www.jnsd.tums.ac.ir

Original Article Open Access

Orthosiphon stamineus: an Asian tea with substantial anticancer properties

Ariyo Movahedi[a], Rusliza Basir[b], Asmah Rahmat[a], Mohammad Charaffedine[c], Fauziah Othman[*b]

[a] Department of Nutrition & Dietetics, Faculty of Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[b] Department of Human Anatomy, Faculty of Medicine, University Putra Malaysia, Serdang, Selangor, Malaysia
[c] Diseased Tissue Pathology Lab, Tyre, Lebanon

Article History
Received:
19/04/2014
Revised:
21/5/2014
Accepted:
15/6/2014

Keywords:
Cell proliferation,
Glucocorticoid,
Cell receptors,
Phytochemicals,
Caspase

A B S T R A C T

Background: Liver cancer is one of the deadly cancers with high prevalence in the East Asia. Likewise most of diseases, herbs and herbal medicine could be an easy and cost effective tool in prevention and possible cancer treatment. The present study investigated the ability of *Orthosiphon stamineus* Benth decoction to protect liver against hepatocellular carcinoma in carcinogenesis-induced animal model.

Methods: Forty male Sprague Dawley rats (age: 8±1 weeks, weight: 248.1±7.21g) were obtained and 10 rats were kept as normal group. Hepatocellular carcinoma was induced for the rest 30 of rats by means of intraperitoneal injection of 200mg/kg diethyl nitrosamine (DEN) dissolved in corn oil. Induced cancer rats were under hepatocarcinogenesis promoter diet made from a mixture of standard rat diet (AIN-76) with 2-acetylaminofluorene (0.02% AAF) for two weeks. Two weeks after this diet, left over rats were divided to two groups as control and treatment. Treatment group, were forced feed daily with 0.7 ml *O. stamineus* decoction.

Results: After 28 weeks treatment with *O. stamineus* decoction, serum biochemical markers including alpha fetoprotein (AFP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), corticosteroid binding globulin (CBG), gamma glutamyl transferase (GGT), homocysteine (HCY), tumor necrosis factor alpha (TNF-α), and alpha 2 macroglobulin (α2MG) have been regulated favorably. Total antioxidant status (TAS) also has been increased drastically. Liver lesion score in treated groups were reduced and glucocorticoid activity has been amplified significantly.

Conclusion: Our results indicate that *O. stamineus* decoction might prevent or subdue liver cancer development.

Introduction

Herbal teas and their decoctions have a long history in human life, especially in East Asia. Different types of herb s are an important part of medical history and have been widely used to cure or prevent diseases. *Orthosiphon stamineus* Benth or Cat's Whiskers (family: *Lamiaceae*) is commonly used as Java Tea. It is a medicinal plant, native in South East Asia (Malaysia, Indonesia, and Thailand) and some part of Tropical Australia [1]. *O. stamineus* is popularly

Corresponding author:
Dr Fauziah Othman, PhD.
Address: Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor, Darul Ehsan, Malaysia.
Email: fauziah@upm.edu.my

consumed as herbal tea and is one of the popular traditional folk medicine extensively used in Southeast Asia for the treatment of wide range of diseases including diabetes, kidney and urinary disorders, high blood pressure and bone or muscular pain [2–5]. In the present study, we studied possible anticancer properties of *O. stamineus* decoction on hepatocellular carcinoma in animal model. Moreover, apart from different cancer markers which have been assessed, due to importance of glucocorticoids in cancer, effect of *O. stamineus* decoction on serum glucocorticoid as well as liver cell glucocorticoid receptors were investigated.

Methods

Animals and experimental protocols

The present study was designed as a preclinical study. The protocol of the rat hepatocarcinogenesis in this study was based on Solt and Farber method [6]. University Putra Malaysia (UPM) Animal Research Ethics Committee has approved this study under approval No: UPM/FPSK/PADS/BR-UUH/00448. Forty male Sprague Dawley rats, 8±1 weeks old, with average weight 246.3±6.2 g, were obtained from UPM Veterinary Faculty and housed in individual plastic bottom cages and maintained in a room at 22 ºC temperature, humidity 60±5% relative with a 12h light/dark cycle. All rats had free access to the standard rat food pellet based on AIN-76A [7], and tap water during the study. Hepatocarcinoma were induced in 30 of the rats by single intraperitoneal injection of 200mg/kg body weight diethyl nitrosamine (DEN) dissolved in corn oil and then were kept for 2 weeks on cancer promoter food, which was mixed with 2-acerylaminofluorene (0.02% AAF) as a promoter of hepatocarcinogenesis without partial hepatectomy to promote hepatocarcinogenesis. The rats were then left for 2 weeks. A group of 10 rats served as normal group with no DEN injection or hepatocarcinogenesis promoter diet. After cancer initiation period, the left over rats were weighed again and divided randomly to two groups with no significant differences in their weight. Both control and *O. stamineus* group were allowed free access to AIN76 and water *ad libitum* for 28 weeks, but rats in *O. stamineus* group were force feed by 0.7ml/100g Body weight/day of *O. stamineus* decoction.

