ASIAN JOURNAL OF PHARMACOGNOSY *Research Article*
Asian J. Pharmacogn 3(3):36-42

eISSN-0128-1119

Evaluation of developmental toxicity and teratogenicity of *Quercus infectoria* galls (Manjakani) aqueous extract in Sprague Dawley rats

M.K. Nurul Ajilah[a], A. Hasmah[b], M.F Wan Ezumi[a]*

[a]*Programme of Biomedicine, School of Health Sciences, Universiti Sains Malaysia Health Campus, Kota Bharu, Malaysia.*
[b]*Programme of Environmental and Occupational Health, School of Health Sciences, Universiti Sains Malaysia Health Campus, Kota Bharu, Malaysia.*
[a*]*Programme of Biomedicine, School of Health Sciences, Universiti Sains Malaysia Health Campus, Kota Bharu, Malaysia.*

*For correspondence: Wan Ezumi : wanezumi@usm.my

Abstract: *Quercus infectoria* galls (QIG) or locally known in Malaysia as manjakani is commonly used by reproductive-age women to restore uterine elasticity and increase sexual pleasure but it is not consumed during pregnancy and early confinement period. However, scientific reports about to its possible effects particularly on the progression of developing embryo are very limited. Thus, the present study was conducted to provide evidence on the potential effects of QIG aqueous extract on embryonic development including teratogenicity in pregnant Sprague Dawley rats. QIG extract at the doses of 0 (control), 125, 250, 500 or 1000 mg/kg/day were orally administered to 30 experimental rats during pre-mating, mating and up to gestation periods of day 16 while sacrificed on day 20 of pregnancy. Results obtained revealed that there were no substantial effects on the number of corpora lutea, implantation sites, percentages of pre-implantation loss and post-implantation death, gravid uterine weight, number of life fetuses and fetal body weight in all experimental animals. Similarly, no correlation and distinguishable diversities of fetal sex ratio were observed among all groups. Gross examination of external and internal organs of fetuses did not indicate evidence of QIG-related alterations as all fetuses displayed normal physical appearances. These findings suggest that the aqueous extracts of QIG of up to 1000 mg/kg/day exerted no developmental toxicity or teratogenic effect in rats.

Keywords: Developmental toxicity; *Quercus infectoria*; Manjakani; Teratogenicity

INTRODUCTION

Herbal medicine is a growing field with long anecdotal information involving the use of plant-derived materials and their preparations for therapeutic purposes or other human health benefits. *Quercus infectoria* gall (QIG) or locally known as manjakani is widely utilized for generations. The galls are formed from a perforation made in the bark by gall wasps *Cynips gallae*

ASIAN JOURNAL OF PHARMACOGNOSY *Research Article*
Asian J. Pharmacogn 3(3):36-42

eISSN-0128-1119

tinctoriae to deposit their eggs (Kaur et al., 2004; Shrestha et al., 2014). The ethnomedicinal practices of this herb have been existed till present days and even being modernised into diverse commercial formulations and herbal products (Singh et al., 2013). Water decoction of the galls is traditionally used among Malay women to treat women's disease, tighten vaginal muscle (Ansari Shaiqua, 2016; Nahid, 2016) thus restoring feminine sexuality and as post-partum medication (Fan et al., 2014). Nevertheless, the usage of this herb during pregnancy and early confinement period is conventionally not recommended by old folks but without scientific evidence (Singh, 2015). However, Noureddini et al., (2018) had recently reported that QIG exhibited contraction effects on uterine muscles of rats which might support this traditional belief across generations.

Due to its popularity, various research aspects have been conducted on this herb with numerous documented journals are widely available reporting its pharmacological properties). Remarkably, QIG is known to accumulates tannins (50-70%) (Ansari Shauqua et al., 2016; Rina et al., 2011; Shrestha et al., 2014;), gallic acid and ellagic acid (Rina et al., 2011; Ansari Shauqua et al., 2016). However, there is still limited information on the safety profiles of this herb while its toxicity potential should not be neglected. There were few cases prevailed that women who used QIG to increase sexual pleasure, however, continued the usage into the early pregnancy without aware of the pregnancy itself (Rahman et al., 2008). Hence, this present study was conducted to investigate the potential effects of QIG aqueous extract on embryonic development including teratogenicity in pregnant Sprague Dawley rats. Moreover, this present study is the first to evaluate the pregnancy outcome with repeated dosing of QIG via the most common human route of administration. The results of this study were expected to provide an evidence-based on data on the safety of this herb specifically on the embryonic development and teratogenic effects in an animal model. These concurrently would increase public awareness regarding the appropriate usage of herbal medicine during pregnancy.

