Journal of
Medicinal Plant Research
Volume 8 Number 44, 25 November, 2014
ISSN 2009-9723

Academic Journals

ABOUT JMPR

The Journal of Medicinal Plant Research is published weekly (one volume per year) by Academic Journals.

The Journal of Medicinal Plants Research (JMPR) is an open access journal that provides rapid publication (weekly) of articles in all areas of Medicinal Plants research, Ethnopharmacology, Fitoterapia, Phytomedicine etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMPR are peerreviewed. Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jmpr@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Medicinal Plant Research will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Prof. Akah Peter Achunike
Editor-in-chief
Department of Pharmacology & Toxicology
University of Nigeria, Nsukka
Nigeria

Associate Editors

Dr. Ugur Cakilcioglu
Elazıg Directorate of National Education
Turkey.

Dr. Jianxin Chen
Information Center,
Beijing University of Chinese Medicine,
Beijing, China
100029,
China.

Dr. Hassan Sher
Department of Botany and Microbiology,
College of Science,
King Saud University, Riyadh
Kingdom of Saudi Arabia.

Dr. Jin Tao
Professor and Dong-Wu Scholar,
Department of Neurobiology,
Medical College of Soochow University,
199 Ren-Ai Road, Dushu Lake Campus,
Suzhou Industrial Park,
Suzhou 215123,
P.R.China.

Dr. Pongsak Rattanachaikunsopon
Department of Biological Science,
Faculty of Science,
Ubon Ratchathani University,
Ubon Ratchathani 34190,
Thailand.

Prof. Parveen Bansal
Department of Biochemistry
Postgraduate Institute of Medical Education and Research
Chandigarh
India.

Dr. Ravichandran Veerasamy
AIMST University
Faculty of Pharmacy, AIMST University, Semeling - 08100,
Kedah, Malaysia.

Dr. Sayeed Ahmad
Herbal Medicine Laboratory, Department of Pharmacognosy and Phytochemistry,
Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, 110062,
India.

Dr. Cheng Tan
Department of Dermatology, first Affiliated Hospital of Nanjing Univeristy of
Traditional Chinese Medicine.
155 Hanzhong Road, Nanjing, Jiangsu Province,
China. 210029

Dr. Naseem Ahmad
Young Scientist (DST, FAST TRACK Scheme)
Plant Biotechnology Laboratory
Department of Botany
Aligarh Muslim University
Aligarh- 202 002,(UP)
India.

Dr. Isiaka A. Ogunwande
Dept. Of Chemistry,
Lagos State University, Ojo, Lagos,
Nigeria.

Editorial Board

Prof Hatil Hashim EL-Kamali
Omdurman Islamic University, Botany Department,
Sudan.

Prof. Dr. Muradiye Nacak
Department of Pharmacology, Faculty of Medicine,
Gaziantep University,
Turkey.

Dr. Sadiq Azam
Department of Biotechnology,
Abdul Wali Khan University Mardan,
Pakistan.

Kongyun Wu
Department of Biology and Environment Engineering,
Guiyang College,
China.

Prof Swati Sen Mandi
Division of plant Biology,
Bose Institute
India.

Dr. Ujjwal Kumar De
Indian Vetreinary Research Institute,
Izatnagar, Bareilly, UP-243122
Veterinary Medicine,
India.

Dr. Arash Kheradmand
Lorestan University,
Iran.

Prof Dr Cemşit Karakurt
Pediatrics and Pediatric Cardiology
Inonu University Faculty of Medicine,
Turkey.

Samuel Adelani Babarinde
Department of Crop and Environmental Protection,
Ladoke Akintola University of Technology,
Ogbomoso
Nigeria.

Dr.Wafaa Ibrahim Rasheed
Professor of Medical Biochemistry National Research Center
Cairo
Egypt.

Journal of Medicinal Plants Research

Table of Contents: Volume 8 Number 44, 25 November, 2014

ARTICLES

Research Articles

Oral Ricinus communis oil exposure at different stages of pregnancy impaired hormonal, lipids profile and histopathology of reproductive organs in Wistar rats 1289
Shakiru Ademola Salami1* and Yinusa Raji

Effect of different concentrations of plant growth regulators on micropropagation of Lantana camara 1299
Ehsan Naderi Samani, Zohreh Jabbarzadeh, Syrus Ghobadi and Marzieh Motamedi

academicJournals

Vol. 8(44), pp. 1289-1298, 25 November, 2014
DOI: 10.5897/JMPR2014.5625
Article Number: 467EC6A48944
ISSN 1996-0875
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Oral *Ricinus communis* oil exposure at different stages of pregnancy impaired hormonal, lipids profile and histopathology of reproductive organs in Wistar rats

Shakiru Ademola Salami[1]* and Yinusa Raji[2]

[1]Department of Physiology, Lagos State University, College of Medicine, Ikeja, Lagos State, Nigeria.
[2]Department of Physiology, University of Ibadan, Ibadan, Oyo State, Nigeria.

Received 15 September, 2014; Accepted 13 November, 2014

Ricinus communis **oil (RCO) has been used and shown to possess laxative, contraceptive, labour inducing, cosmetics and estrogenic capabilities. Despite these, there is paucity of studies on the effects of maternal RCO exposure at different stages of pregnancy. This study investigated effects of RCO exposure on maternal biochemical, hormonal and histopathology of reproductive organs. RCO was prepared by cold extraction using methanol and subjected to physicochemical analysis, gas chromatography (GC) and mass spectrometry (MS). Acute oral toxicity was done by limit test procedure. Twenty five pregnant rats randomly assigned to 5 equal groups were treated with distilled water (control, group 1), RCO (950 mg/kg p.o) during gestation days (GD) 1 to 7, 7 to 14, 14 to 21 and 1 to 21 (groups 2 to 5), respectively. Maternal hormonal, biochemical, and histopathology of reproductive organs were determined. Data were analyzed using Student's t-test and ANOVA. RCO showed no lethality up to 5000 mg/kg body weight. Serum alanine aminotransferase of GD 7 to 14 and 14 to 21 decreased significantly when compared with control. Aspartate aminotransferase decreased significantly in GD 1 to 7, 7 to 14, and 1 to 21. Total cholesterol, triglyceraldehyde and high-density lipoprotein increased while progesterone and estrogen levels decreased significantly in RCO treated groups. There were no lesions in the histology of the ovary of all treated groups; however, GD 1 to 7 and 7 to 14 showed resorption and ballooning of the uterine epithelial tissues, respectively. Exposure to RCO at early gestation periods impacted negatively on reproductive hormones, lipid profiles and histopathology of the uterus.**

Key words: *Ricinus communis oil*, estrogenic, gas chromatography, gestation days, lipid profiles, uterine damage.

INTRODUCTION

Castor oil plant is a member of the spurge family of plants (Euphorbiaceae). Greek physicians of the first century AD regarded the oil as suitable only for external application until the 18th century when it was listed in several

*Corresponding author. E-mail: piety1424@yahoo.com. Tel: +2348036190270.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

pharmacopoeias as a purgative (Cosmetic Ingredient Review Expert Panel, 2007). According to the International Cosmetic Ingredient Dictionary and Handbook (Gottschalck and McEwen, 2004), *Ricinus communis* (castor) seed oil is defined as the fixed oil obtained from the seeds of *R. communis*. Extracts from plants have been reported to contain a multitude of boil-ogically active compounds (Gustafsson, 2008). Ricinoleic acid accounts for 87 to 90% of the fatty acyl groups in RCO with oleic acid (2 to 7%), linoleic acid (3 to 5%), palmitic acid (1 to 2%), stearic acid (1%), dihydrostearic acid (1%), and trace amounts of other fatty acyl (TNO BIBRA International Ltd. 1999). Other sources reported 2.4% lauric acid (Larsen et al., 2001), 2 to 5% linoleic acid (Maier et al., 1999), and globulin, cholesterol, lipase, vitamin E, and *β*-sitosterol (Scarpa and Guerci, 1982). Naturally occurring phytosterols have been reported to bear tremendous similarity to synthetic steroids like corticosterone and hydrocorticosterone. National Toxicological Programme, NTP (1992) reported that groups of rats and mice fed diets containing 0.62, 1.25, 2.5, 5.0, and 10% castor oil, respectively, continuously for 13 weeks showed a slight decrease in epididymal weight (6 to 7%). Studies by Raji et al. (2006) reported a significant decrease in weight of reproductive organs, sperm functions, and serum level of testosterone in *R. communis* extract treated male rats in a dose dependent manner. Clinically, the use of *R. communis* oil as a labour inducer has been extensively reported (Davis, 1984; Mitri et al., 1987; Steingrub et al., 1988; Garry et al., 2000; Boel et al., 2010). The oil was also reported to have abortifacient activity when taken orally by pregnant women (Sani and Sule, 2007). Extracts of the seed have been tested in women and found to produce long-term contraception (Okwuasaba et al., 1991). Okwuasaba et al. (1991) also evaluated anticonceptive and estrogenic effects of a methanol extract of *R. communis* var. *minor* seeds in rabbits and rats. Increased occurrence of reproductive disorders has continued to raise concerns regarding the impact of endocrine disrupting chemicals (EDC) on reproductive health (Savabieasfahani et al., 2006). EDCs are hormonally active, synthetic, or natural compounds that are present within the environment and food sources at concentrations that can interfere with the normal activity of endocrine systems, most notably the reproductive endocrine axis (IPCS, 2002). Endocrine disrupting chemicals that can interact with estrogen receptors have received considerable attention because they can modulate signaling by native estrogen, a key regulator of several physiologic functions including repro-duction. *R. communis* oil has been shown to possess laxative, contraceptive, labour inducing and estrogenic properties. Studies have also linked agents with estrogenic properties to having endocrine disrupting capabilities with likely harmful effect (Bergstrom et al., 1996; Kavlock, 1999; Leonida et al., 2007). There is paucity of studies targeting effects of RCO in females at different gestation periods. This study investigates the effects of RCO exposure at different gestation periods on maternal biochemical, hormonal, and histopathology of reproductive organs.

