg and 250 mg/kg *Gynura procumbens* respectively compared to carcinogen group. Immunohistochemical staining of ACF showed suppressed azoxymethane induced colonic cell proliferation and Bcl-2 expression. Glutathione-S-transferase and superoxide dismutase activities were higher in treated rats compared to carcinogen groups.

**Conclusion:** *Gynura procumbens* reduced the incidence of AOM induced ACF. The findings showed that *Gynura procumbens* may have antiproliferative and antioxidative properties. Moreover, *Gynura procumbens* possesses the medicinal properties to prevent colon cancer.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Colon cancer is the third-leading cause of cancer deaths worldwide (Yamashita et al., 1994). It is estimated that about one million new cases of colorectal cancer (CRC) are diagnosed, and about half a million deaths caused by colon cancer occurred each year worldwide (Tenesa and Dunlop, 2009). In 2012, there were approximately 103,170 cases of colon cancer and 40,290 cases of rectal cancer are reported (Saslow et al., 2012). Recently, the use of herbal medicines for complementary treatments of some diseases has been popular and researchers have shown that medicinal plants are commonly used by cancer patients to manage their diseases (Riboli and Norat, 2003; Van Duijnhoven et al., 2009). *Gynura procumbens* (Lour.)Merr. Compositae is an annual evergreen shrub with a fleshy stem and purple tint. In Malaysia (Lour.) Merr. Medicinal plant is called as *Sambung nyawa*, and it is commonly used in South-East Asia, particularly in Malaysia, Indonesia, and Thailand. It has been widely used for the remedy of rash, eruptive fevers, migraines, kidney disorder’s, constipation, mellitus, diabetes hypertension, and cancer (Perry and Metzger, 1980). Pharmacologic studies have shown that *Gynura procumbens* possesses anti-herpes simplex virus, (Nawawi et al., 1999), anti-inflammatory (Iskander et al., 2002), anti-ulcerogenic activities (Mahmood et al., 2010) and anticancer properties (Agustina et al., 2006). The advantages
of the traditional medicinal use of *Gynura procumbens* have supported by the investigation of possible pharmacologically active ingredients containing sapnins, flavonoids and terpenoids (Akowuah et al., 2002). Previous studies reported that consumption of the ethanol extract of *Gynura procumbens* leaves inhibits tongue carcinogenesis in rats (Agustina et al., 2006). Moreover, it showed an inhibitory effect on the carcinogenicity of mice lung tumour induced by benzo (α)pyrene (BAP) (Nisa et al., 2012). A previous in vitro study showed that the acetate fraction of *Gynura procumbens* leaves had a cytotoxic effect on breast cancer cells (Jenie et al., 2007). Studies about the effects of *Gynura procumbens* on colon cancer are scarce, particularly dietary chemoprevention studies with respect to colon carcinogenesis. The major problem of chemotherapy treatment its side effect which may result in other undesirable effects to human body such as hairfall, skin getting darker (Valeriote et al., 2002; Jiang et al., 2004). This problem has lead to the use of medicinal plants that have less side effects (Sugiyanto et al., 2003). The purpose of the present study was to investigate the chemopreventive properties of *Gynura procumbens* leaves on azoxymethane (AOM)-induced aberrant crypt foci (ACF) in Sprague-Dawley rats. This may help in the development of subsequent research in cancer prevention.

2. Materials and methods

2.1. Plant sample and extract preparation

Fresh leaves of *Gynura procumbens* plant were purchased from Ethno Resources SdnBhd, Malaysia, and the voucher specimen was deposited at the Herbarium of Rimbailumu, University of Malaya, Kuala Lumpur. The leaves were washed by tap water and then dried up; ground into a powder by electrical blender. One hundred grams of the powder were soaked in 900 mL of ethanol (95%) for 48 h. Subsequently, the ethanol extract was filtered using (Whatman No. 1) filter paper and evaporated to obtain the crude extract using (Buchi) type rotary evaporator. The percent yield of *Gynura procumbens* ethanol extracts was 4.0% (w/w). The ethanol extract was dissolved in 10% Tween 20 and feed by oral route to the rats in a dosage of 250 and 500 mg/kg body weight respectively.