MATERIALS AND METHODS

Aqueous extract of QIG: The QIG powder was obtained from a local herbal market in Kota Bharu, Kelantan, Malaysia. It was then authenticated in the laboratory-based on its macroscopic features. The mixture of coarse and fine, with white color, absence of odor and bitter astringent taste confirmed the correct plant material. The aqueous extract was prepared by reflux extraction technique (Hapidin et al., 2015) by immersing 400 g of QIG powder with distilled water at a ratio of 1:5. The mixture was subsequently heated using a heating mantle at 60 °C for 24 hours and refluxed simultaneously. The resulting mixture was allowed to cool at room temperature before filtration. The extract was then refiltered by filter funnel under reduced pressure and further subjected to freeze-dried for 3 days to obtain a lyophilised powder. Additionally, 20 g of the powder was sent to National Poison Centre (NPC), USM Main Campus, Pulau Pinang for a toxicological screening of heavy metal and unknown poison.

Dosage preparation: The selection of doses was determined based on our previous study of QIG and the consideration of dose translation from human to animal. The dose determination was also based on the established guidelines by the Organisation for Economic Co-operation and Development (OECD) that recommends a maximum dose for the repeated study is 1000 milligram (mg)/kilogram (kg)/day (OECD, 2001). Therefore, the doses selected for this study were 0 (control), 125, 250, 500 and 1000 mg/kg/day. The QIG powder was then weighed and reconstituted with distilled water to a fixed volume of 1 mL before oral administration to animals were made.

Experimental animals: Thirty virgin female Sprague Dawley rats of 7-10 weeks old with bodyweight of 150-180 g and adult fertile male rats (for the mating purpose only) were procured from the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia Health

ASIAN JOURNAL OF PHARMACOGNOSY *Research Article*
Asian J. Pharmacogn 3(3):36-42

eISSN-0128-1119

Campus, Kelantan. Female rats were housed individually in cages with wood chips bedding and were maintained under standard laboratory condition of 12 h light/dark cycle (lights on from 0700 to 1900 hrs). The animals were fed with standard commercial rat pellet diet (Atromin, Germany) and water *ad libitum*. Animal were employed into the study after a week of acclimatization. This study has obtained its animal ethics approval from the Animal Ethics Committee of Universiti Sains Malaysia (USM/ Animal Ethics Approval/ 2015/ 637).

Guidelines and protocol: This developmental toxicity study was carried out by adopting principles of the OECD Guidelines No. 422 (OECD, 2015) and No. 414 (OECD, 2001). QIG extract was continuously administered to female rats at three important stages; non-pregnancy, cohabitation and pregnancy phases.

Treatment: Following acclimatization, the examination of estrous cycle were performed daily approximately for 10 days prior to oral gavaging (pre-treatment period). This was to confirm the stability of animal cyclicity prior to introduction with QIG extract, by which those showing irregular cycle were excluded from the study. Dosing was subsequently commenced to all females by oral gavage on the first day of diestrus of the pre-mating period for approximately 10-days duration and continuously throughout mating period (maximum 14 days) and lasted of up to D16 of pregnancy period. All female rats were randomly assigned into five groups (6 rats in each group). The treatment groups received lyophilized QIG powder diluted in distilled water at the selected doses (125, 250, 500, and 1000 mg/kg/day) while the control group received distilled water as vehicle.

Mating and pregnancy: Female rats with regular estrous cycle were compulsory to mate with males at proestrus phase in 1:1 basis to induce pregnancy. Vaginal plug from each female was examined for the presence of sperm in the following morning. A sperm positive vaginal smear was designated as day zero (D0) of pregnancy or post-coitus (pc).

General observation: Animals were routinely monitored for changes in body weight, general health, morbidity, moribundity, mortality, or any reaction to the treatment throughout the study period. Any signs of toxicity or abnormal occurrences in behavioral status, respiratory signs, locomotion, skin, eyes, and excretory production were documented.

Maternal gross examination: On day D20 pc, external gross examination of dams were carried out and later their visceral organs were grossly examined by a dissecting up the abdominal cavity to expose important organs (uteri, ovaries, liver, kidney, spleen, adrenal glands and gastrointestinal tract). Only gravid uterus and ovaries were removed from the body, cleared of adhering fat and weighed. The isolated uterus was then cut open, the sacs were carefully ruptured one at a time to record the number and position of the implantations, and early or late resorption. Uteri of apparently non-pregnant females were slit opened and immersed in 0.5% of ammonium sufide solution for approximately 5 to 10 minutes under fume hood as to examine the presence of any implantation sites. While for ovaries, the number of ripening corpora lutea were counted to further calculate the percentage of pre-implantation loss.