Plant material and Preparation of the O. stamineus decoction

O. stamineus was prepared from University Agriculture Park of UPM. *O. stamineus* leaves were dried under shadow, weighed and washed 3 times with tap water and then put into a 10 liter beaker. For each 100g of dried herb, 4000 ml of distilled water was added. Then the mixture was heated up to 70˚C to decrease the water content to 1000 ml through evaporation. After these steps, the residues were filtered. The liquids were cooled and kept in the fridge at 4˚C in clean bottles until used. This process has been done weekly until end of the study.

Chemicals and Biochemical analyses

Alpha-Fetoprotein tumor marker (AFP), tumor necrosis factor alpha (TNF-α), homocysteine (HCY), corticosteroid binding globulin (CBG), alpha 2 macroglobulin (α_2MG) were analyzed using standard commercial ELISA kit (Cusabio Biotech, China). Gamma glutamyl transpeptidase (GGT) were tested by using Colorimetric Assay Kit (BioVision, USA). Alanine aminotransferase (ALT/SGPT), aspartate aminotransferase (AST/SGOT), alkaline phosphatase (ALP), and total antioxidant status (TAS) were analyzed by Chemical pathology lab at FMHS, UPM using Roche Cobas® C-311 analyzer.

Histopathological examinations

Half of the liver tissue of each samples were fixed in 10% formalin and then the paraffin blocks were prepared. The sections from blocks were stained with hematoxylin-eosin. The histopathological evaluations were performed blindly by an expert pathologist using a scoring system. The lesion scoring was obtained according to the modified method of Batts and Ludwig by Stevens *et al.* [8,9]. The rest of livers were kept in -80˚C for liver glucocorticoid receptor analysis. Fluorescent *in situ* hybridization (FISH) was used to analyze glucocorticoid receptor RNA activity by QuantiGene® ViewRNA ISH Tissue 2-Plex Assay kit (Affymetrix® Inc, USA). For positive control ACTB, GAPD were used as housekeeping genes. The frozen tissues were cut using rotary cryo microtome (Leica 1850 UV) at 4-8 micron and pasted on a slide for FISH test. Slides were observed under confocal laser microscope (Olympus FV10, Japan). For Fast Red Substrate Cy3/TRITC (filter set: Excitation: 530±20 nm, Emission: 590±20 nm, Dichroic:

Table 1. Effect of *O. stamineus* on serum biochemical markers as compared to normal and control groups

Marker	Normal (n=10)	Control (n=8)	*O. stamineus* (n=9)
ALP (IU/L)	38.86±1.72 [a]	77.92±3.74 [b]	59.00±6.70 [c]
ALT (U/L)	25.89±1.66 [a]	67.33±4.92 [b]	41.07±3.80 [c]
AST (U/L)	56.73±2.53 [a]	156.49±10.66 [b]	106.19±16.62 [c]
AST/ALT Ratio	2.25±0.08	2.48±0.25	2.60±0.34
CBG (µg/ml)	10.76±0.26 [a]	11.52±0.35 [b]	12.53±0.45 [c]
HCY (nmol/ml)	0.57±0.03 [a]	1.42±0.14 [b]	0.94±0.04 [c]
TNF-α (pg/ml)	24.08±1.00 [a]	49.17±1.12 [b]	43.97±1.63 [c]
α2MG (ng/ml)	0.71±0.04 [a]	1.43±0.09 [b]	1.23±0.16 [c]
AFP (pg/ml)	47.6±1.05 [a]	102.05±2.86 [b]	83.99±4.10 [c]
TAS (mmol/l)	9.86±0.35 [a]	1.09±0.16 [b*]	8.98±0.18 [c]
GGT (mU/ml)	0.68±0.01 [a]	1.14±0.04 [b]	0.84±0.015 [c]

[abc] *Values in the same row with the different superscripts are significantly different at p<0.05 based on one way ANOVA, Duncan's post hoc test.*

[*] *Value is significantly lower than other groups at p<0.01 based on one way ANOVA, Duncan's post hoc test.*

562 nm), for Fast Blue Substrate, Cy7-B/Alexa 750 (custom filter set: Excitation: 630±20 nm, Emission: 775±25 nm, Dichroic: 750 nm), and for DAPI filter set (Excitation: 387/11 nm, Emission: 447/60 nm) were used.

Statistical analyses

Data were expressed as Means±SEM. Statistical differences between Normal, treated and control groups were determined using one way repeated measures analysis of variance (ANOVA) followed by Dunkan's multiple range as post hoc test. Differences between groups were considered significantly different when the P value was less than 0.05.