2.2. Chemicals

Azoxymethane (AOM) is a colon carcinogen and commonly used to induce aberrant crypt foci in the rat colon (Sigma-Aldrich, Switzerland) in 100 mg vial and stored at −20 °C until further use. Before administration, it was diluted with normal saline to 10 mL and given subcutaneously at 15 mg/kg body weight to the rats once a week for two weeks (Andersson et al., 2008). 5-Fluourouracil (Sigma Chemical Co., St. Louis, MO, USA) as standard drug was dissolved in normal saline and injected intraperitoneally in the rats at a dosage of 35 mg/kg body weight (Tanaka et al., 2001).

2.3. Antioxidant measurement in vitro

2.3.1. Total phenolic content

(TPC) of the plant extract was assessed according to the Folin–Ciocalteu spectrophotometric method with slight modifications (Gan et al., 2010). Briefly, the measurement was performed by mixing equal volumes of sample (1 mg/mL DMSO) and 10% Folin–Ciocalteu reagent in a 96-well plate, incubated for 5 min then 10% sodium carbonate solution was added. After 90 min incubation, the TPC was evaluated at 750 nm and the measurement of TPC was compared to a standard curve of Gallic acid (GA) solution, and TPC was expressed as milligrams of Gallic acid equivalents (GAE) per gram of plant extract (mg GAE/g db) (AOAC, 1995).

2.3.2. Total flavonoids measurement was assessed by using the aluminum chloride colorimetric protocol

Sample was prepared 1 mg/mL DMSO and mixed with equal volume of 0.2% AlCl₃. After 10 min incubation the supernatant placed on 96-well plate and read at 510 nm. The measurement of total flavonoids was expressed as quercetin equivalents in mg (QE)/g of plant extract. The assay was carried out in triplicates (Dowd, 1959).

2.4. Acute toxicity study

To evaluate a safe dosage for the *Gynura procumbens*, a total of 36 healthy Sprague Dawley (SD) rats (18 females and 18 males) were purchased from the animal experimental unit, Faculty of Medicine. They were allocated equally into three groups and treated with vehicle (Tween20), 2 g/kg or 5 g/kg of *Gynura procumbens* preparation, respectively. After overnight fasting, the rats were administered appropriate doses, and observed for 30 min, 2, 4, 8, 24 and 48 h for behavioural and toxicological signs. No death occurred during the period of 14 days. Animals were fasted on day 14th and sacrificed on day 15th under overdose of ketamine and xylazine. Biochemistry and histology parameters were examined according to standard protocol.

2.5. Experimental animals for colon chemoprotective

Adult healthy male SD male rats (120–150 g weight) were purchased from animal experimental unit. Thirty rats (six-weeks old) were randomly allocated into 5 groups of 6 rats each. The animals were housed individually in separate cages and were fed with rat’s pellet and allowed excess of tap water. The protocol of this experiment was accepted by committee members’ ethics for laboratory animal experimentation Ethic No. PM/07/05/2012/ MMA (b) (R).

2.5.1. Experimental protocols

A total of 30 healthy male SD rats were divided randomly into 5 groups. Group 1 rats, which served as the control/normal group received subcutaneous injections of normal saline (5 mL/kg) and orally administered with (5 mL/kg) 10% Tween 20 daily for 10 weeks.

Group 2 received subcutaneous injections of AOM at a dosage of 15 mg/kg body weight, once per week, for two consecutive weeks (Robles et al., 2010). They were daily gavage with 10% Tween 20 (5 mL/kg) daily for 10 weeks and served as carcinogen groups.