MATERIALS AND METHODS

Animals

Adult Wistar male (weighing, 180 to 200 g) and female rats (200 to 250 g) obtained from the Central Animal House, Lagos State University, College of Medicine, Ikeja, Nigeria, were used for the experiments. Females were nulliparous and males used for mating were certified fertile by the isolated mating technique. Animals were allowed to acclimatize for three weeks to laboratory conditions, housed singly in cages, and fed with rats' cubes (Ladokun Feeds Limited, Ibadan, Nigeria) and water *ad libitum* for the entire duration of the study. A 12 h dark-light period was maintained throughout the study. Ethical approval on use of animal in this study was certified by the College of Medicine, University of Ibadan Animal House Committee.

Plant

Seeds of *R. communis* plant were collected from Oyo town, Oyo State, South Western Nigeria between July and August, authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan and assigned voucher number 106878.

Extraction, physicochemical analysis, gas chromatography and mass spectrometry of *R. communis* seed

Seeds of *R. communis* were air dried to a constant weight. Mortar and pestle were used to crush the beans into a paste in order to release castor fat for extraction. Pulverized seeds (1.5 kg) were extracted with 5 L of methanol by cold extraction. The pulverized seeds were soaked for 72 h after which the mixture was filtered to remove the marc. The mixture separated into 3 layers RC_A, RC_B and RC_C. RC_A was a golden coloured oily layer (368.9 g), with a yield of 24.6%, RC_B was a brownish resinous substance (20 g), and yield of 1.3%, while RC_C was a dusty brown substance (residue, 55 g), and yield of 3.7%. The separated mixtures obtained by filtration were evaporated of the solvent in a rotatory evaporator at 37°C and stored at 0°C prior to further analysis (Cosmetic Ingredient Review Expert Panel, 2007). RC_A was the major component of interest and was thus subjected to further physicochemical analysis.

Physicochemical screening, gas chromatography and mass spectrometry (GCMS) of oily fraction RC_A of *Ricinus communis* seed oil

Physicochemical analysis on the oil comprising saponification value, acid value, specific gravity and GCMS was as reported by Nkpa et al. (1989) and Ibironke et al. (2004), respectively.

Gas chromatography and mass spectrometry on RCO

Gas chromatography and mass spectrometry (GCMS) analysis of the oil was done using an Agilent Technologies 6890GC interfaced to an Agilent 5973N mass selective detector. HP-5MS column with diameter of 30×0.25 mm×1.0 µm was used with helium as carrier

gas at a flow rate of 22 ml/min. The gas chromatography oven temperature was initially held at 50°C for 5 mins then increased at 2°C/min to 250°C. The injector temperature was at 250°C with a split ratio of 1:30 and MS detector at 280°C. Percentage compositions were then obtained from electronic integration measurement using flame ionization detector at 280°C. The peak numbers and relative abundance of the chemical components with their retention time were then determined. Individual constituents of the oil were identified on the basis of their retention indices determined with a reference to a homologous series of n-alkanes and by comparison of their mass spectral fragmentation patterns (Ibironke et al., 2004).

Acute oral toxicity experiment on RCO in rats

Sequential limit test of the Organization for Economic Cooperation and Development (OECD, 2001) protocol was utilized for this study. Male and female rats were tested at both 2000 and 5000 mg/kg.

Experimental protocol

Twenty-five mature nulliparous female albino rats (10 weeks old) with normal estrous cycle were used. Male rats for mating were certified fertile by isolated mating technique and mating was confirmed by the presence of a sperm positive vaginal smear according to Stump et al. (2007). Day after which sperm positive vaginal smear was found was taken as gestational day 1 (Foster et al., 2011). Pregnant rats were then randomly assigned to treatment groups in a manner that provided for comparable body weight across groups. There were five animals per group and dosage for all groups was 950 mg/kg, which is the recommended therapeutic dose in adult humans (Drugstore.com, Inc., 2004) via oral dosing syringe except group 1 (control) which received distilled water. Group 1, control animals received distilled water, group 2 were administered RCO between gestation days (GD) 1 and 7, group 3 were administered RCO between GD 7 and14, group 4 were administered RCO between GD 14 and 21 and group 5 were administered RCO between GD 1 and 21. These treatment regimens were chosen in order to target all the critical period of intrauterine life (that is, early, mid, late and entire gestation periods). The animals were subsequently sacrificed on GD 21. Blood samples were collected via the orbital sinus for hormonal and biochemical indices. Weights of the animals were also taken once every other day; the ovary and uterus were also taken for histopathological screening.

Collection of serum from pregnant rats

Pregnant rats were bled from the orbital sinus at gestation day 21. Blood (2 ml) was then collected into polyethylene tubes and allowed to clot at 4°C for 1 h. The blood samples were then centrifuged at 3500 g for 15 min at 4°C. Serum samples were then kept at -10°C until assayed for biochemical and hormonal parameters. Animals were subsequently sacrificed via cervical dislocation.

Determination of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Serum ALT and AST were assayed according to the methods of Reithman and Frankel (1957).

Determination of lipid and lipoprotein

Total cholesterol, triglyceride, low density lipoprotein cholesterol and high density lipoprotein cholesterol levels were determined as described by Rifai et al. (1999).

Organ collection

The animals were sacrificed by cervical dislocation and dissected (from the abdominal cavity) to collect organ of interest; kidney, liver, ovary and uterus. The organs were cleared of adherent tissues, fats and then weighed immediately with an electronic weighing balance, model DT 300 with a capacity of 0.01 to 300 g.

Histopathology of the ovary and uterus

After weighing the ovary and uterus were fixed in Bouin solution, embedded in paraffin, sliced thin, stained using hematoxylin and eosin (HE), and observed under a light microscope. Photomicrographs of the slides were then taken.

Assay of estrogen and progesterone

Serum estrogen and progesterone was measured using the ELISA Test Kit (Endocrine Technologies, Newark, NJ).

Statistical analysis

Mean values, standard error of mean (Mean ± SEM), test of significance between two groups and for more than two groups by the analysis of variance (ANOVA) were all determined using Graph Pad Prism V 5.01.

RESULTS

Physicochemical analysis on RCO

The physicochemical analysis of *R. communis* oil gave an acid value of 0.154 mg KOH/g, saponification value of 139.7, percentage free-fatty acid value of 0.077, density of 0.95 g/ml at 25°C and a pale yellow viscous liquid.

Gas chromatography and mass spectrometry on RCO

Figures 1A & 1B showed the results of the gas chromatography and mass spectrometry on RCO showed the results of the gas chromatography and mass spectrometry of fixed oil isolated from the *R. communis* seed showing the relative abundance of the chemical constituents with retention time. Overall, four major constituents were identified in the fixed oil; 5.90% of 9, 12 octadecadienic acid at retention time of 15.369 s, 12.99% of 9,17 octadecadienal at retention time of 15.666 s, 46.68% of 9 octa 12 hydroxydecanoic acid at retention time of 16.626 s and finally 34.41% of ricinoliec acid at 17.049 s retention time.

Table 1. Acute oral toxicity effect of administering *Ricinus communis* oil at 2000mg/kg body weight and 5000mg/kg body weight.

No. of animal (Female)	Survival (%)	Death (%)
1st	100	0
2nd	100	0
3rd	100	0
4th	100	0
5th	100	0

Table 2. Effects of *Ricinus communis* oil on body weight of pregnant rats at different gestation periods.

Group (By gestation days) N= 5	Body weight (g)		
	1st Week	2nd Week	3rd Week
Control	204±10.30	219±14.18	232±13.93
GD 1 to 7	202±11.14	227±12.61	250±12.65
GD 7 to 14	200±7.07	229±13.64	240±20.98
GD 14 to 21	202±11.14	223±10.44	238±16.25
GD 1 to 21	202±13.93	213±12.41	230±14.14

Table 3. Biochemical parameters in pregnant rats treated with RCO at different gestation period.