Results

During the intervention study some of the rats in both groups died. *O. stamineus* treated group showed non-significantly lower mortality rate as compared to the control group (25% *vs* 33.3%, $p < 0.05$). After 28 weeks of treatment, based on the present findings, rats in the control group showed higher but no significant weight gain as compared to *O. stamineus* group 491.2±17.9 g *vs* 461.1±15.0 g ($p > 0.05$). Although the control group showed significantly higher liver weight (12.28±0.31g) as compared to both normal (10.50±0.38g) and *O. stamineus* group (10.94±0.63g) ($p < 0.05$), no significant liver weight ratio was observed 2.47±0.06 *vs* 2.60±0.17 and 2.28±0.10 respectively ($p > 0.05$).

The effect of different treatments on serum biochemical biomarkers has been demonstrated in Table 1. Both normal and *O. stamineus* groups showed significantly lower serum ALP ($p < 0.05$) as compared to the control group. Similar outcomes were also found for AST, ALT, HCY, and α_2MG ($p < 0.05$). In spite of lower level of

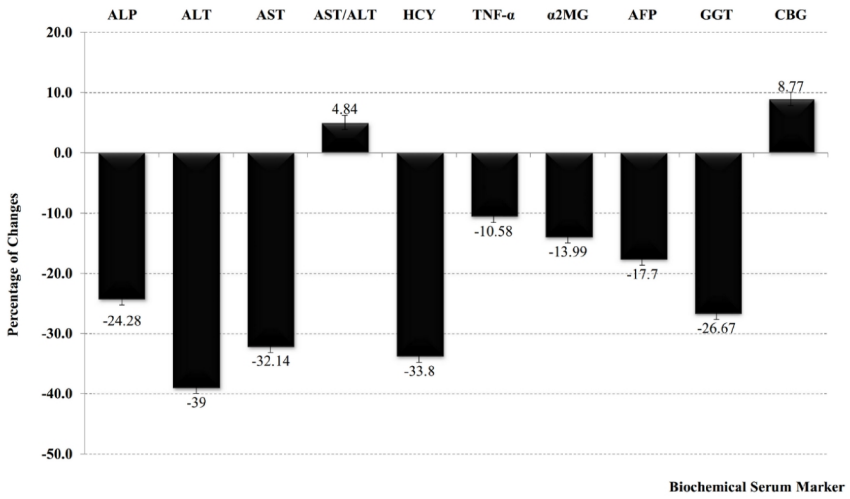

Figure 1. Percentage of changes of different serum biochemical markers in O. stamineus group as compared to the control

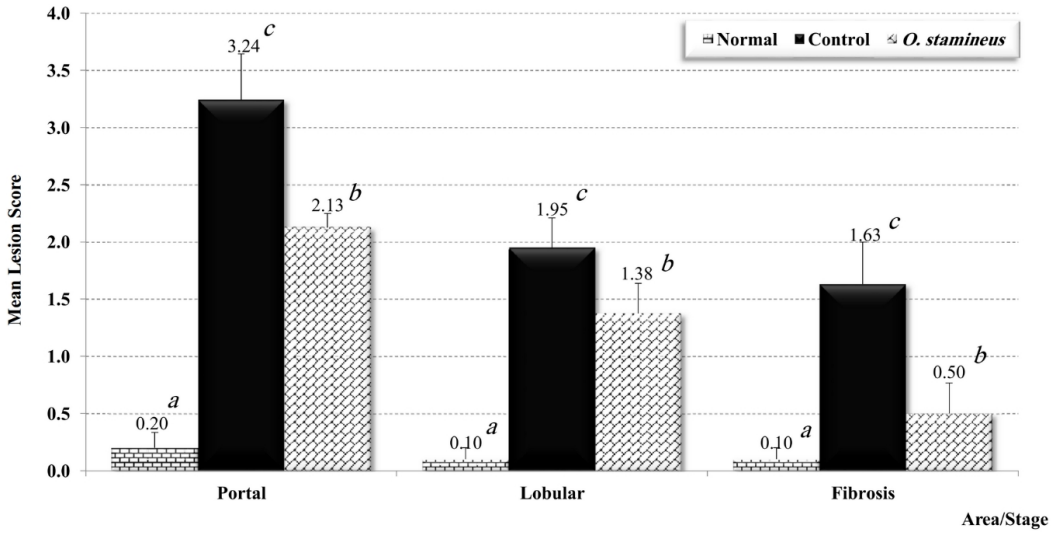

Figure 2. Mean lesion score of rats liver tissue in different groups

[abc] Values in each area/stage with the different superscripts are significantly different at p<0.05, based on one way ANOVA, Duncan's post hoc test.