Fetal gross examination: All fetuses were thoroughly examined for the teratogenic parameters such as number of live or dead fetuses, body weight, sex ratio (male: female), and occurrence of external malformations. Head, eyes, palate, nares, limbs, neck, spine, chest, abdomen, orifices, tail and genitals were closely inspected under dissecting stereo microscope to confirm for any abnormalities.

Statistical analysis: All data were first checked for normality and Levene's tests to check for equality of variances. The analysis number of corpora lutea, implantation sites, live foetuses, and

ASIAN JOURNAL OF PHARMACOGNOSY *Research Article*
Asian J. Pharmacogn 3(3):36-42

eISSN-0128-1119

fetal body weights were analyzed using the one-way ANOVA followed by Bonferroni post-hoc test when appropriate.

Additionally, post-implantation death was analyzed using non-parametric of Kruskal-Wallis test followed by Mann-Whitney *U* test when appropriate. Comparisons between fetal body weight and gender were analyzed using the independent *t*-test. Finally, analysis of fetal gender differences was performed using Chi-square test. All parametric data were expressed as mean ± standard error of the mean (SEM) or ratio while the non-parametric data were expressed as median (interquartile range) (IQR).

RESULTS AND DISCUSSION

As the benefits of herbal medicine are well accepted, the safety of edible product preparations must be seriously scrutinized. QIG for instance, with absence of reported adverse effects does not imply that this herb is definitely safe. Thus, the toxicological assessment is crucial to be carried out in experimental animal model to grant evidence of its potential toxicity. This current experiment has investigated the aqueous extract of QIG on the potential developmental toxicity and teratogenicity in Sprague Dawley rats. Oral gavage was selected for this study as the most relevant route of administration considering that it is similar to human consumption of QIG.

Heavy Metal & Unknown Poison Screening: The toxicological analysis of heavy metal and unknown poison screening resulted that concentration of arsenic (As), cadmium (Cd) and mercury (Hg) were below the quantitation limit, while plumbum or lead (Pb) was detected as 1.368 part per million (ppm). This value was considered acceptable under Food 1983 Act and Poison Act 1952.

Health status and fates of females: Administration of QIG extract produced no treatment related mortality or aberrant physical characteristic associated with the adverse effect in rats at any dose level tested. However, a few animals that received high dose of QIG (1000 mg/kg/day) displayed weird circling motion in the cages soon after the dose administration were made particularly on the first week of dosing. This was believed to occur due to bitter taste of QIG which was considered as indirect effect and no physical injuries were detected on the respective animals.

Maternal visceral changes and organ weights: There were no dose related changes seen in all dams of any groups during autopsy (Figure 1). All visceral organs inducing the reproductive organs of dams displayed normal appearances. Furthermore, there were no significant changes ($p>0.05$) in the weights of ovaries, vagina, fallopian tubes and gravid uterus among all groups of animals (Table 1). With regards to the increased pattern of gravid uterine weight, several factors could play a role thus were not considered to be toxicologically important because it may be due to the variations of fetal body weights, sizes of the internal organ and the number of live fetuses (Lee et al., 2015; Okamura et al., 2011; Wan Ezumi, 2009).

Number of corpora lutea and implantation sites: Animals received QIG (500 mg/kg/day) exhibited the highest number of corpora lutea and implantation sites when compared to the control and other treated groups. These differences however were not significant ($P>0.05$) (Table 1).

Pre- implantation loss and post-implantation death (resorption): Percentage of pre-implantation loss was noted higher in all treated groups compared to the control group, though no significant differences ($P>0.05$) found. Further, there were no significant differences in the percentage of post implantation death in all groups. Although several litters with late resorption were observed in treated groups dams, this result was not statistically affected by the QIG treatment (Table 1).

ASIAN JOURNAL OF PHARMACOGNOSY *Research Article*
Asian J. Pharmacogn 3(3):36-42

eISSN-0128-1119

Number of live fetuses: At caesarean section, 300 fetuses were found in 30 litters from all control and treated dams. Viability was confirmed when fetuses responded to any physical induction. Litters in animals received QIG (500 mg/kg/day) exhibited the highest number of live fetuses when compared to the control. Likewise, no significant difference was noted in any of the group. Hence, the QIG administration did not affect the number of embryo-fetuses and their viability rates.