Parameter	Control	GD 1 to 7	GD 7 to 14	GD 14 to 21	GD 1 to 21
ALT (U/L)	16.2±1.16	17.8±1.69	5.8±1.16***	12.0±0.84*	16.0±3.60
AST (U/L)	109.6±2.01	62.2±2.22**	49.2±5.93***	102.0±5.32	91.8±5.62*
Total cholesterol (mmol/L)	2.8±0.10	3.9±0.10***	3.4±0.13**	3.9±0.13***	3.8±0.09***
Triglyceraldehyde (mmol/L)	0.5±0.03	0.9±0.03***	0.8±0.05**	0.9±0.05***	0.7±0.02*
HDL (mmol/L)	0.7±0.13	1.4±0.13**	1.3±0.20*	1.6±0.21**	1.6±0.19**
LDL(mmol/L)	1.8±0.06	2.2±0.18	1.3±0.4**	2.0±0.16	2.0±0.11

*p< 0.05, **p<0.01 ***p< 0.001; ALT: Alanine amino transferase; AST: Aspartate amino transferase; HDL: High density lipoprotein; LDL: Low density lipoprotein.

Acute oral toxicity study

After more than 14 days observatory period, there was no death or visible physical damage (Table 1). Animals were without any visible and identifiable abnormality or mortality even at 5000 mg/kg body weight. Female rats were used though the experiment was also repeated in male rats. The results obtained for both sexes were the same.

Effect of maternal exposure to RCO on gestational weight in pregnant rats

There were no significant differences in the mean weights of pregnant rats (Table 2) treated with RCO at different gestation period when compared with control for the three weeks gestation period.

Effects of maternal exposure to RCO on serum ALT and AST

There was a significant decrease ($p<0.05$) in serum level of ALT in pregnant rats treated with RCO between gestation days 7 to 14 and 14 to 21 when compared to the control (Table 3). Serum level of ALT increased in pregnant rats treated with RCO between gestation days 1 to 7 and 1 to 21, the increase (Table 3) was however not statistically different when compared with the control. There were statistically significant decreases from control (Table 3) in the serum AST levels of pregnant rats treated with RCO between gestation days 1 to 7, 7 to 14, and 1 to 21($p<0.01$). Pregnant rats treated with RCO between gestation days 14 and 21 also showed decrease in serum aspartate aminotransferase. The decrease was however not statistically different from the control.

Table 4. Serum estrogen and progesterone levels in pregnant rats treated with RCO at different gestation periods.

Serum level	Control	GD 1 to 7	GD 7 to 14	GD14 to 21	GD 1 to 21
Estrogen (ng/ml)	24.5	19.1*	15.7**	15.9**	14.7***
Progesterone (ng/ml)	52.0	45.0*	11.0***	40.0*	33.0**

*p<0.05, **p<0.01, ***p<0.001.

Table 5. Organ weight in pregnant rats treated with RCO (950 mg/kg) at different gestation periods (GD= gestation days).

Organ weight (g)	Control (N=5)	GD 1 to 7 (N=5)	GD 7 to 14 (N=5)	GD 14 to 21 (N=5)	GD 1 to 21 (N=5)
Ovary	0.03±0.00	0.02±0.07	0.03±0.00	0.03±0.01	0.06±0.01
Uterus	0.09±0.02	0.02±0.07	0.14±0.02	0.07±0.02	0.13±0.03
Kidney	0.28±0.03	0.26±0.01	0.30±0.01	0.3±0.07	0.22±0.01
Liver	3.80±0.60	3.10±0.10	3.30±0.20	3.60±0.40	3.00±0.20

*P<0.05, **p<0.01, ***p<0.001

Table 6. Numbers of life/dead fetuses at sacrifice in pregnant rats treated with RCO at different gestation periods.

Group	Life	Dead
Control	7	0
GD 1 to 7	1	4
GD 7 to 14	1	3
GD 14 to 21	2	4
GD 1 to 21	1	5

Effects of maternal RCO exposure on serum lipid profile

There were statistically significant increases (Table 3) in the serum levels of total cholesterol in pregnant rats treated with RCO between gestation days 1 to 7, 7 to 14, 14 to 21 and 1 to 21 ($p<0.01$). As shown in Table 3, there were statistically significant increases when compared with the control in the serum triglyceraldehyde content in pregnant rats treated with RCO at gestation days 1 to 7, 7 to 14, 14 to 21 and 1 to 21 ($p<0.01$). There were also statistically significant increases when compared with the control (Table 3) in the serum high density lipoprotein level in pregnant rats treated with RCO at gestation days 1 to 7, 7 to 14, 14 to 21 and 1 to 21 ($p<0.05$ and 0.01). There was a statistically significant decrease when compared with the control (Table 3) in serum low density lipoprotein levels in pregnant rats treated with RCO between gestation days 7 and 14 ($p<0.05$). However, increases in low density lipoprotein levels were not statistically significant for pregnant rats treated between gestation days 1 to 7, 14 to 21 and 1 to 21 (Table 3).

Effects of maternal exposure to RCO on serum progesterone and estrogen level

There were significant decreases ($p<0.05$) in the progesterone and estrogen levels in all RCO treated groups (Table 4).

Effects of maternal exposure to RCO on absolute mean organ weights of treated pregnant rats

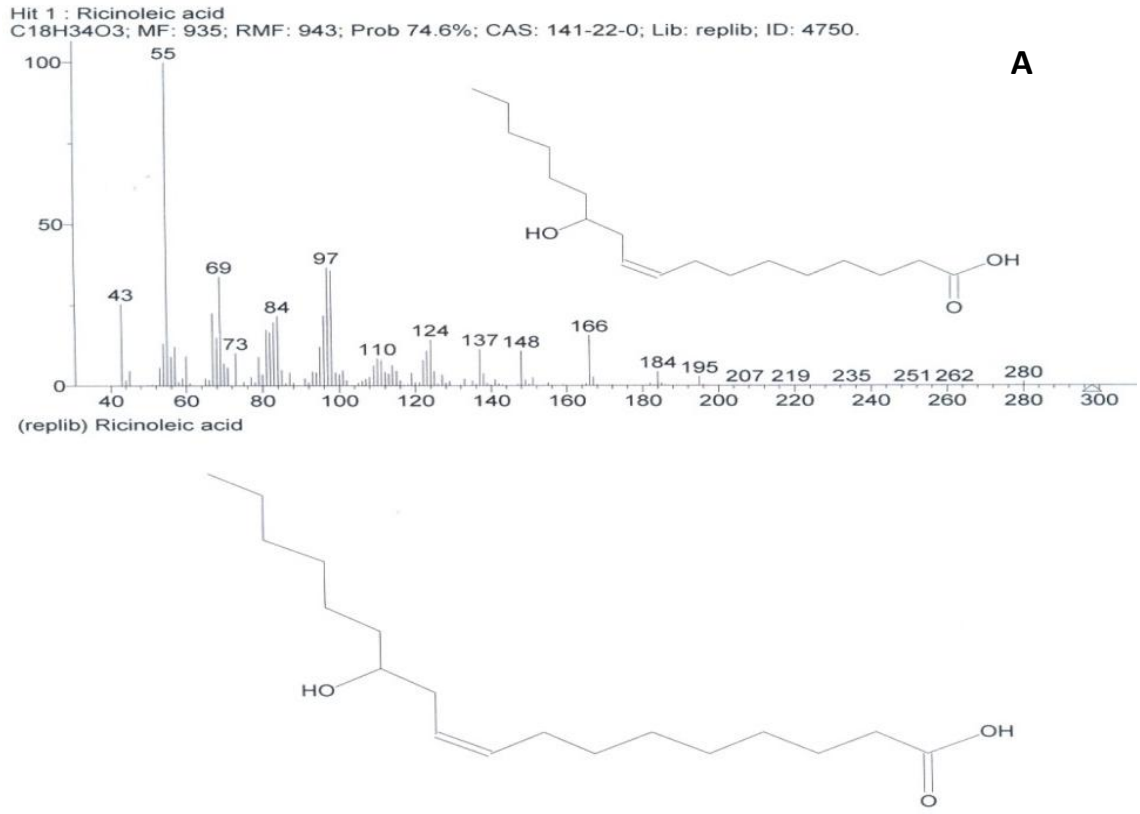
As shown in Table 5, there were no significant changes in the mean organ weight between treated pregnant rats and untreated pregnant control.

Effects of maternal exposure to RCO on histology of the ovary/uterus and life death numbers of fetus at sacrifice in treated rats

There were no lesions in the ovary of female rats from control and RCO treated groups as shown in photomicrographs A to E (Figure 2). However, histology of uterus of female rats from gestation days 7 to 14 showed ballooning of the uterine epithelia cells (Figure 3, plate C) and implantation sites with resorptions for female rats from GD 1 to 7 (plate B) and GD 14 to 21 plate D (Figure 3). Table 6 showed the number of life/dead fetuses. Treated groups with RCO had more dead fetuses with control having none.