AST/ALT ratio in normal group, no significant difference was found between groups (p> 0.05). Promising considerably higher level of CBG was found in *O. stamineus* group and the lowest value was found in normal group (p< 0.05). *O. stamineus* showed significantly higher level of TNF-α, α_2MG, AFP, and GGT as compared to normal (p< 0.05) but significantly lower than control group (p< 0.05). *O. stamineus* group showed significantly higher TAS as compared to the normal group (p< 0.05), and both were considerably higher than control group (p<0.01).

As Figure 1 indicates, after 28 weeks of treatment with *O. stamineus* decoction most of liver function and cancer markers have improved and fallen significantly as compared to the control (p< 0.05). ALP was reduced by 24.28±1.79 %, ALT, AST, and HCY decreased by 39±0.77%, 32.14±1.56%, and 33.8±0.29% respectively. AST/ALT ratio was increased non-significantly -4.84±1.36, (p> 0.05). TNF-α, α_2MG, AFP and GGT were also dropped (10.58±1.46%, 13.99±1.78%, 17.7±1.43%, 26.67±0.38% respectively). Unlike other markers, CBG has elevated significantly (8.77±1.29%, p< 0.05). Moreover, *O. stamineus* decoction was able to upsurge TAS drastically by 723.85±1.13%.

Histopathological findings

Lesion score of different group is shown in Figure 2. Accordingly, rats in control groups showed significantly higher lesion score in both portal and lobular region as well as fibrosis stage

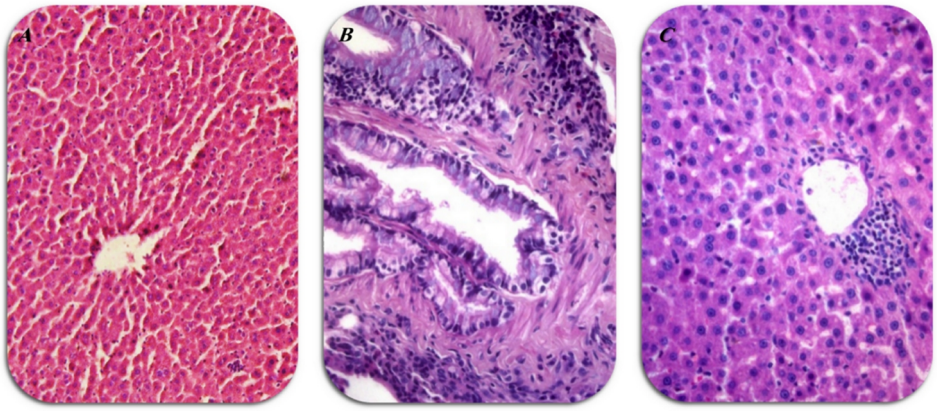

Figure 3. Light micrograph of liver cells in different groups.
A: Normal Liver Cell at the Lobular Region of Normal Group. Lesion score: 0. B: HCC in Control Group. Lesion score: 4. C: Hepatitis in *O. stamineus*. Lesion score: 2 H&E, ×400

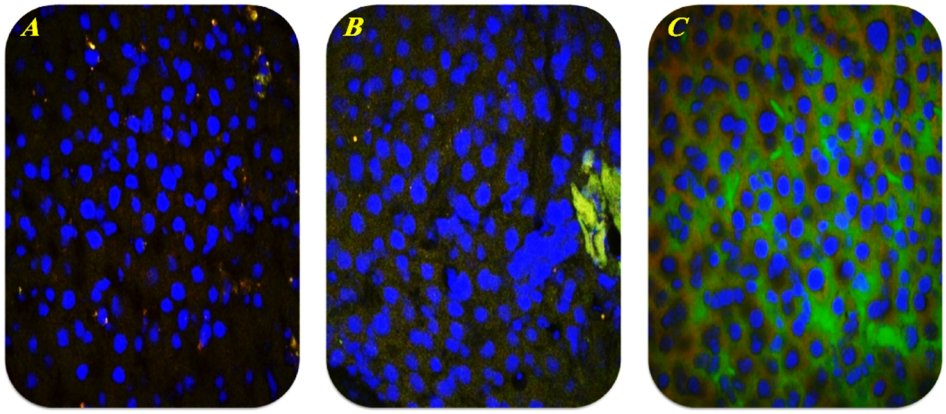

Figure 4. Fluorescent *in situ* hybridization micrograph of lobular region of protein expression of glucocorticoid receptors in the cytoplasm.
A: Normal, B: Control. C: *O. stamineus* treated group. Frozen section, ×600 Magnification. More green color express more GR.

compared to *O. stamineus* and normal groups ($p < 0.05$). Obviously, normal group had the lowest lesion score ($p < 0.05$) due to rats health status. *O. stamineus* group showed significantly lower lesion score in all sites as compared to the control group ($p < 0.05$) (Figure 3).

