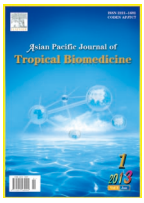
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:

Effects of *Ficus asperifolia* on normal rat estrus cyclicity

Esther Ngadjui, Pierre Watcho*, Telesphore Benoit Nguelefack, Albert Kamanyi

Department of Animal Biology, Faculty of Science, University of Dschang, Box 67 Dschang, Cameroon

PEER REVIEW

Peer reviewer

Prof. Miguel Carro-Juárez, Laboratorio de Comportamiento Reproductivo Escuela de Medicina Veterinaria y Zootecnia Universidad Autónoma de Tlaxcala, Mexico.
Tel: 2464624143
Fax: 2464624143
E-mail: miguel_carro@hotmail.com

Comments

This study contributes to the understanding of the local effects of *Ficus asperifolia* upon the reproductive tract of females. The hypothesis of the authors was ascertained and results provided good evidence to support its traditional use in reproductive medicine of Cameroon folk. Other possibilities of medicinal use of *Ficus asperifolia* could be potentially analyzed with more precise techniques. Aims of the present study were covered but potential changes in vaginal smears should indicate subtle changes in vaginal cells that commonly indicate major changes in the periphery and induced by the plant fractions such as carotenes and alkaloids. I suggest its publication in the present form.
(Details on Page 56)

ABSTRACT

Objective: To evaluate *Ficus asperifolia* (Moraceae) (*F. asperifolia*) effecting on regular estrus cycle of Wistar rats. **Methods:** Air-dried fruits of *F. asperifolia* were extracted using water. Prior to the test, vaginal smear was monitored daily for a 3-week period to select females with normal (regular) estrous cycle. Those with regular estrus cycle weighing between 150-170 g were randomized into three sets of 15 animals each. Each set was then divided into three groups: Group 1 (control) was orally administered with distilled water (10 mL/kg body weight) once a day for 1 week starting from the proestrus stage. Groups 2 and 3 were respectively treated with 100 and 500 mg/kg body weight of the plant aqueous extract. The two other sets of 15 animals each were similarly treated as the first set for 3 weeks and 6 weeks respectively. Estrus cycle pattern was monitored before and during plant extract application whereas lipid profile, ovary, uterus and liver growth indices were determined at the end of each treatment. **Results:** *F. asperifolia* did not disrupt (0%) the order of appearance of normal estrus cycle stages, namely, proestrus, estrus, metestrus and diestrus. Short-term treatment (1 week duration) exhibited high frequency of appearance of proestrus and estrus stages while mid- (3 weeks) and long-term (6 weeks) treatments revealed constancy in the frequency of all stages irrespective to animal groups. The plasma and organ lipid profile, as well as ovary, uterus and liver growth remained unchanged when compared to distilled water-treated animals. Following long-term administration of plant extract (6 weeks), no adverse effect was noticed. **Conclusions:** Our data partially support the use of *F. asperifolia* in common medicine.

KEYWORDS

Ficus asperifolia, Estrus cycle, Rats.

1. Introduction

During preclinical investigations into the safety of drugs and chemicals, many are found to interfere with the reproductive function of the female[1,2]. This interference is commonly expressed as a change in normal components of vaginal smear or disruption in the frequency of particular stages of the estrus cycle[3]. In this light, alternative treatments with plant extracts are required to have more specific pharmacological profile[4,5]. The importance of plants as a source of fertility drugs has been emphasized by many researchers[6]. Fertility agents obtained from indigenous medicinal plants would be of immense benefit, especially to inhabitants of developing countries since the cost of these drugs would be within their means[7,8]. *Ficus*

*Corresponding author: Prof. Pierre Watcho, Department of Animal Biology, Faculty of Science, University of Dschang, Box 67 Dschang, Cameroon.
Tel: +237 77516130; 96137604
E-mail: pwatcho@yahoo.fr

Article history:
Received 12 Aug 2012
Received in revised form 20 Aug, 2nd revised form 25 Aug, 3rd revised form 29 Aug 2012
Accepted 10 Oct 2012
Available online 28 Jan 2013

asperifolia (Moraceae) (*F. asperifolia*), known as "Ntchach lum" in the western region of Cameroon, is used to reverse some cases of sterility/infertility in women. Our earlier studies have shown that this plant, especially its aqueous extract, possesses real pro-implantation, pro-development, uterotrophic, and uterotonic-like activities[9,10]. These preliminary findings motivated us to find out whether an accidental intake of *F. asperifolia* by females with regular estrus cycle could be of any damage on their estrus cycle. In the present study, we examined the effects of a short-, mid-, and long-term oral administration of aqueous extract of *F. asperifolia* (the most efficient extract from our pilot study) on the regularity and frequency of appearance of rat estrus cycle stages.