Fetal sex ratio: The overall male to female fetal sex ratio of all groups was 1.01:1. Male sex distributions were slightly higher in the control and the highest dose (1000 mg/kg/day QIG) groups. To reflect this, some essential point worth to discuss is that QIG has long being used in traditional practices as medical product for sex selection in North India. This herb is incorporated as one of the main ingredients and mixed with some other herbal formulation that is used among Indian women to favour male offspring (Bandyopadhyay & Singh, 2007). Meanwhile, female sex distributions were higher in dams receiving QIG (250 and 500 mg/kg/day) as shown in Table 1 even though no correlation and distinguishable diversities of sex ratio among all groups of fetuses based on Chi-square test.

Fetal body weight: Dams that received the highest dose of QIG (1000 mg/kg/day) acquired the highest value of fetal body weight as compared to the control and other treated groups. The small increases in fetal body weight among treated groups were marginal and no significant differences were observed for all groups of animals. Furthermore, the analysis by Independent T test indicated that there were no significant differences of mean fetal body weight between male and female fetuses in all groups (Table 1). This study suggested that QIG exhibit no statistical significance in embryo-fetal toxicity even though the fetal body weights of treated dams were increasing dependently with the doses. The body weight variation was possibly due to the weight of male fetuses that slightly heavier than females (Almeida and Lemonica, 2000; Wan Ezumi, 2009) albeit fetal sex ratio was not significant. Furthermore, the U-shaped uterine horns also caused fetuses to acquire different size and weight where fetuses positioned in the middle horn are heavier compared to those located at both ends of the uterine horn (Chauhoud & Paumgartten, 2005).

Fetal external evaluation: External evaluation was performed on 61 and 239 fetuses of the control and treated group animals respectively exhibited normal physical characteristics. No alterations were observed on the head (cranium) and facial organs such as eyes, nose, jaw and mouth as these appeared to be normal in shape and size. The tongue was in normal colour with no protrusion. Furthermore, no signs of cleft were seen in lips and palate. Limbs of fetuses were also normal in shape, size and position. In fact, the depth of digital furrows with five digits on each forepaws/ hind paws were typically regular. Besides, the position of tail with slightly curved towards the end were comparable among all fetuses (Figure 2-4). Thus, it was suggested that no teratogenic potential upon administration of QIG at any dose level during gestation period from D0 until D16 of pc.

ASIAN JOURNAL OF PHARMACOGNOSY *Research Article*
Asian J. Pharmacogn 3(3):36-42

eISSN-0128-1119

Table 1 Maternal and fetal variables upon administration of QIG from pre-mating until day 16 of gestation periods

Parameters	Dose (mg/kg/day)					P-value
	0 (Control)	125	250	500	1000	
No. of pregnant rats	6	6	6	6	6	n/s
Pregnancy index (%)	100	100	100	100	100	n/s
Gravid uterine weight (g) [a]	62.35 ± 4.03	52.99 ± 6.72	57.08 ± 5.60	67.39 ± 2.94	68.08 ± 9.95	n/s
No. of corpora lutea/litter [a]	11.83 ± 0.31	13.00 ± 1.28	14.50 ± 1.05	14.67 ± 0.89	12.17 ± 1.47	n/s
No. of implantations sites/litter [a]	10.17 ± 0.87	9.33 ± 3.14	9.83 ± 2.56	11.50 ± 2.17	10.17 ± 3.60	n/s
Pre- implantation loss/litter (%) [b]	3.85 (35.42)	24.01 (40.39)	32.56 (22.56)	18.96 (29.28)	15.38 (15.82)	n/s
Post- implantation loss/litter (%) [b]	0	0 (4.55)	0 (2.78)	0	0 (10.31)	n/s
Litters with early resorption	0	0	0	0	0	n/s
Litters with late resorption	0	2	1	0	3	n/s
No. of live fetuses/litter [a]	10.17 ± 0.87	9.00 ± 1.24	9.67 ± 1.09	11.50 ± 0.89	9.67 ± 1.41	n/s
Fetal body weight (g) [a]	4.10 ± 0.29	3.71 ± 0.20	3.93 ± 0.10	3.88 ± 0.12	4.21 ± 0.37	n/s
Sex ratio (male: female)	1.44 : 1	1.07 : 1	1 : 1.45	1 : 1.46	1.52 : 1	n/s

Data are expressed as [a]Mean ± SEM (parametric data) or [b]Median (IQR) (non-parametric data).
n/s Statistically not significant (P>0.05) among all groups