Groups 3 and 4 (*Gynura procumbens* treatment groups) received subcutaneous injections of AOM15 mg/kg body weight once per week, for two consecutive weeks and administered orally with the *Gynura procumbens* ethanol extract 500 mg/kg and 250 mg/kg body weight, respectively, daily for 10 weeks. Group 5 (reference group) rats were injected subcutaneously with AOM 15 mg/kg once per week, for two consecutive weeks and injected intraperitoneally with 35 mg/kg body weight daily of 5-FU for five consecutive days (Tanaka et al., 2001). All rats were weighted biweekly during the experiment.

2.5.2. Gross evaluation of clone mucosa

The rats were sacrificed after 10 weeks under high dose of Xylazine (5 and 10 mg/mL) and Ketamine (50 and 100 mg/mL) anaesthesia. Complete autopsies were performed and the colons from caecum to rectum were immediately removed, gently flushed
with PBS, and open longitudinally. Ultimately, the tissue samples were fixed in phosphate buffered formalin and stained with methylene blue (0.2%) in phosphate buffered saline for 20 min at room temperature and rinsed twice in phosphate buffered saline, using a light microscope to score based on the number of ACF, which are defined as foci containing more than two aberrant crypts. The number of ACF per colon and the number of aberrant crypts in each focus were determined with the aid of a light microscope. ACF were readily identified from zone relative to normal crypts by their swelling and noticeable pericryptal zone (Kawamori et al., 1994). Scoring was based on number of ACF identified in colon.

2.5.3. Histopathological examination

Colon tissues were cut, and samples were fixed in 10% buffered formalin for 24 h and processed by programmed tissue processing automated machine. The colon sections were embedded in paraffin and tissue blocks sectioned at thickness of 5 μm and stained with routine hematoxylin and eosin to evaluate tumor histology.

2.5.4. Immunohistochemical staining

Immunohistochemical analysis was performed using the commercial kit of streptavidin–biotin and peroxidase protocol according to the manufacturer’s structure (Dako ARK™ USA) to detect proliferating cell nuclear antigen (PCNA), and Bcl-2 protein. Tissue sections were fixed with 10% phosphate buffered formalin, dehydrated using series grade of ethanol, and cut at 5 μm sections. These sections were deparaffinised and rehydrated in graded series of ethanol. Then, incubated in a microwave for antigen retrieval using (10 mM sodium citrate buffer). The processed tissues were then rinsed in phosphate buffered saline (PBS) and endogenous peroxidase, and were blocked using 0.3% H₂O₂ for 20–30 min. Tissue samples were washed gently using wash buffer and incubated with PCNA (1:100) and Bcl-2 (1:100) biotinylated primary antibodies for 15 min and washed with PBS. Then, the samples were kept in buffer bath in a humid chamber. Streptavidin-peroxidase (streptavidin conjugated to horseradish peroxidase in PBS containing an anti-microbial agent) was added and then incubated for 15 min followed by gentle rinsing. Diaminobenzidine (DAB)-substrate chromagen was added to the samples, then incubated for over 5 min followed by rinsing with phosphate buffered saline (PBS) and endogenous peroxidase, and were blocked using 0.3% H₂O₂ for 20–30 min. Tissue samples were washed gently using wash buffer and incubated with PCNA (1:100) and Bcl-2 (1:100) biotinylated primary antibodies for 15 min and washed with PBS. Then, the samples were kept in buffer bath in a humid chamber. Streptavidin-peroxidase (streptavidin conjugated to horseradish peroxidase in PBS containing an anti-microbial agent) was added and then incubated for 15 min followed by gentle rinsing. Diaminobenzidine (DAB)-substrate chromagen was added to the samples, then incubated for over 5 min followed by rinsing with phosphate buffered saline and immersed in hematoxylin for 5 s. The slides were rinsed then dipped for 10 times in 0.037 M/L of ammonia. Negative control sections were processed similarly but with the omission of the primary antibodies. The slides were rinsed in a bath of deionized water for 2–5 min. Positive antigens given brown staining under optical microscope. The PCNA labeling index (PI) was calculated as the [(number of positive cells)/(total number of cells)] × 100 for each field, which is averaged to get the PI for each section (Yamashita et al., 1994).