DISCUSSION

The route of administration of RCO in this study was in accordance with the route of possible human exposure during pregnancy when used as a laxative or labour



B

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.369	5.92	C:\DATABASE\NIST08.L			
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	132273	000112-63-0	99
			8,11-Octadecadienoic acid, methyl ester	132258	056599-58-7	99
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	132274	000112-63-0	99
2	15.666	12.99	C:\DATABASE\NIST08.L			
			9,12-Octadecadienoic acid (Z,Z)-	121228	000060-33-3	99
			9,12-Octadecadienoic acid (Z,Z)-	121227	000060-33-3	95
			9,17-Octadecadienal, (Z)-	108922	056554-35-9	93
3	16.626	46.68	C:\DATABASE\NIST08.L			
			Methyl ricinoleate	145844	000141-24-2	94
			9-Octadecenoic acid, 12-hydroxy-, methyl ester, (Z)-	145871	127062-53-7	87
			Methyl 12-hydroxy-9-octadecenoate	145850	1000336-28-8	83
4	17.049	34.41	C:\DATABASE\NIST08.L			
			Ricinoleic acid	135272	000141-22-0	91
			Ricinoleic acid	135270	000141-22-0	90
			Ricinoleic acid	135271	000141-22-0	83

Figure 1. (a) Fragmentation pattern and structure of ricinoliec acid for RCO from gas chromatography and mass spectrometry, (b) Nomenclature of individual constituents of RCO from gas chromatography and mass spectrometry.

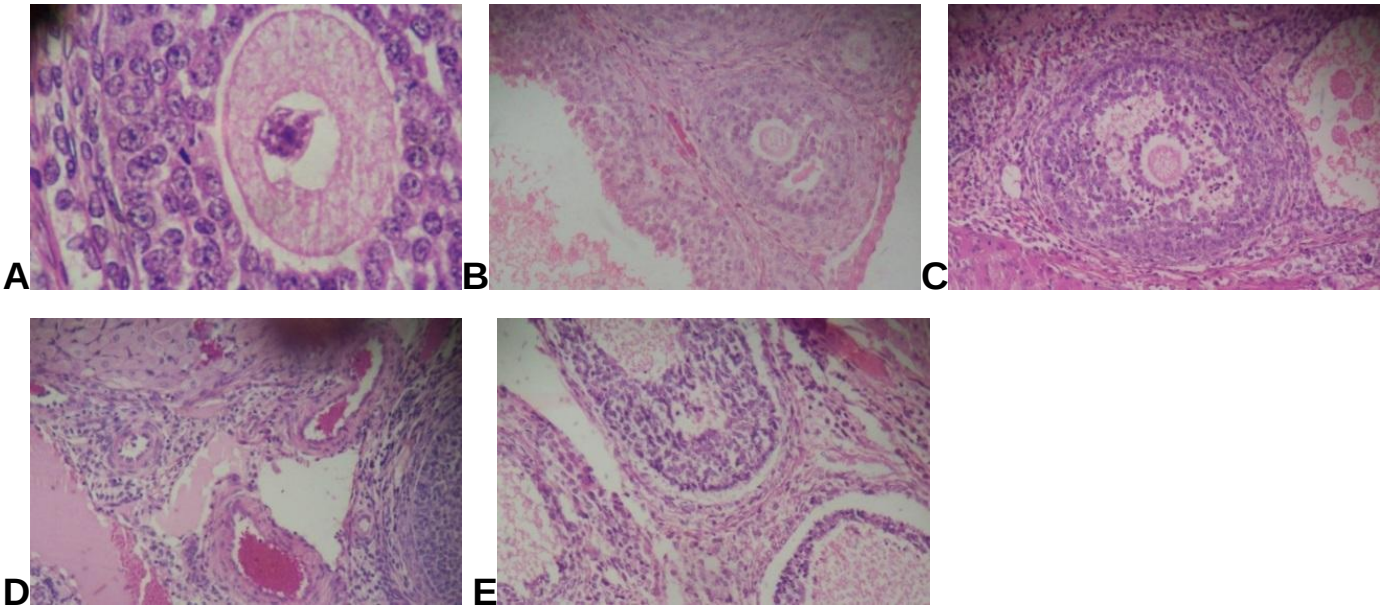


Figure 2. Photomicrographs of the ovary from A, control pregnant rats, no visible lesion, B, exposed between gestation days (GD) 1 to 7, no visible lesion, C, exposed between gestation days (GD) 7 to 14, no visible lesion, D, exposed between gestation days (GD) 14 to 21, no lesions and E, exposed between gestation days (GD) 1 to 21, no visible lesions (magnification X 400).

this route of administration has been extensively delineated and found to be the same in human and rats (Paul and McCay 1942; Watson and Gordon, 1962; Thompson, 1980; Ihara-Watanabe et al., 1999). Dosage used in this study was also according to recommended therapeutic human dose (Drugstore.com, Inc 2004). Data obtained from physicochemical properties, gas chromatography and mass spectrometry were within the range of those reported from previous studies (Kato and Yamaura, 1970; Larsen et al., 2001; National Toxicological Programme, NTP, 2003) except saponification value and percentage of Ricinoleic acid which is quite lower in this study than reported values. This could be due to the fact that geographical distribution and individual soil characteristics have been found to influence percentage availability of individual constituents of plant (Rafieiolhossaini et al., 2008). The seed oil used in this study was from South-West Nigeria with peculiar weather and soil distribution as compared to seed oil from geographical locations of other studies. To the best of our knowledge, this study was the first on the physiochemical and GCMS characteristics of a typical *R. communis* plant seed from South-Western Nigeria.

For the current study, RCO satisfied the criteria for the use of limit test of the up and down procedure of the Organization for Economic Cooperation and Development (OECD 425, 2001). There was no lethality when animals were treated at limit dose of 2000 and 5000 mg/kg body weight showing a wide safety margin for RCO when ingested orally. Testing at 5000 mg/kg body weight was discouraged except for a strong likelihood that such result would have direct relevance for protecting human/animal health and environment (OECD 425, 2001). Availability of RCO as “over the counter drug” (OTC) and the possibility of abuse propelled the test at 5000 mg/kg body weight in this study. An acute oral LD_{50} greater than 10 g/kg was reported by Allegri et al. (1981) for hydrogenated castor oil. In other studies involving incorporation of up to 10% RCO in diets (Masri et al., 1962; NTP, 1992; Ihara-Watanabe et al., 1999) and intravenous administration of 0.1 ml/kg body weight of RCO (Lorenz et al., 1982), no gross abnormalities or significant effects were observed on survival of groups of male and female rats. There were no significant differences in the body weight of pregnant rats treated with RCO at different gestation periods when compared with control. Though there was weight gain, it was not statistically different from that of control. This could be attributed to the fact that feeding habits between RCO treated pregnant rats and control were not different throughout the duration of gestation. This corroborates studies of Masri et al. (1962) and NTP (1992), where 10% castor oil fed male and non-pregnant female rats for 5 and 13 weeks, respectively led to no significant differences in food consumption and mean body weights between test and control groups.

Serum ALT and AST decreased significantly ($p<0.05$) in pregnant rats exposed to RCO at gestation days (7 to14, 14 to 21) and (1 to 7 and 1 to 21) for ALT and AST, respectively. Elevated ALT and AST levels have been implicated in most liver diseases while AST have been found to be mostly of extra hepatic origin with its level rising