CONCLUSION

The current study presented that continuous oral exposure of QIG extract lasted until gestation period of D16 did not produce any significant developmental and teratogenic effects towards a substantial number of parameters assessed in this study. Further study is however necessary to be conducted hence to establish a mechanism of action of QIG and to understand the facts that involves in the developmental toxicity. Thus, the non-observable adverse effect level for QIG in this study was determine at 1000 mg/kg/day.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial supports from the Universiti Sains Malaysia (USM) Research University Individual Grant Scheme (1001/PPSK/813061) and the research facilities by the School of Health Sciences, USM Health Campus.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

eISSN-0128-1119

1. Kaur G, Hamid H, Ali A, Alam MS, Athar M (2004) Antiinflammatory evaluation of alcoholic extract of galls of *Quercus infectoria*. *J* Ethnopharmacol. 90(2): 285-292.
2. Shrestha S, Kaushik VS, Eshwarappa RSB, Subaramaihha SR, Ramanna LM, Lakkappa DB (2014) Pharmacognostic studies of insect gall of *Quercus infectoria* Olivier (Fagaceae). Asian Pac. J. Trop. Biomed. 4(1): 35-39.
3. Singh S, Kumar R, Kaur H (2013) Undetermined teratogenic risks by traditional sex selection drugs. Int J Med and Dent Sci. 2(2): 239-241.
4. Ansari Shaiqua AH, Wasim A, Rizwan MK, Azhar H (2016) Ethnopharmacology of *Quercus infectoria* Olivier – Galls: A review. Hippocratic Journal of Unani Medicine. 11(3): 105-118.
5. Nahid L, Friba S, Maryam S, Nastaran K (2016) The effect of extract of oak gall for vaginal tightening and rejuvenation in women with vaginal relaxation. Open J Obstet Gynecol. 6: 879-887.
6. Fan SH, Ali NA, Basri DF (2014) Evaluation of analgesic activity of the methanol extract from the galls of *Quercus infectoria* (Olivier) in rats. Evid Based Complement Alternat Med. 2014: 1-6.
7. Jagdev Singh (2018) Quercus infectoria (Manjakani, Majuphal) benefits, uses and side effects. Ayur Times. Available online https://www.ayurtimes.com/quercus-infectoria-manjakani-majuphal-benefits-uses-side-effects/.
7. Noureddini M, Nadali M, Banafshe HR (2018) Effect of aqueous extract of *Quercus Infectoria gall* on the basic contractility, frequency and strength of isolated virgin rat uterus smooth muscle. Heath Biotechnol. *Biopharma.* 1(4):56-65.
8. Rina R, Rafiquzzaman M, Hasmah A (2011) Spectrophotometer determination of total phenol and flavanoid content in manjakani (Quercus infectoria) extracts. Health Environ J. 2(1): 9-13.
9. Rahman AA, Sulaiman SA, Ahmad Z, Daud WNW, Hamid AM (2008) Prevalence and pattern of use of herbal medicines during pregnancy in Tumpat district, Kelantan. Malays J Med Sci. 15(3): 40-48.
10. Hapidin H, Rozelan D, Abdullah H, Hanaffi WNW, Soelaiman IN (2015) *Quercus infectoria* gall extract enhanced the proliferation and activity of human fetal osteoblast cell line (hFOB 1.19). Malays J Med Sci. 22(1): 12-22.
11. Organization for Economic Co-operation and Development (2001) Guideline for the testing of chemicals No 414. Prenatal developmental toxicity study. OECD; p. 1-13.
12. Organization for Economic Co-operation and Development (2015) OECD Guideline for the testing of chemicals No. 422. Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test. OECD; p. 1-14.
13. Lee JS, Kim YH, Kim DB, Shin GH, Lee JH, Cho JH, et al. (2015) Acute and subchronic (28 days) oral toxicity studies of *Codonopsis lanceolata* extract in Sprague–Dawley rats. Regul Toxicol Pharmacol. 71(3): 491-497.
14. Okamura T, Suzuki S, Ogawa T, Kobayashi J, Kusuoka O, Hatayama K, et al. (2011) Background data for general toxicology parameters in RccHanTM: WIST rats at 8, 10, 19 and 32 weeks of age. J. Toxicol. Pathol. 24: 195-205.
15. Wan Ezumi MF (2009) Standardised aqueous extract of *Labisia pumila var. alata* (Biolabisia)- Study of its reproductive, developmental and female toxicities in rats. PhD [Dissertation]. Kubang Kerian: Universiti Sains Malaysia.
16. Bandyopadhyay S, Singh A (2007) Sex selection through traditional drugs in rural north India. Indian J Community Med. 32(1): 32-34.
17. Almeida FCG, Lemonica IP (2000) The toxic effects of *Coleus barbatus* B. on the different periods of pregnancy in rats. J Ethnopharmacol. 73(1-2): 53-60.
18. Chahoud I, Paumgartten F (2005) Relationships between fetal body weight of Wistar rats at term and the extent of skeletal ossification. Braz J Med Biol Res. 38(4): 565-575.