2.6. Antioxidant activity

One gram of colon tissue was mixed with 10 mL of phosphate buffer solution (10% w/v), then tissue homogenized and centrifuged at 4000 rpm for 10 min at −4 °C. The colon homogenate supernatant was used in the measurement of thiobarbituric acid reactive substances TBARS assay that measured malondialdehyde (MDA) for lipid peroxidation levels. Glutathione-S-transfer (GST) and superoxide dismutase (SOD) were also assessed in colon tissue homogenate. These enzymes were selected due to their role in chemoprevention (Asiamah et al., 2011). All measurements were performed by using commercial kits (Cayman Chemical Company, U.S.A).

2.7. Biochemical analysis

Blood were collected from animals in gel activating tubes and centrifuged at 3400 rpm for 10 min. The sera was separated and sent for the determination of glucose, albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatinine and urea levels. These enzymes were measured by standard automated techniques in the Central Diagnostic Laboratory.

2.8. Statistical analysis

Statistical analysis of the values was performed using SPSS (version 19). One-way ANOVA followed by Tukey’s post-hoc test were used to compare the measurements between groups. The data were examined for normality and reported as the mean ± standard deviation of the mean. All values at P < 0.05 were considered significant.

3. Results and discussion

3.1. Antioxidant in vitro result

Total phenolic content of the ethanolic extract of Gynura procumbens were estimated by the Folin–Ciocalteu colorimetric method using gallic acid to generate the standard curve and was determined to be 64.7 mg GAE/g of ethanol extracts (standard curve equation: y = 0.0077x + 0.0036, R² = 0.994). At the same time, flavonoids were 40.7 ± 1.94 mg (Quercetin equivalents) per g of ethanol extracts) and a ratio flavonoids/phenolics of 0.62. Thus, phenolic compounds were the predominant antioxidant constituents in Gynura procumbens extracts, which lead to more potent radical scavenging effects. Phenolic may inhibit cancer through different mechanisms such as antioxidant effects, activation of detoxifying enzymes and increasing cell to cell communication (Malin et al., 2003). In the current study, was showed that Gynura procumbens has phenolic content, indicating that it may play a role in the cancer prevention.

3.2. Acute toxicity test

No mortality was observed in the animals that were orally administered Gynura procumbens extract at doses 2 g/kg and 5 g/kg. In addition, there were no visible manifestations of hepatotoxic and
These histopathological data between carcinogen group and treated groups. Kidney were weighed after rats were sacrificed. Even at the highest dosage administered. These findings indicated that the oral gavage of Gynura procumbens extract was concluded to be safe, and there were no kidney and liver toxicity. Based on outward abnormal behavior at these doses. Thus there were no significant differences in biochemical and histopathological data between carcinogen group and treated groups. These findings indicated that the oral gavage of Gynura procumbens extract was concluded to be safe, and there were no kidney and liver toxicity detected even at the highest dosage administered.

3.3. Body and organs weight

Body weight of all rats were taken at the beginning (0 week) of the experiment then weekly up to 10 weeks. Colon, liver and kidney were weighed after rats were sacrificed. Although there are differences in weight between groups, but these differences were not statistically significant.

3.4. Effects of Gynura procumbens on the incidence of ACF in male rats

The incidence of aberrant crypt foci is shown in Table 1. Rats fed with Gynura procumbens showed significantly lower ACF numbers compared with carcinogen groups (P < 0.001). In all experimental groups, ACF incidence in the distal colon was significantly higher compared with proximal colon (P < 0.001). However, the number of ACF in both the distal and proximal colon in carcinogen group (130.60 ± 21.93) were significantly higher compared to the 500 mg/kg Gynura procumbens, 250 mg/kg Gynura procumbens and reference groups (21.40 ± 7.09,23.20 ± 4.7, and 32.0 ± 2.2, respectively; P < 0.001). There are about 83.6% and 82.2% reduction in the total crypts in the groups fed with 500 mg/kg and 250 mg/kg Gynura procumbens, respectively, compared to carcinogen group. Previous studies have approved the use of ACF as a biomarker indicator in colon cancer (Boateng et al., 2007). ACF has been reported by Bird in 1987 in rodents injected with AOM, and similar lesions were described in humans in 1991 by Pretlow. Since then, the AOM-induced ACF model has been the most widely used animal model for colon cancer (Zhang et al., 2000). Due to the presence of phytochemical content in Gynura procumbens extract, this plant might be able to reduce the incidence of ACF and prevent cancer development (Agustina et al., 2006).