2. Materials and methods

2.1. Plant collection and preparation of aqueous extract

Fresh fruits of *F. asperifolia* were collected during the month of February in 2011, from trees in Dschang, Cameroon. Botanical identification was performed in the Cameroon National Herbarium in comparison with the existing specimen number 338/15240/HNC. The fruits were shade-dried for 5 d and ground into powder. To obtain an aqueous extract similar to the traditional recommendation, a total of 1 kg of *F. asperifolia* were soaked in distilled water (5 L) and the mixture boiled for 15 min. The heated decoction was taken and allowed to cool at room temperature, filtered and oven-dried to give 46.67 g of dried aqueous extract (yield of extraction, 4.66%) used in the study.

2.2. Phytochemical screening

Qualitative phytochemical evaluation was performed on aqueous extract of *F. asperifolia* to determine the existence of sterol and triterpenes (Libermann Buchard test), flavonoids (test of Shinoda), and saponins (Hostettmann test)[11].

2.3. Animals

Healthy non-pregnant adult female Wistar rats between 10 and 12 weeks of age weighing 150-170 g were used in this study. They were housed in groups (five per group in polypropylene cages) and maintained under uniform husbandry conditions with natural photoperiod, humidity, temperature (26±2) °C and free access to food and water. The Local Committee of Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established by the European Union on Animal Care and Experimentation (CEE Council 86/609).

2.4. Experimental design

2.4.1. Estrus cycle monitoring

Three weeks prior to the treatment, vaginal smears were collected and observed each morning (8-10 a.m.) to determine estrus cyclicity of each animal. This involved sampling the cells of the vaginal canal with sterile saline using a glass pipette. The recovered solution containing cells was placed on microscope slides, fixed with methanol, stained with methylene blue, dried and examined microscopically. Cell descriptions were used to classify rats based on the stages of the estrus cycle (proestrus, estrus, metestrus and diestrus)[2].

2.4.2. Animal's treatment

A total of 45 females with regular estrus cycle were randomized into three sets of 15 animals each and treated for 1 (set I), 3 (set II) and 6 weeks (set III). Each set was then divided into three groups and treated as follows: Group 1 (control) was orally administered with distilled water (10 mL/kg body weight) once a day starting from proestrus stage. Group 2 and group 3 were respectively treated with 100 and 500 mg/kg body weight of aqueous extract of *F. asperifolia*. Estrus cycle pattern was monitored daily and the frequency of appearance of each stage determined. At the end of each treatment period, animals were euthanized under anaesthesia (diazepam/ketamine). Blood was collected by cardiac puncture and the plasma prepared and stored at -20 °C for biochemical analysis. Ovaries, uterus and liver were removed, blotted, weighed and kept at -20 °C.

2.4.3. Preparation of ovarian and uterus supernatants

Ovaries and uterus of each animal were homogenized in sterile saline at 1% and 5% respectively (0-4 °C) using a potter homogenizer. The homogenate was then centrifuged at 3 500 rpm for 15 min (Techmel & Techmel, USA). The supernatant was collected and kept frozen overnight at -20 °C before being used for various biochemical assays. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were determined in plasma, ovary and uterus using available commercial biochemical kits.

2.5. Statistical analysis

Data were expressed in mean±SEM. One-way ANOVA followed by post-hoc Fisher's LSD were used to analyze statistical difference among groups using STATISTICA, Statsoft, Inc. (2008), data analysis software system, version 8.0. www.statsoft.com. Comparisons with $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Preliminary phytochemical analysis

The fresh aqueous and methanol extracts of *F. asperifolia* gave a positive reaction to alkaloids, saponins, sterols and triterpens.

3.2. Effects of F. asperifolia on the regularity of the estrus cycle

Figure 1 shows the results of the aqueous extract of the dried fruits from *F. asperifolia* on adult female rats after 1, 3 and 6 weeks of treatment. When compared to control, there was no significant difference recorded in females receiving the aqueous extract of *F. asperifolia*, irrespective of the dose and duration of treatment. All female rats showed a normal sequence of changes in the cytological elements of the vaginal smear. Further, the order of appearance of various phases and the duration of the estrus cycle remained unchanged after treatment.

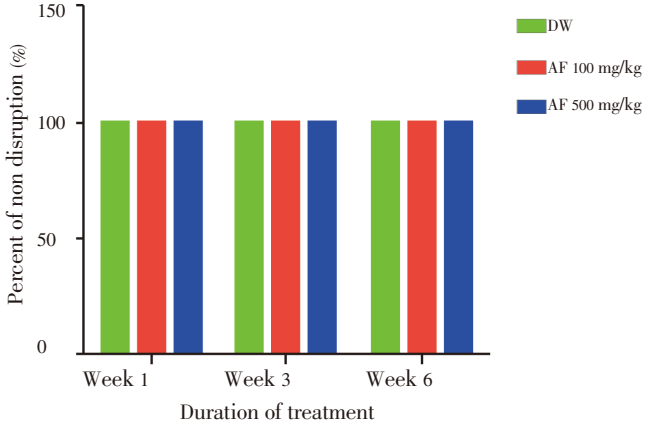


Figure 1. Effect of the aqueous extract of *F. asperifolia* on the regularity of the rat estrus cycle.
DW=distilled water; FA=*F. asperifolia* aqueous extract.