Discussion

In this study, the control group had the highest mortality rate, which was expected. Although gross histology of *O. stamineus* group showed no hepatic nodules in this group unlike the control ones, quite similar mortality rate was a disappointing outcome. Although significantly higher weight in control group was found which was in contrast with previous studies [10], the relationship between HCC and body weight is still questionable and there are many different opinions on this topic. Usually weight loss would be seen in critical level of liver cancer [11]. There might be many possible underlying variables, which can affect weight loss or gain in liver cancer. Disease acquaintance and severity of the disease are two main factors which mainly dominate weight changes. On the other hand mortality rate in groups might affected the real result of body weight in the present study. The result of liver weight of *O. stamineus* was also in contrast with previous studies in close field [10]. Differences in study design as well as duration of the studies might be the reason of these differences. It has been shown that ALP among liver function tests, in addition to other tumor characters, were independent factors for disease-free survival and overall survival. Recent studies have suggested that preoperative ALP levels could be utilized to monitor and predict recurrence in high risk HCC patients [12]. Both Normal and *O. stamineus* groups showed significantly lower serum ALP ($p < 0.05$) which was similar to previous studies (10,13]. Significant elevation of serum AST and ALT activities were seen in a variety of liver conditions, cirrhosis, non-alcoholic steatohepatitis (NASH), drug toxicity, liver tissue degeneration and necrosis [14]. AST elevations frequently predominate in patients with cirrhosis and even in liver diseases that classically have an increased ALT [15]. Both normal and *O. stamineus* groups showed significantly lower serum AST and ALT ($p<0.05$). Advantageous effect of *O. stamineus* on liver enzymes including AST and ALT has been reported previously [10,13,16] and the present study supported the previous claims as well. Concerning AST/ALT ratio which has been reported a more clinical utility than assessing individual elevated levels [17], non-significant lower ratio was found among *O. stamineus* group. Absence of significant result might be due to higher mean standard error among control group.

The role of TNF-α in liver cancer has dual capabilities. TNF-α is a pleiotropic cytokine that can induce both cell death and cell proliferation [18]. It is related to all steps engaged in tumorigenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [19]. Deregulated TNF-α expression within the tumor microenvironment seems to favor malignant cell tissue invasion, migration and finally metastatic formation. On the other hand, TNF-α clearly possesses antitumor properties not only in

preclinical models but also in the clinical setting [20]. Overall, based on the present study, *O. stamineus* can decrease serum TNF-α either due to direct effect of its two main compounds eupatorin and sinensetin, which inhibit TNF-α production [21] or indirect mechanism by suppressing HCC.

Animal studies showed that α_2MG is an important novel cytochemical marker to identify hepatocellular preneoplastic and neoplastic lesions, particularly amphophilic cell foci, undetectable by established cytochemical markers and tightly linked to rat hepatocarcinogenesis [22]. Furthermore, a number of authors have reported up-regulation of serum α_2MG in association with HCC in humans, being significantly raised as compared to liver cirrhosis and amoebic liver abscess [23]. In the present study, *O. stamineus* treated group showed significantly lower level of α_2MG. As cancer cells produce and secrete large amounts of α_2MG, which seems to be linked with their tumorigenicity [24], therefore *O. stamineus* might decrease α_2MG secretion in cancer suppression mechanism.

Concerning HCY outcome, the present results are supported by a few studies which have found high levels of HCY in different types of cancer [25] and liver disorders [26]. Both epidemiological and experimental studies found a link between hyperhomocysteinemia and a wide range of impaired liver function like cirrhosis and chronic alcohol consumption [27]. To our knowledge the present study was the first attempt to investigate the effect of treated herb on HCY level, therefore there was no pros and cons evidence to the present results.

AFP is one of the old, yet the most widely used blood marker test for liver cancer. High level of AFP among control group of the present study was similar to previous ones. Many of the studies in last four decades have shown that AFP was elevated in hepatocarcinogenesis, embryonic carcinomas [28,29]. Beneficial effect of *O. stamineus* in the present study might be due to antioxidant activity and flavonoids in *O. stamineus* [30–32].

It is well established that the elevated serum GGT activity could be found in diseases of the liver, biliary system, pancreas, and different types of cancers including HCC [33]. In the present study, significantly lower level of GGT was found in *O. stamineus* group as compared to control group ($p< 0.05$). It has been shown that GGT is associated with antioxidants and oxidative stress [34]. Extremely high level of TAS in treated group could be one of the master keys in revealing possible cancer suppressor capabilities of *O. stamineus*. It has been shown that excessive reactive oxygen species (ROS) cause oxidative damages to biomolecules and lead to cellular alterations and ultimately tumorigenesis and neoplastic transformation [35]. Therefore, high level of TAS could not only act as excess ROS protector, but might also indirectly affect other cancer risk factors which have been tested.