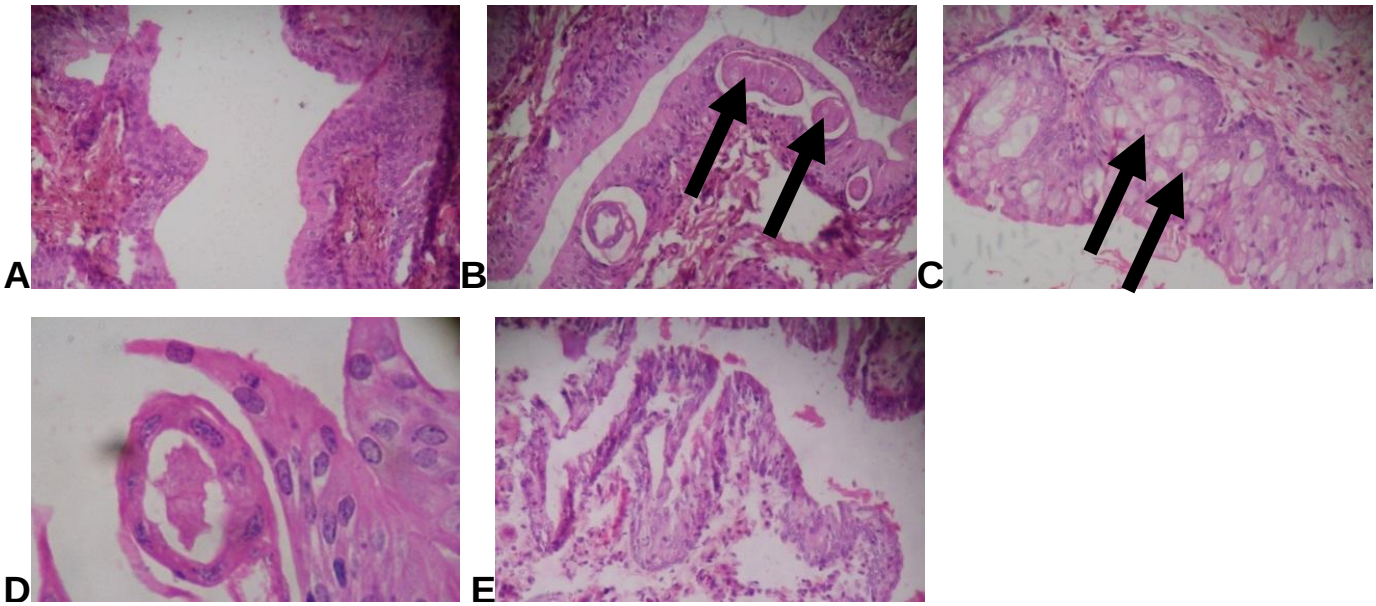


Figure 3. Photomicrographs of the uterus from A, control pregnant rat, no visible lesion, B, rat exposed between gestation days (GD) 1 to 7, with implantation sites present but no lesions, C, rats exposed between gestation days (GD) 7 to 14, with ballooning of some of the epithelial cells, D, rats exposed between gestation days (GD) 14 to 21 with no lesions, fewer implantation sites with foci, and E, rats exposed between gestation days (GD) 1 to 21 with no visible lesion (magnification × 400).

rising in heart disease (Pradumna et al., 2009). The fact that these two enzymes that are markers of hepato-cellular injury were not elevated showed that RCO possibly has no hepato-cellular toxic effect. Coupled with this was the fact that liver weight of treated and control rats also showed no significant difference (Table 5). Studies (Masri et al., 1962; Lorenz et al., 1982; NTP, 1992) have shown that RCO has no acute intravenous, short term oral or sub chronic oral toxicity.

In this study, serum cholesterol levels were significantly elevated ($p<0.05$) in pregnant rats treated with RCO when compared with control (Table 3). Hypercholesterolaemia observed in this study could partly account for the decreased estrogen and progesterone in this study. Hypercholesterolaemia has been implicated as a possible factor for hormonal imbalance as reported by Kenji and Nikolaos (1998) in their study where cholesterol enriched diet causing hypercholesterolaemia was found to impair peripheral Leydig cell testosterone responses to testicular stimulation with human chorionic gonadotropin. Similarly, elevated levels of total lipids observed in this study corroborated the study of Kenji and Nikoloas (1998) where hypercholesterolaemia was also associated with elevated level of total lipids. Pregnant rats exposed to RCO in this study also showed significantly reduced serum levels of progesterone and estrogens when compared with control. Major hormones produced by the feto-placental unit are progesterone, estradiol, estriol, human chorionic gonadotrophin and human placental lactogen (Lording and De Kretser, 1972). The major estrogen produced during human pregnancy is estriol and elevated estriol levels indicate fetal wellbeing. Progesterone and estrogen have numerous beneficial functions in ensuring the survival of the developing fetus throughout gestation period. Estrogen enhances fetal organ development, stimulates maternal hepatic protein production, increase mass of breast and adipose tissue and also increase the size of the uterus and uterine blood flow which are critical in the timing of implantation of the embryo (Rodney and Bell, 2009). Progesterone is essential for maintaining the uterus and early embryo, inhibits myometrial contraction and suppresses maternal immunological responses to fetal antigens (Rodney and Bell, 2009). The significantly reduced serum levels of estrogen and progesterone of treated pregnant rats in this study with the attendant compromise of their normal functions might be responsible for the impaired maternal histology of the uterus, fetal resorptions and deaths in RCO treated rats. The reduced estrogen and progesterone could however be as a result of RCO induced damage to the uterine wall (as shown in Figure 3) with a subsequent impairment in the normal secretory functions of the uterus that in conjunction with the foetus form the feto-placental unit.

Histology of the uterus in pregnant rats exposed to RCO at gestation days 1 to 7 and 7 to 14 showed ballooning of the uterus, uterine tissues disruption, and resorption with implantation sites indicating possible compromise of the uterine support for the developing embryo. These findings could be responsible for reduced litter size and weight experienced in litters from this group

in the follow-up study. Fowden et al. (2006) posited that changes that could impair intra uterine availability of nutrients, oxygen and hormones usually program tissue development leading to abnormalities later at adulthood. The timing, duration, severity and type of insult during development have also been found to be contributing factor to the type of physiological outcome.

In conclusion, pregnant rats exposed orally to RCO in this study particularly during early gestation periods showed impaired lipid profiles, hormonal balance and uterine histology. These may be due to ricinoliec acid and sterols which from gas chromatography and mass spectrometry analysis in this study constitute greater percentage of the fixed oil of *R. communis* seed. Thompson (1980) has delineated the pathway of enzymatic degradation of RCO by reporting that pancreatic lipase acts on RCO to liberate glycerol and ricinoliec acid. The ricinoliec acid is then rapidly metabolized. Onwuluri and Anekwe (2001) have also attributed that the presence of sterols in RCO is important in that such sterols as steroid alcohol can act as intermediate in the synthesis of related steroids. More so some steroids have been found to be convertible into animal steroids hormone in the presence of relevant enzymes *in-vivo* (Green et al., 1995). Hence, the effects of RCO observed in this study are probably due to sterols and ricinoliec acid as identified in the gas chromatography and mass spectrometry analysis of the oil.

Conflict of Interest

Authors have not declared any conflict of interest.

REFERENCES

Allegri R, Deschel GC, Filippi V, Gibellini M, Portioli E, Veneri D (1981). Proposal for the pharmacopoeia: hydrogenated castor oil translation. Boll. Chim. Farm. 120:557-558.

Bergstrom R, Adami HO, Mohner W, Zatonski W, Storm H, Ekbom A, Tretli S, Teppo A, Akre A, Hakulinen T (1996). Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. J. Natl. Cancer Inst. 88:727-733.

Boel ME, Lee SJ, Rijken MJ, Paw MK, Pimanpanarak MT, Saw O, Singhasivanon P, Nosten F, McGready R (2010). Castor Oil for Induction of Labor: Not Harmful, Not Helpful. Obstet. Gynecol. Surv. 65(2):77-78.

Cosmetic Ingredient Review Expert Panel (2007). Final Report on the Safety Assessment of *Ricinus communis* (Castor) seed Oil, Hydrogenated Castor Oil, Ricinoleic Acid, Potassium Ricinoleate, Sodium Ricinoleate, Zink, and Octyldodecyl Ricinoleate. Glyceryl Ricinoleate, Methyl Ricinoleate, Cetyl Ricinoleate, Ethyl Ricinoleate, Glycol Ricinoleate, Isopropyl Ricinoleate. Int. J. Toxicol. 26(suppl. 3):31-77.

Davis L (1984). The use of castor oil to stimulate labor in patients with premature rupture of membranes. J. Nurse Midwifery 29:366-370.

Drugstore.com Inc (2004). Rite Aid Castor oil, stimulant laxative U.S.P. Package details: Directions. http://www.drugstore.com.

Foster WG, Lagunova A, Anzarb M, Sadeua JC, Khanb MIR, Bruina JE, Woynillowicza AK, Buhrc M, Hollowaya AC (2011). Effect of *in utero* and lactational nicotine exposure on the male reproductive tract in peripubertal and adult rats. Reprod. Toxicol. 31:418-423.

Fowden AL, Giussani DA, Forhead AJ (2006). Intrauterine Programming of Physiological Systems: Causes and Consequences. Physiology 21(1):29-37.

Garry DR, Figueroa J, Guillaume P, Cucco V (2000). Use of castor oil in pregnancies at term. Altern. Ther. Health Med. 6:77-79.

Gottschalck TE, McEwen GN Jr (2004). International Cosmetic Ingredient Dictionary and Handbook,10th ed. Washington, DC: CTFA.

Green NPO, Stout GW, Taylor DJ (1995). Biological Sciences 2nd Edition. Cambridge University Press.

Gustafsson JA (2008). Plant derived ligands of nuclear receptors and their biological effects. Plant Med. 74:1-338.

Ibironke A Ajayi, Rotimi A Oderinde, Victor O Taiwo and Emmanuel O Agbedana (2004) Dietary effects on growth, plasma lipid and tissues of rats fed with non-conventional oil of *Telfairia occidentalis*. J. Sci. Food Agric. 84:1715-1721.

Ihara-Watanabe M, Shiroyama T, Konda G, Umekawa H, Takahashi T, Yamada T, Furuichi Y (1999). Effects of castor oil on lipid metabolism in rats. Biosci. Biotechnol. Biochem. 63:595-597.

International Programme on Chemical Safety (IPCS) (2002). Global assessment of the state-of-the-science of endocrine disruptors. Geneva: World Health Organization.

Kato A, Yamaura Y (1970). A rapid gas chromatographic method for the determination of fatty acid compositions of soybean oil and castor oil. Chem. Ind. 39:1260.