Table 2

Effects of Gynura procumbens on the crypt multiplicity in male rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of foci containing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Crypt</td>
</tr>
<tr>
<td>15 mg/kg AOM (carcinogen group)</td>
<td>13.0 ± 7.4</td>
</tr>
<tr>
<td>AOM + 500 mg/kg Gynura procumbens</td>
<td>3.0 ± 1.9*</td>
</tr>
<tr>
<td>AOM + 250 mg/kg Gynura procumbens</td>
<td>2.4 ± 1.7*</td>
</tr>
<tr>
<td>AOM + (5-FU) 35 mg/kg</td>
<td>8.0 ± 4.3*</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation. * Significant difference at a level of (P < 0.001) (ANOVA, Tukey).

4.1. Effects of Gynura procumbens on the crypt multiplicity in male rats

The carcinogen group had a higher incidence of ACF with larger crypts compared to the experimental groups (Table 2).

3.5. Effects of Gynura procumbens on the antioxidant in vivo in AOM-induced colon cancer rats

3.5.1. Effects of Gynura procumbens on the GST in AOM-induced colon cancer rats

Glutathione-S-transferase is a phase II detoxification enzyme. Our result showed that colon GST activity (nmol/min/mL) was significantly high in the rats fed with 500 mg/kg and 250 mg/kg Gynura procumbens followed with the carcinogen group fed rats; 34 ± 5.8, 26.398 ± 0.92, 16.65 ± 4.57, respectively, P < 0.05 (Fig. 1). One of the most common known chemopreventive activities, the indirect defensive mechanism of phytochemicals is the induction of phase II metabolizing enzymes such as glutathione-S-transferase (GST). One of the strategies for polyphenol to prevent cancer is that might be interact with phase I and phase II enzyme systems, phase II enzymes catalyze the conjugation reactions between glutathione and Phase I reactive electrophilic intermediates to facilitate their elimination from the body (Wilkinson and Clapper, 1997; Moskaug et al., 2005). Our results are also consistent with previous studies where GST activity was significantly induced when the rats were administrated with (prebiotics + SM), flax seed...
meal and silymarin (Kohno et al., 2002; Williams et al., 2007; Gourineni et al., 2011).

3.5.2. Effects of Gynura procumbens on the SOD in AOM-induced colon cancer rats

Superoxide dismutase activity (U/mL) ranged from a high of 11.55 ± 0.331 in rats fed with Gynura procumbens 250 mg/kg and 8.41 ± 0.941 in rats fed with Gynura procumbens 500 mg/kg to low 5.87 ± 0.311 in carcinogen group rats fed with AOM (Fig. 2). The results showed that rats fed with treatment diets showed significantly higher superoxide dismutase activity (SOD) (U/mL) compared with the carcinogen group (Fig. 2). Superoxide dismutase catalyzes the dismutation of superoxide anion radical, which are potent carcinogens (Hei and Filipic, 2004). They are converted to hydrogen peroxide (H₂O₂) and molecular oxygen. SOD work in sync in the prevention of ACF formation, where the H₂O₂ produced by the SOD is further reduced to molecules of water by catalase. The results of current study were similar to the previous studies that showed a significant lower activity of SOD enzyme in different tumor cell lines (Oliva et al., 1997; Sanchez et al., 2006).