Glucocorticoids (GCs) are frequently used to support patients suffering from a various type of cancers. Their key therapeutic role is based on GC receptor (GR)-mediated mechanisms that activate cell death however this differs depending on type of cancer [36]. GC might directly activate the apoptotic machinery by regulating components of either the 'extrinsic' or 'intrinsic' pathways or both. Studies using the caspase 8 inhibitor cytokine response modifier A (CrmA) in transgenic mice [37] and human cell lines [38] suggested that GC-induced apoptosis may not critically depend on the extrinsic pathway. GC might induce apoptosis indirectly by gene (de)regulations that entail distress and cellular damage. This category might include the regulation of genes affecting metabolic pathways [39,40], general transcription and/or translation [41], production of/or response to oxygen radicals [42,43]. Moreover, glucocorticoids prevent prostaglandin synthesis at the level of phospholipase A2 as well as at the level of cyclooxygenase/PGE isomerase (COX-1 and COX-2) [44]. The latter effect is similar to non-steroidal anti-inflammatory drugs (NSAIDs), which potentiate the anti-inflammatory effect [45,46]. COX-2-dependent activity is an essential element for cellular and molecular mechanisms of cancer cell motility and invasion. COX-2 activity also modulates the expression of matrix metalloproteinase (MMP), which may be a part of the molecular mechanism by which COX-2 promotes cell invasion and migration [46]. Many studies on different type of cancer have shown that cyclooxygenase suppression would decrease cancer cells [45]. Therefore, cyclooxygenase suppression by glucocorticoids might decrease risk of cancer or control its metastasis. Glucocorticoids inhibit hepatocellular proliferation and modulate the expression of oncogenes and tumor suppressor genes via mechanisms involving the glucocorticoid receptor. Glucocorticoids also

produce a receptor-mediated inhibitory effect on both basal and hormone-stimulated expression of molecules important for shutting off cytokine action as well as different caspase pathways [47]. Based on the present study, *O. stamineus* has glucocorticoids stimulation activity, which might have positive effect on cancer prevention or treatment. The results of confocal microscopy of fluorescent *in situ* hybridization of liver cells helped us to have a better answer for our findings in both light microscopy as well as biochemical results. As FISH results shown in Figure 4, high level of glucocorticoid receptor activity was illustrated in *O. stamineus* treated group. Therefore, higher activity of GC receptors and higher level of serum CBG which have been found in the present study could also explain the possible anti-cancer or cancer suppressor competences of *O. stamineus*. Overall, as the present study is the first study in its field further studies would help us to have better view on mechanism of action of these herbs on glucocorticoids stimulation.

Lesion score evaluation of rat livers also showed *O. stamineus* decoction successfully reduced the score of inflammation or necrosis at the portal and lobular as compared to control group ($p< 0.05$) which was similar to previous studies [10,13].

Conclusion

These beneficial effects of *O. stamineus* could be explained by significantly high level of flavonoid compounds and antioxidant capability of the *O. stamineus*. In order to find out the active compound(s) that play major role(s) in producing these effects, further studies are necessary.

Acknowledgements

The authors would like to thank Mrs. Safarina Ismuddin, Mrs. Zamzarina Bajari, Mr. Norazmie Ramly, Mr. Syed Hasbullah Syed Kamaruddin, Mr. Mohd Khairil, and Mr. Ramli Suhaimi for their technical assistance. This study was funded by Universiti Putra Malaysia under RUGS-9367600 research grant.

Conflicts of interest: The authors of this article declare that they have no conflict of interest in the subject matter or materials discussed in this manuscript.