Kavlock RJ (1999). Overview of endocrine disruptor research activity in the United States. Chemosphere 39:1227-1236.

Kenji S, Nikolaos S (1998). Effects of hypercholesterolaemia on testicular function and sperm physiology. Yonago acta Medica 41:23-29.

Larsen SW, Rinvar E, Svendsen O, Lykkesfeldt J, Friis GJ, Larsen C (2001). Determination of the disappearance rate of iodine-125 labelled oils from the injection site after intramuscular and subcutaneous administration to pigs. Int. J. Pharm. 230:67-75.

Leonida F, Daniele DS, Francesco D-F (2007). Altered reproductive success in rat pairs after environmental-like exposure to xenoestrogen. Proc. R. Soc. B. 274:1631-1636.

Lording DW, De Kretser DM (1972). Comparative ultrastructural and histochemical studies of the interstitial cells of the rat testis during fetal development and postnatal development. J. Reprod. Fert. 29:261-269.

Lorenz W, Schmal A, Schult H (1982). Histamine release and hypotensive reactions in dogs by solubilizing agents and fatty acids: Analysis of various components in cremophor El and development of a compound with reduced toxicity. Agents Actions 12(1-2):64-80.

Maier M, Staupendahl D, Duerr HR, Refior HJ (1999). Castor oil decreases pain during extracorporeal shock wave application. Arch. Orthop. Trauma Surg. 119:423-427.

Masri MS, Goldblatt LA, DeEds F, Kohler GO (1962). Relation of cathartic activity to structural modifications of ricinoleic acid of castor oil. J. Pharm. Sci. 51:999-1002.

Mitri F, Hofmeyr GJ, Van Geldren CJ (1987). Meconium during labor-self-medication and other associations. SAMJ 431-433.

National Toxicology Program (NTP) (1992). NTP technical report on the toxicity studies of castor oil (CAS No. 8001-79-4).

National Toxicology Program (NTP) (2003). NTP Chemical Repository. Castor Oil. Research Triangle Park, NC: NTP.

Nkpa NN, Arowolo TA, Akpan HJ (1989). Quality of Nigerian palm oil after bleaching with local treated clays. J. Am. Oil Chem. Soc. (JAOCS) 66(2):218-222.

OECD guideline for testing of chemicals acute oral toxicity-up-and-down procedure 425 adopted: 17th December 2001.

Okwuasaba FK, Osunkwo UA, Ekwenchi MM (1991). Anticonceptive and estrogenic effects of a seed extract of *Ricinus communis* var. minor. J. Ethnopharmacol. 34:141-145.

Paul H, McCay CM (1942). The utilization of rats by herbivora. Arch. Biochem. 1:247-253.

Pradumna J, Amir A, Tarun G, Philip B (2009). Liver function test and pregnancy. J. Matern-Fetal Neonatal Med. 22(3):274-283.

Rafieiolhossaini M, Adams A, De Kimpe N, Van Damme P (2008). Influence of soil nitrogen level and plant spacing on essential oil content and composition of German chamomile (*Matricaria chamomilla*). Planta Medica 74:310.

Raji Y, Oloyo AK, Morakinyo AO (2006). Effect of methanol extract of *Ricinus communis* seed on reproduction of male rats. Asian J. Androl. 8(1):115-121

Reithman S, Frankel S (1957). A colorimetric method for determination of serum glutamate oxaloacetate and pyruvate transaminase. Am. J. Clin Pathol. 28: 56-63.

Rifai N, Bachorik PS, Albers JJ (1999). Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER (Eds.), Tietz Textbook of Clinical Chemistry. WB Saunders, Philadelphia, Pennsylvania. pp. 809-861.

Rodney AR, Bell DR (2009). Medical Physiology Principles for clinical Medicine 3rd Edition Lippincott Williams and Wilkins. pp. 688-725.

Sani UM, Sule MI (2007). Anti-Fertility activity of Methanol extracts of Three different seed varieties of *Ricinus Communis* Linn (Euphorbiaceae). Nig. J. Pharm. Sci. 6:78-83.

Savabieasfahani M, Kurunthachalam K, Olga A, Neil PE, Vasantha P (2006). Developmental Programming: Differential Effects of Prenatal Exposure to Bisphenol-A or Methoxychlor on Reproductive Function. Endocrinology 147(12):5956-5966.

Scarpa A, Guerci A (1982). Various uses of the castor oil plant (*Ricinus communis* L.). A review. J. Ethnopharmacol. 5:117-137.

Steingrub JS, Lopez T, Teres D, Steingart R (1988). Amniotic fluid embolism associated with castor oil ingestion. Crit Care Med16:642-643.

Stump DG, Joseph FH, Sandra RM, Craig HF, Bruno S, Motoki S (2007). An oral two generation reproductive toxicity study of S-111-S-WB in rats. Reprod. Toxicol. 25:7-20.

Thompson WG (1980). Laxatives: Clinical pharmacology and rational use. Drugs 19:49-58.

TNO BIBRA International Ltd. (1999). Toxicity profile. Castor oil. Surrey, United Kingdom: TNO BIBRA International Ltd. p. 9.

Onwuluri VA, Anekwe GE (2001). Amino acids and other Biochemical components of *Ricinus communis* (Variety minor), an Anti-conceptive seed. Pak. J. Biol. Sci. 4(7):866-868.

Watson WC, Gordon RS Jr (1962). Studies on the digestion, absorption and metabolism of castor oil. Biochem. Pharmacol. 11:229-236.

academicJournals

Vol. 8(44), pp. 1299- 1303, 25 November, 2014
DOI: 10.5897/JMPR2014.5517
Article Number: 6822B5448945
ISSN 1996-0875
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Effect of different concentrations of plant growth regulators on micropropagation of *Lantana camara*

Ehsan Naderi Samani[1], Zohreh Jabbarzadeh[1]*, Syrus Ghobadi[2] and Marzieh Motamedi[3]

[1]Department of Horticultural Science, College of Agriculture, Urmia University, Orumieh, Iran.
[2]Department of Horticultural Science, College of Agriculture, Isfahan University of Technology, Isfahan, Iran.
[3]Department of Agronomy, College of Agriculture, Isfahan University of Technology, Isfahan, Iran.

Received 16 July, 2014; Accepted 13 October, 2014

***Lantana camara* is a plant with numerous medicinal properties and belongs to Verbenaceae family. Too few studies on micropropagation of this plant exist in the literature. This study was designed and conducted to investigate the effective factors on *in vitro* shoot proliferation of *L. camara*. This experiment was conducted in Isfahan University of Technology, Isfahan, Iran. In this study, nodal segments of *L. camara* were used as explants and woody plant medium (WPM) as culture medium. The explants were placed in culture medium of WPM containing 6-benzyladenine (BA) and thidiazuron (TDZ) in various concentrations for shoot proliferation and MS medium containing different concentrations of indole-3-acetic acid (IAA) for rooting. After four weeks, different indices of shoot proliferation and rooting were investigated. The experiments' data were analyzed by Statistical Analysis System (SAS) software and the means were compared using Duncan's multiple range test (DMRT) at 5% probability level. The results indicated that the highest shoot and internode length, fresh and dry weight and the maximum number of leaves were obtained in control treatment. The maximum number of shoots was observed in 8 mg/L BA and the highest fresh weight was in 4 mg/L BA. Cytokinins, particularly BA, in high concentrations cause apical dominance to be overcome through declining auxin effect, and proliferation was increased. Therefore, shoot and internode length was decreased with increasing BA concentration. The most number of roots was also obtained in treatment with 0.5 mg/l IAA. The increase in roots' number occurs because of hydrolysis of foods and their transfer to the sprouts.**

Key words: Nodal segments, shoot proliferation, thidiazuron, woody plant medium.

INTRODUCTION

Lantana camara is an evergreen shrub and has heart shaped leaves with rough surface and serrated leaves. Tropical and subtropical regions are suitable for *L. camara* growth. In traditional medicine, it is used for treating leprosy, influenza, asthma, bronchitis and some other diseases. Antifungal, antibacterial, insecticidal and

*Corresponding author. E-mail: Z.jabbarzadeh@urmia.ac.ir.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

anticancer activity of *L*. *camara* secondary metabolites has been demonstrated by different researchers (Srivastava et al., 2011; Affonso et al., 2007; Passos et al., 2009). The germination percentage of *L. camara* seeds is very low. Layering and cutting are other ways of propagation of *L. camara* which have their own problems; such that a few plants were produced with these propagules. Micropropagation is one of the ways to mass propagate of plants and much study about *L. camara* has not been done. This experiment was conducted to study the effects of various concentrations of growth regulators to increase proliferation and rooting ability of *L. camara* for medicinal use.