3.5.3. Effects of Gynura procumbens on the MDA in AOM-induced colon cancer rats

Malondialdehyde level (MDA) in carcinogen group was significantly higher compared to treatment groups Gynura procumbens 250 mg/kg and 500 mg/kg (Fig. 3). TBARS assay indicated the level of lipid peroxidation that is present by measuring the level of MDA in all groups. In this study, there was a significant reduction of MDA in groups treated with Gynura procumbens ethanol extract both doses compared to the untreated AOM-induced group (carcinogen group). This reduction in the MDA level of extract-treated rats was comparable with 5-FU treated group, suggesting that the Gynura procumbens reduce lipid peroxidation and protected colon cells as 5-FU. A reduced lipid peroxidation is in turn associated with a reduction in oxidative stress. Oxidative stress play a significant role in the molecular mechanism of CRC initiation as well as progression (Seril et al., 2003). The increase of the oxidation products MDA in tumor tissue is accompanied by a significant decrease of the antioxidant enzyme SOD (Oliva et al., 1997; Sanchez et al., 2006).

3.6. Detection of ACF by methylene blue stain

The aberrant crypt foci were visualized and counted using methylene blue (0.2%). The ACF were slightly elevated above the surrounding epithelial mucosa and demonstrated characteristic oval or slit-like opening. Some ACF were stained more densely by methylene blue and detected as almost normal-sized lesions (Fig. 4).

3.7. Histopathological study

The histological features of dysplastic ACF and normal colon cells have also been illustrated by H&E staining. Histology section of normal colon and treated groups showed normal architecture of the mucosal and submucosal layers. AOM-induced ACF group showed epithelial mucosal glands proliferation with severe dysplastic alteration changes which represent transformation to carcinoma (Fig. 5).
3.8. Immunohistochemical staining

PCNA and Bcl-2 staining of colons: The proliferating cell nuclear antigen (PCNA) was evaluated as an important marker for cell proliferation in the colon sections, and the results showed that the carcinogen group was much higher expression of PCNA protein than treated groups (Fig. 6). The percentage of PCNA-positive cells of the colon sections in the carcinogen group were 48.83%, whereas PCNA-positive cells (%) from the dose *Gynura procumbens* fed 500 mg/kg, 250 mg/kg and reference groups were 17%, 20% and 10%, respectively. Immunohistochemical staining of Bcl-2 protein demonstrated that rats in the treated groups had low expression of Bcl-2 protein with respect to AOM group that showed higher expression (Fig. 7). The cellular proliferation rate plated a significant prognostic marker for variety of cancer disorders, including colon cancer (Violette et al., 2002; Yamaguchi et al., 2003). The immunohistochemical staining assay of PCNA, and Bcl-2 proteins was carried out to assess the proliferation and apoptotic alterations of colorectal mucosa. Our results showed that *Gynura procumbens* ethanol extract suppressed the expression of PCNA. Most of the possible chemopreventive drugs used against chemically induced colon carcinogenesis inhibit the proliferation of cell activity through PCNA index (Tanaka et al., 2001). Previous studies reported such findings during the administration of AOM (Yamashita et al., 1994; Tanaka et al., 1999). Our findings also showed increased expression of PCNA, and this may reflect an increased cell proliferation in colon tumors.

The anti-apoptotic protein Bcl-2 was down-regulated in all treated groups. Enhanced Bcl-2 expression has been observed in rats with chemically induced colonic adenocarcinomas. The anti-apoptotic function of bcl-2 oncogene may be played important role in tumorigenesis by raising the threshold for apoptosis (Adams and Cory, 1998). Our findings are in agreement with similar studies
on bcl-2 expression in human normal mucosa and colorectal cancer (Maurer et al., 1998).

3.9. Effect of Gynura procumbens on biochemical parameters

There were no statistically significant differences in the level of urea, creatinine, total protein and (ALT, AST, ALP) for the AOM group compared with the normal and treated groups (Tables 3 and 4).

4. Conclusion

According to the results of this study, it can be expected that Gynura procumbens may have antiproliferative and antioxidative properties. Reduction in ACF incidence could be due to the direct effects of treatment diets indirect mechanism such as stimulating of detoxifying (GST) and antioxidative enzymes or by acting as antiproliferative. Gynura procumbens proves to be a medicinal plant which possesses the medicinal properties to prevent colon cancer as results from this study have made obvious.