References

1. Abdelwahab SI, Mohan S, Mohamed Elhassan M, Al-Mekhlafi N, Mariod AA, Abdul AB, et al. Antiapoptotic and Antioxidant Properties of Orthosiphon stamineus Benth (Cat's Whiskers): Intervention in the Bcl-2-Mediated Apoptotic Pathway. Evid Based Complement Alternat Med. 2011;156765.
2. Adam Y, Somchit MN, Sulaiman MR, Nasaruddin AA, Zuraini A, Bustamam AA, et al. Diuretic properties of Orthosiphon stamineus Benth. J Ethnopharmacol. 2009; 124(1):154–8.
3. Bwin DM, Gwan US. Burmese indigenous medicinal plants: I. Plants with reputed hypoglycemic action. Burma Medical Research Institute. Burma Medical Research Institute; 1967.
4. Sumaryono W, Proksch P, Wray V, Witte L, Hartmann T. Qualitative and Quantitative Analysis of the Phenolic Constituents from Orthosiphon aristatus. Planta Med. 1991; 57(2):176–80.
5. Tezuka Y, Stampoulis P, Banskota AH, Awale S, Tran KQ, Saiki I, et al. Constituents of the Vietnamese medicinal plant Orthosiphon stamineus. Chem Pharm Bull (Tokyo). 2000 ;48(11):1711–9.
6. Solt D, Farber E. New principle for the analysis of chemical carcinogenesis. Nature 1976;263(5579): 701–3.
7. AIN. Report of the American Institute of Nutrition ad hoc Committee on Standards for Nutritional Studies. J Nutr. 1977;107(7):1340–8.
8. Stevens A, Lowe JS, Young B, Wheater PR. Wheater's Basic Histopathology: A Colour Atlas and Text. Churchill Livingstone; 2002.
9. Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. Am J Surg Pathol. 1995;19(12):1409–17.
10. Alshawsh M a, Abdulla MA, Ismail S, Amin Z a. Hepatoprotective Effects of Orthosiphon stamineus Extract on Thioacetamide-Induced Liver Cirrhosis in Rats. Evid Based Complement Alternat Med. 2011;103039.
11. NCI. Understanding Cancer Series: Cancer - National Cancer Institute. National Institutes of Health. 2013.
12. Yu MC, Chan KM, Lee CF, Lee YS, Eldeen FZ, Chou HS, et al. Alkaline phosphatase: does it have a role in predicting hepatocellular carcinoma recurrence? J Gastrointest Surg. 2011;15(8): 1440–9.
13. Chin J, Hussin A, Ismail S. Hepatoprotective effect of Orthosiphon stamineus Benth against acetaminophen intoxication in rats. J Nat Remedies. 2009;9(2):177–84.
14. Yang R-Z, Park S, Reagan WJ, Goldstein R, Zhong S, Lawton M, et al. Alanine aminotransferase isoenzymes: molecular cloning and quantitative analysis of tissue expression in rats and serum elevation in liver toxicity. Hepatology. 2009;49(2):598–607.
15. Green RM, Flamm S. AGA technical review on the evaluation of liver chemistry tests.