Affonso et al. (2007) used Murashige and Skoog (MS) medium for *L*. *camara* micropropagation and reported 4.4 µmol/L 6-benzyladenine (BA), accompanied with 0.44 µmol/L thidiazuron (TDZ), which caused a significant decrease in shoot length and root formation, but 4.4 µmol/L BA alone caused increase in shoot and nodes number of each explant. In a research, woody plant medium (WPM) was used for *Ulmus parvifolia* Jacq. And it was reported that using 0.5 mg/L BA in this medium was suitable for growing and propagating the shoots obtained from nodal segments of this plant. Also, 0.5 mg/L TDZ and 2 mg/L N-(2-chloro-4-pyridyl)-N-phenylurea (4-CPPU) caused increase in nodal development in this plant. WPM containing 1 mg/L 1-naphthalene acetic acid and sucrose is the best treatment for rooting of this plant (Thakur and Karnosky, 2007).

A research on *in vivo* culture of a guava species offered woody plant medium (WPM) supplemented with 2 mg/L BA as the best medium for shoot induction while WPM with 1 mg/L BA was suitable for proliferation and shoot length, and the effect of MS, WPM, 1/2 MS and B5, as culture media, was found to be significant on shoot quality (Meghwal et al., 2003). Babu et al. (2003) used WPM culture containing active charcoal and various auxin and cytokinin hormones for *in vitro* proliferation of *Cinnamomum camphora*.

Direct shoot regeneration from nodal segments, internode, hypocotyl and embryo of *Withania somnifera* was investigated in MS medium. The required concentration and the type of cytokinin is different and dependent on the type of the explant: nodal segments in BA (0.1 to 5.0 mg/L) and TDZ (0.2 and 0.3 mg/L), internode explants in BA (1 and 5 mg/L), hypocotyl explants in BA (0.5 mg/L) and embryonic explants in TDZ (0.2 and 0.3 mg/L) generated shoot. The generated shoots, rooted in MS medium containing BA (0.01 mg/L) or 1/2 MS with no plant growth regulators (Kulkarni et al., 2000).

For *Vitex trifolia* treated with BA, Kin, 2-iP, TDZ and adenine, the highest number of shoots in each explants (nodal segments) was reported for treatment with 5 mg/L BA in MS medium (Hiregoudar et al., 2006). For *Tectona grandis*, the best regeneration of calli was obtained from internodes in MS medium containing 10 mg/L BA and 1 mg/L gibberellic acid. For proliferation of the generated shoots, they were subcultured in MS medium containing 10 mg/L BAP (Widiyanto et al., 2005). In a study, the effect of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) was investigated on root propagation of Gisela 5' from *Prunus* genus, reporting that IBA inhibited callus formation in initial days of root formation and produced stronger roots in larger numbers. Both growth regulators caused the increase in rooting percentage as compared to the control treatment (Stefanicic et al., 2005). The treatment with 0.5 mg/L IAA has been offered as an appropriate treatment for *Digitalis lamarckii* rooting (Verma et al., 2011). Rathore et al. (2008) reported that 1 mg/L IBA was the most appropriate treatment for rooting of *Terminalia bellerica*.

MATERIALS AND METHODS

This experiment was conducted in the Horticultural Sciences Department of Isfahan University of Technology, Isfahan, Iran. Plant material was supplied from Shahre Golha greenhouse of Isfahan. The nodal segments (10 mm in length) of the current year's shoots were excised and washed using dishwashing liquid in 2/1000 ratio and placed under running water for four hours. For disinfection of plant materials, 20% sodium hypochlorite for twenty minutes and 70% alcohol for one minute were used and then explants were rinsed three to six times using sterile distilled water. After cutting the damaged parts caused by the disinfectant solution using sterile scalpel, the single buds were cultured in the medium as explants. To identify the best medium for proliferation, WPM containing different concentrations of TDZ and BA (0, 2, 4 and 8 mg/L) and to find the best medium for rooting, MS containing different concentrations of IAA (0, 0.25 and 0.5 mg/L) was used. The culture was kept at 24°C and 1000-lux light for 30 days. To assess proliferation in different treatments, the number and length of shoots, number of nodes, internode length, number of leaves, fresh and dry weight of shoots were measured at the end of period; also, the number of primary and secondary roots, root length, primary root diameter, fresh and dry weight of roots were measured at the end of period to assess rooting. The proliferation experiments were based on a factorial design with 2 factors: type of cytokinin with 2 levels (BA and TDZ) and concentration of cytokinin with 4 levels (0, 2, 4 and 8 mg/L) and the rooting experiments were conducted on a completely randomized design. These experiments were conducted with 3 replications and 3 observations for each replication. The experiments' data were analyzed by SAS (version 9.1) software and the means were compared using Duncan's multiple range test (DMRT) at 5% probability level.

RESULTS AND DISCUSSION

Shoot proliferation

As shown in Table 1, BA caused increase in number of shoots and some shoot proliferation factors, but TDZ acted as an inhibitory agent in all factors. The most number

Table 1. Comparison of mean effect of different BA concentrations on proliferation indices.

PGRs type	Concentration of PGRs (mg/l)	Number of shoots	Shoot length	Number of nodes	Internode length	Number of leaves	Fresh weight	Dry weight
BAP	0	2.22[c*]	1.04[a]	3.11[a]	0.33[a]	7.52[a]	0.46[b]	0.08[a]
	2	4.22[b]	0. 8[b]	2.90[b]	0.27[b]	4.46[c]	0.47[b]	0.05[c]
	4	4.44[b]	0.61[c]	2.57[c]	0.23[c]	4.80[b]	0.50[a]	0.06[b]
	8	4.77[a]	0.60[c]	3.13[a]	0.19[e]	7.45[a]	0.41[c]	0.04[d]
TDZ	0	2.22[c]	1.04[a]	3.11[a]	0.33[a]	7.52[a]	0.46[b]	0.08[a]
	2	1.00[d]	0.25[d]	1.16[d]	0.21[d]	3.33[d]	0.02[d]	0.002[e]
	4	0.46[e]	0.14[e]	0.91[e]	0.13[f]	1.63[f]	0.01[d]	0.001[f]
	8	0.45[e]	0.13[e]	0.83[f]	0.12[f]	2.31[e]	0.01[d]	0.001[f]

*In each column dissimilar letters indicate significant difference at 5% level.

of shoots and nodes was noted in 8 mg/L BA treatment, the highest shoot length, internode length, the highest dry weight and the most number of leaves was observed in control treatment and the highest fresh weight in 4 mg/L BA treatment. The least value of the indices was seen in 8 mg/L TDZ treatment. Cytokinins in appropriate concentration caused a considerable increase in DNA and RNA and subsequently increase in protein synthesis (Mok and Mok, 2001). Elimination of lateral bud dormancy, induction of adventitious bud formation, lateral bud growth and control of cell division cycle are some other functions of cytokinins inside the plant (Gaspar et al., 2003). Cytokinins, particularly BA, in high concentrations cause apical dominance to be overcome through declining auxin effect. This could be one of the reasons for decreased length of shoot and internode through increasing in BA concentration in the present study. On the other hand, the competition among shoots for nutrients could be the reason for decreased shoot growth as BA concentration increases.

The findings of this study are consistent with those of others, indicating that the difference in BA concentration could be due to genetic factors and laboratory conditions. Throughout some research conducted by Affonso et al. (2007) on *L. camara*, introduction of 4.4 µmol/L BA caused increase in the number of shoots and nodes per explant. On the other hand, addition of 4.4 µmol/L BA and 0.44 µmol/L TDZ into MS culture medium caused a significant decrease in length of shoots and formation of the roots, indicating the importance of BA in inducing proliferation and cellular division in the cultured explants and formation of limbs in BA-treated tissues. 5 and 10 mg/L BA caused increase in number of shoots in *Adhatoda vasica* after two weeks but inhibited the growth of explants (Abhyankar and Reddy, 2007).

Dziedzic (2008) employed lateral buds of *Lonicera caerulea in vivo* and reported that the fresh weight obtained from the growth of explants and the number of formed shoots in culture medium depended on the plant genotype, the type of culture medium and BA concentration. One of the effects by cytokinins is cellular division and the young, proliferated cells have a large vacuole and contain lots of water (Opik and Rolfe, 2005). Increase in BA concentration causes increase in cellular division and since these cells do not grow and do not possess the remaining thick wall, their water is evaporated throughout drying process and hence dry weight declines.

Several researchers have used very low concentrations of TDZ for woody plants' proliferation (Rai, 2002; Sharma and Shahzad, 2008). The type and condition of the explant, the domestic hormones' levels and growth regulators' application and interactions with healthy tissues are among the factors contributing to *in vivo* regeneration and shoot generation (Lakshmi et al., 2010; Kesari et al., 2012). Lateral buds naturally have large amounts of cytokinin (because of the cytokinin accumulation made in the root and moving upward) (Zulfiqar et al., 2009). On the other hand, TDZ effect could be attributed to its ability to induce cytokinin accumulation inside the tissue (Victor et al., 1999). Large amounts of endogenous cytokinin in lateral buds of *L. camara* and its interaction with TDZ could explain the TDZ inhibitory effect on *L. camara* proliferation in the present work. According to the reports by some researchers, TDZ is not appropriate in some plants for rooting and causes highly decreased length in shoots in high concentrations. Use of TDZ in guava micro-propagation caused short, ungrown shoots accompanied with yellow leaves, exacerbated as TDZ concentration increased (Ning et al., 2007; Techado and Lim, 2000). The results of the present study are in agreement with these reports.