Acknowledgments

The authors express their gratitude to the staffs of the Faculty of Medicine Animal House for the care and supply of rats. This research was supported by the University of Malaya Grant (PV046/2012) and University of Malaya High Impact Research Grant (UM/M0HE/HIR Grant E000045-20001).

Table 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total bilirubin (μmol/L)</th>
<th>Total protein (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 20 (10%)</td>
<td>49.6 ± 3.3</td>
<td>168 ± 2.2</td>
<td>130.6 ± 3.8</td>
<td>2.8 ± 0.20</td>
<td>64.2 ± 0.66</td>
</tr>
<tr>
<td>Gynura procumbens 500 mg/kg</td>
<td>61.00 ± 12.97</td>
<td>189.4 ± 3.6</td>
<td>149.2 ± 10.2</td>
<td>2.6 ± 0.24</td>
<td>67.6 ± 1.0</td>
</tr>
<tr>
<td>Gynura procumbens 250 mg/kg</td>
<td>61.6 ± 12.9</td>
<td>161.8 ± 26.7</td>
<td>161 ± 26.6</td>
<td>2.4 ± 0.24</td>
<td>70 ± 0.83</td>
</tr>
<tr>
<td>15 mg/kg AOM (carcinogen group)</td>
<td>47.6 ± 8.2</td>
<td>203 ± 23.43</td>
<td>106.8 ± 24.7</td>
<td>2.4 ± 0.54</td>
<td>64.4 ± 1.8</td>
</tr>
<tr>
<td>AOM + (5-FU) 35 mg/kg</td>
<td>53.2 ± 7.25</td>
<td>163.8 ± 29.1</td>
<td>135.4 ± 13.7</td>
<td>2.4 ± 0.55</td>
<td>66.4 ± 3.7</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation. No significant difference at ( P < 0.05) (ANOVA, Tukey).

Table 4

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sodium (mmol/L)</th>
<th>Potassium (mmol/L)</th>
<th>Urea (mmol/L)</th>
<th>Creatinine (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 20 (10%)</td>
<td>144.6 ± 1.1</td>
<td>4.62 ± 0.002</td>
<td>5.06 ± 0.22</td>
<td>28.8 ± 4.66</td>
</tr>
<tr>
<td>Gynura procumbens 500 mg/kg</td>
<td>140.4 ± 3.8</td>
<td>4.46 ± 0.12</td>
<td>6.04 ± 0.4</td>
<td>29.6 ± 3.17</td>
</tr>
<tr>
<td>Gynura procumbens 250 mg/kg</td>
<td>141 ± 0.4</td>
<td>4.85 ± 0.15</td>
<td>5.98 ± 0.52</td>
<td>28.8 ± 2.6</td>
</tr>
<tr>
<td>15 mg/kg AOM (carcinogen group)</td>
<td>138 ± 3.7</td>
<td>4.5 ± 0.16</td>
<td>5.01 ± 0.51</td>
<td>28.8 ± 6.01</td>
</tr>
<tr>
<td>AOM + (5-FU) 35 mg/kg</td>
<td>140.8 ± 3.5</td>
<td>4.3 ± 0.27</td>
<td>5.5 ± 0.7</td>
<td>32.6 ± 2.5</td>
</tr>
</tbody>
</table>

Values expressed as mean ± standard deviation. No significant difference at ( P < 0.05) (ANOVA, Tukey).

Fig. 7. Expression of Bcl-2 protein in the colon tissue of rats. Expression of Bcl-2 protein in the colon tissue of rats. (a) Normal group, (b) carcinogen group (c) 5-FU treated group (d), and (e) high and low doses treated groups. Immunohistochemical analysis of Bcl-2 protein showed down-expression of Bcl-2 protein in rats treated with 5-FU and plant extract comparing to carcinogen group.
References


Silymarin, a naturally occurring polyphenolic antioxidant protocatechuic acid. Cancer Sci. 85, 686–691.