Gastroenterology. 2002;123(4):1367–84.
16. Maheswari C. Hepatoprotective activity of "Orthosiphon stamineus" on liver damage caused by paracetamol in rats. Jordan J Biol Sci. 2008; 1(3):6673.
17. Gowda S, Desai PB, Hull V V, Math AAK, Vernekar SN, Kulkarni SS. A review on laboratory liver function tests. Pan Afr Med J. 2009;3:17.
18. Kucharczak J, Simmons MJ, Fan Y, Gélinas C. To be, or not to be: NF-kappaB is the answer--role of Rel/NF-kappaB in the regulation of apoptosis. Oncogene. 2003;22(56):8961–82.
19. Sethi G, Sung B, Aggarwal BB. TNF: a master switch for inflammation to cancer. Front Biosci. 2008;13:5094–107.
20. Mocellin S, Nitti D. TNF and cancer: the two sides of the coin. Front Biosci. 2008;13:2774–83.
21. Laavola M, Nieminen R, Yam MF, Sadikun A, Asmawi MZ, Basir R, et al. Flavonoids eupatorin and sinensetin present in Orthosiphon stamineus leaves inhibit inflammatory gene expression and STAT1 activation. Planta Med. 2012;78(8):779–86.
22. Sukata T, Uwagawa S, Ozaki K, Sumida K, Kikuchi K, Kushida M, et al. alpha(2)-Macroglobulin: a novel cytochemical marker characterizing preneoplastic and neoplastic rat liver lesions negative for hitherto established cytochemical markers. Am J Pathol. 2004;165 (5):1479–88.
23. Kotaka M, Chen GG, Lai PBS, Lau WY, Chan PKS, Leung TWT, et al. Analysis of differentially expressed genes in hepatocellular carcinoma with hepatitis C virus by suppression subtractive hybridization. Oncol Res. 2002;13(3):161–7.
24. Smorenburg SM, Griffini P, Tiggelman AB, Moorman AF, Boers W, Van Noorden JF. alpha2-Macroglobulin is mainly produced by cancer cells and not by hepatocytes in rats with colon carcinoma metastases in liver. Hepatology. 1996;23(3):560–70.
25. Wu LL, Wu JT. Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker. Clin Chim Acta.;322(1-2):21–8.
26. De Carvalho SCR, Muniz MTC, Siqueira MDV, Siqueira ERF, Gomes AV, Silva KA, et al. Plasmatic higher levels of homocysteine in non-alcoholic fatty liver disease (NAFLD). Nutr J. 2013;12:37.
27. Sakuta H, Suzuki T. Alcohol consumption and plasma homocysteine. Alcohol. 2005;37(2):73–7.
28. Hu Z, Zhao W. Novel insights into the molecular mechanisms of α-fetoprotein expression and malignant phenotypes of hepatocellular carcinoma. Cell Mol Immunol. 2012;9(1):7–8.
29. Ruoslahti E, Seppälä M. Studies of carcino-fetal proteins. 3. Development of a radioimmunoassay for -fetoprotein. Demonstration of -fetoprotein in serum of healthy human adults. Int J Cancer. 1971;8(3):374–83.
30. Yam MF, Lim CP, Fung Ang L, Por LY, Wong ST, Asmawi MZ, et al. Antioxidant and Toxicity Studies of 50% Methanolic Extract of Orthosiphon stamineus Benth. Biomed Res Int. Hindawi Publishing Corporation.2013:1–10.
31. Loon YH, Wong JW, Yap SP, Yuen KH. Determination of flavonoids from Orthosiphon stamineus in plasma using a simple HPLC method with ultraviolet detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2005;816(1-2):161–6.
32. Pan Y, Abd-Rashid BA, Ismail Z, Ismail R, Mak JW, Pook PCK, et al. In vitro effects of active constituents and extracts of Orthosiphon stamineus on the activities of three major human cDNA-expressed cytochrome P450 enzymes. Chem Biol Interact. Elsevier Ireland Ltd; 2011;190(1):1–8.
33. Hu G, Tuomilehto J, Pukkala E, Hakulinen T, Antikainen R, Vartiainen E, et al. Joint effects of coffee consumption and serum gamma-glutamyltransferase on the risk of liver cancer. Hepatology. 2008;48(1):129–36.
34. Lim J-S, Yang J-H, Chun B-Y, Kam S, Jacobs DR, Lee D-H. Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress? Free Radic Biol Med. 2004;37(7):1018–23.
35. Cooke MS, Evans MD, Dizdaroglu M, Lunec J. Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J. 2003 Jul;17(10):1195–214.
36. Schlossmacher G, Stevens A, White A. Glucocorticoid receptor-mediated apoptosis: mechanisms of resistance in cancer cells. J Endocrinol. 2011;211(1):17–25.
37. Smith KG, Strasser A, Vaux DL. CrmA expression in T lymphocytes of transgenic mice inhibits CD95 (Fas/APO-1)-transduced apoptosis, but does not cause lymphadenopathy or autoimmune disease. EMBO J. 1996;15(19): 5167–76.
38. Planey SL, Abrams MT, Robertson NM, Litwack G. Role of apical caspases and glucocorticoid-regulated genes in glucocorticoid-induced apoptosis of pre-B leukemic cells. Cancer Res. 2003;63(1):172–8.
39. Kofler R. The molecular basis of glucocorticoid-induced apoptosis of lymphoblastic leukemia cells. Histochem Cell Biol. 2000;114(1):1–7.
40. Tonko M, Ausserlechner MJ, Bernhard D, Helmberg A, Kofler R. Gene expression profiles of proliferating vs. G1/G0 arrested human leukemia cells suggest a mechanism for glucocorticoid-induced apoptosis. FASEB J. 2001;15(3):693–9.
41. Obexer P, Certa U, Kofler R, Helmberg A. Expression profiling of glucocorticoid-treated T-ALL cell lines: rapid repression of multiple genes involved in RNA-, protein- and nucleotide

synthesis. Oncogene. 2001;20(32):4324–36.
42. Fernandez A, Kiefer J, Fosdick L, McConkey DJ. Oxygen radical production and thiol depletion are required for Ca(2+)-mediated endogenous endonuclease activation in apoptotic thymocytes. J Immunol. 1995;155(11):5133–9.
43. Tome ME, Briehl MM. Thymocytes selected for resistance to hydrogen peroxide show altered antioxidant enzyme profiles and resistance to dexamethasone-induced apoptosis. Cell Death Differ. 2001;8(9):953–61.
44. Goppelt-Struebe M, Wolter D, Resch K. Glucocorticoids inhibit prostaglandin synthesis not only at the level of phospholipase A2 but also at the level of cyclo-oxygenase/PGE isomerase. Br J Pharmacol. 1989;98(4):1287–95.
45. Hoellen F, Kelling K, Dittmer C, Diedrich K, Friedrich M, Thill M. Impact of cyclooxygenase-2 in breast cancer. Anticancer Res. 2011;31(12): 4359–67.
46. Larkins TL, Nowell M, Singh S, Sanford GL. Inhibition of cyclooxygenase-2 decreases breast cancer cell motility, invasion and matrix metalloproteinase expression. BMC Cancer. 2006; 6(1):181.
47. Schmidt S, Rainer J, Ploner C, Presul E, Riml S, Kofler R. Glucocorticoid-induced apoptosis and glucocorticoid resistance: molecular mechanisms and clinical relevance. Cell Death Differ. 2004;11 Suppl 1(S1):S45–55.