Rooting

In this experiment, all IAA concentrations caused increase in rooting index, although this was significant in

Table 2. Comparison of mean effect of different IAA concentrations on proliferation indices.

IAA concentration (mg/l)	Number of primary roots	Number of secondary root	Primary root length	Primary root diameter	Fresh weight	Dry weight
0	0.33[c]	2.22[a]	3.42[b]	0.29[a]	0.038[a]	0.004[a]
0.25	1.00[b]	6.11[a]	3.76[b]	0.62[a]	0.079[a]	0.007[a]
0.5	1.66[a]	9.33[a]	9.39[a]	0.88[a]	0.088[a]	0.026[a]

In each column dissimilar letters indicate significant difference at 5% level.

only some of the indices (Table 2). IAA is necessary in central cylinder to initiate cellular proliferation in the surrounding circle. IAA is necessary to progress cellular proliferation and maintain biological ability of the cells in growing lateral roots (Taiz and Zeiger, 2010). Several reports have appeared on IAA effect on increased number of roots (Stefanicic et al., 2005; Janarthanam et al., 2012). As IAA concentration increased, the number and length of root increased in lemon. The increase in roots' number occurs because of hydrolysis of foods and transferring them to sprouts (Thayamini and Umadevi, 2011). This could be the reason for increased number of primary and secondary roots as IAA concentration increases. In *L*. *camara*, the highest percentage of rooting was obtained in 0.44 µmol/L concentration of IAA (Affonso et al., 2000).

Conclusion

The highest proliferation were obtained with 8 mg/L BA while the highest shoot and internode length, fresh and dry weight and the maximum number of leaves were obtained in control treatment and the highest fresh weight was in 4 mg/L BA. The studies revealed that high concentrations of BA cause plants to overcome the apical dominance by declining IAA effects; thus, proliferation was increased but shoot and internode length was decreased. In this study, the most number of roots was also obtained in MS medium supplemented with 0.5 mg/l IAA.

Conflict of interest

Authors declare that there are no conflicts of interests

REFERENCES

Abhyankar G, Reddy VD (2007). Rapid micropropagation via axillary bud proliferation of *Adhatoda vasica* Nees. From nodal segments. Ind. J. Exp. Biol. 45:268-271.

Affonso VR, Bizzo HR, Lima SS, Esquibela MA, Sato A (2007). Solid phase microextraction (SPME) analysis of volatile compounds produced by *in vitro* shoots of *Lantana camara* L. under the influence of auxins and cytokinins. J. Braz. Chem. Soc. 18(8):1504-1508.

Babu KN, Sajina A, Minoo D, John CZ, Mini PM, Tushar KV, Rema J, Ravindran PN (2003). Micropropagation of camphor tree (*Cinnamomum camphora*). Plant Cell Tiss. Organ Cult. 74:179-183.

Dziedzic E (2008). Propagation of blue honeysuckle (*Lonicera caerulea* var. *kamtschatica Pojark*) in *in vitro* culture. J. Fruit Ornament. Plant Res. 16:93-100.

Gaspar TH, Kevers C, Faivre-Rampant O, Crèvecoeur M, Penel CL, Greppin H, Dommes J (2003). Changing concepts in plant hormone action. *In vitro* Cel. Dev. Biol-Plant 39(2):85-106.

Hiregoudar LV, Morthy HN, Bhat JG, Nayeen A, Hema BP, Hahn EJ, Peak KY (2006). Rapid clonal propagation of *Vitex trifolia*. Biol. Plant 50(2):291-294.

Janarthanam B, Dhamotharan R, Sumathi E (2012). Thidiazuron (TDZ)–induced plant regeneration from intermodal explants of *Santalum album* L. J. Biosci. Res. 3(3):145-153.

Kesari V, Ramesh AM, Rangan L (2012). High frequency direct organogenesis and evaluation of genetic stability for *in vitro* regenerated *Pongamia pinnata*, a valuable biodiesel plant. Biomass Bioenergy 44:23-32.

Kulkarni AA, Thengane SR, Krishnamurthy KV (2000). Direct shoot regeneration from node, internode, hypocotyl and embryo explants of *Withania somnifera*. Plant Cell Tiss. Organ Cult. 62:203-209.

Lakshmi PA, Nandagopalan V, Piramila HM, Prabakaran DS (2010). Standardization of *in vitro* studies on direct and indirect organogenesis of *Trichosanthes cucumerina*. Proc. Sixth Intl. Plant Tiss. Cult. Biotech. Conf. Plant Tiss. Cult. Biotech. pp. 73-77.

Meghwal PR, Singh SK, Sharma HC (2003). Micropropagation of aneuploid guava. Ind. J. Hort. 60:29-33.

Mok DWS, Mok MC (2001). Cytokinin metabolism and action. Plant Mol. Biol. 52:89-118.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiol. Plant 15:473-497.

Ning GG, Fan XL, Huang WJ, Bao MZ, Zhang JB (2007). Micropropagation of six *Prunus mume* cultivars through axilary shoot proliferation and ISSR analysis of cloned plants. Acta Biol. Cracov. Series Bot. 49(1):25-31.

Opik H, Rolfe S (2005). The Physiology of Flowering Plants. (4thed). Cambridge University Press. p. 392.

Passos JL, Meira RMSA, Barbosa LCA (2009). Foliar anatomy of the species *Lantana camara* and L *radula* (Verbenaceae). Planta Danin. Viços. 27(4):689-700.

Rai VR (2002). Rapid clonal propagation of *Nothapodytes foetida* (Wight) sleumer- Athreatened medicinal tree. *In vitro* Cell. Develop. Biol. Plant 38(4):347-351.

Rathore P, Suthar R, Purohi SD (2008). Micropropagation of *Terminalia bellerica* Roxb. From juvenile explants. Ind. J. Biotech. 7:246-249.

Sharma R, Shahzad A (2008). Thidiazuran (TDZ) induced regeneration from cotyledonary node explant of *Abelmoschus moschatus* Medik. L., (A valuable medicinal plant). World J. Agric. Sci. 4(4):449-452.

Srivastava P, Sisodia V, Chaturvedi R (2011). Effect of culture conditions on synthesis of triterpenoids in suspension cultures of *Lantana camara* L. Bioproc. Biosyst. Eng. 34:75-80.

Stefanicic M, Stampar F, Osterc G (2005). Influence of IAA and IBA on root development and quality of Prunus GiSelA5 leafy cutting. Hortic. Sci. 40(7):2052-2055.

Taiz L, Zeiger E, (2010) Plant Physiology, Fifth Edition. Sinauer Associates. Sunderland, MA. P 623.

Techado S, Lim M (2000). Improvement of mangosteen micropropagation through meristematic nodular callus formation from *in vitro*-derived leaf explants. Sci. Hort. 86:291-298.

Thakur RC, Karnosky DF (2007). Micropropagation and germplasm conservation of Central Park Splendor Chinese elm (*Ulmus parvifolia* Jacq. 'A/Ross Central Park') trees. Plant Cell Rep. 26:1171-1177.

Thayamini HS, Umadevi T (2011). Influence of indole acetic acid (IAA) on the establishment of stem cuttings of lemon (*Citrus limon* L.). J. Agric. Res. 49(4):517-524.

Verma SK, Yucesan BB, Sahin G, Gurel S, Gurel E (2011). Direct shoot regeneration from leaf explants of *Digitalis lamarckii*, an endemic medicinal species. Turk. J. Bot. 35:689-695.

Victor JMR, Murthy BNS, Murch SJ, Krishnaraj S, Saxena P (1999). Studies of endogenous purine metabolism in thidiazuron-induced somatic embryogenesis of Peanut (*Arachis hypogea* L.). Plant Growth Regul. 28:41-47.

Widiyanto SN, Erytrina D, Rahmania H (2005). Adventitious shoot formation on teak (*Tectona grandis* L.f.) callus cultures derived from internodal segments. IInd IS on Biotech. of Trop and Subtrop. Species. pp.153-157.

Zulfiqar B, Abbasi NA, Ahmad T, Hafiz IA (2009). Effect of explant sources and different concentrations of plant growth regulators on *in vitro* shoot proliferation and rooting of Avocado (*Persea americana* Mill.) CV. "Fuerte". Pak. J. Bot. 41(5):2333-2346.

Journal of Medicinal Plant Research

Related Journals Published by Academic Journals

- *African Journal of Pharmacy and Pharmacology*
- *Journal of Dentistry and Oral Hygiene*
- *International Journal of Nursing and Midwifery*
- *Journal of Parasitology and Vector Biology*
- *Journal of Pharmacognosy and Phytotherapy*
- *Journal of Toxicology and Environmental Health Sciences*

academicJournals