3.3. Effects of F. asperifolia on the frequency of appearance of different phases in estrus cycle

With regard to each estrus cycle stage, animals treated for 3 and 6 weeks showed constancy in the frequency of appearance of either proestrus, estrus, metestrus or diestrus phase in both control and plant extract-treated female rats. The highest frequency of appearance was obtained with the estrous stage with almost 70% after 6 weeks of treatment. In rats receiving the plant extract, especially the dose 100 mg/kg for 1 week, a tendency to an increase in the appearance of proestrus, estrus and diestrus phases were observed comparatively to distilled water group (Figure 2).

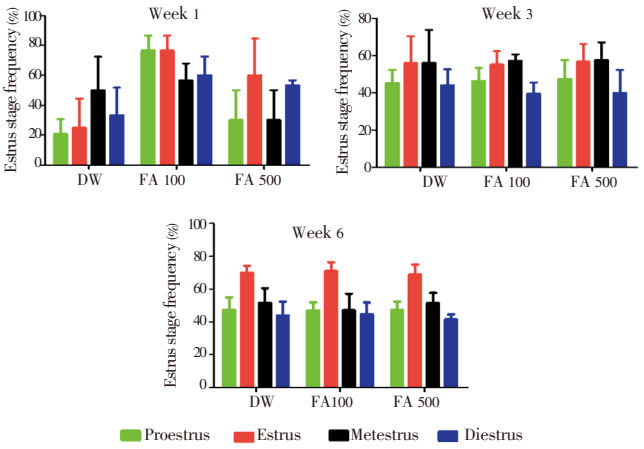


Figure 2. Frequency of appearance of estrus stages after treatment with *F. asperifolia* aqueous extract.
DW: distilled water; FA=*F. asperifolia* aqueous extract.

3.4. Effects of F. asperifolia on lipid profile

Table 1 shows the plasma, ovary and uterine lipid profile. Apart from a significant ($P<0.05$) increase in uterine total cholesterol in animals treated for 1 week, the TC, TG, HDL-C and LDL-C concentrations in plasma and organs remained unchanged in all groups and at all-time points.

3.5. Effects of F. asperifolia on uterine, ovary and liver relative weights

There was no change in the relative weights of uterus, ovary and liver of *F. asperifolia*-treated female rats compared to control (Table 2).

Table 1
Effects of aqueous extract of *F. asperifolia* on lipid profile of control and of *F. asperifolia* treated groups.

Parameters		Week 1			Week 3			Week 6		
		Control	Aqueous extract 100 mg/kg	Aqueous extract 500 mg/kg	Control	Aqueous extract 100 mg/kg	Aqueous extract 500 mg/kg	Control	Aqueous extract 100 mg/kg	Aqueous extract 500 mg/kg
Plasma (mg/dL)	TC	44.604±1.305	44.577±1.670	43.449±3.866	45.771±4.931	47.097±2.851	46.700±2.330	43.980±4.068	45.175±2.432	44.776±6.434
	TG	87.313±31.541	100.299±29.170	100.448±34.220	89.105±15.057	119.552±49.476	164.478±72.546	84.537±12.824	73.188±0.229	121.801±73.870
	HDL-C	13.276±2.221	12.955±2.751	12.776±1.466	13.026±1.568	13.194±2.670	13.381±0.921	14.328±2.529	12.616±0.799	12.981±1.309
	LDL-C	13.862±0.024	12.610±0.021	12.467±0.024	11.925±0.027	9.992±0.025	10.994±0.020	11.921±0.022	12.223±0.024	12.180±0.024
Ovary (mg/g wet tissue)	TC	6.827±1.833	6.986±0.442	6.333±5.559	7.160±2.220	6.640±1.821	7.000±0.632	6.947±0.129	6.200±0.651	6.720±0.601
	TG	3.544±0.212	3.375±0.115	3.739±0.336	3.401±0.228	3.453±0.849	3.244±0.024	3.309±0.073	3.244±0.073	3.234±0.038
	HDL-C	4.853±0.089	5.003±0.084	4.886±0.186	5.316±0.073	4.964±0.201	5.394±0.414	4.749±0.211	4.847±0.066	4.945±0.299
	LDL-C	1.173±0.001	1.308±0.008	0.899±0.005	1.163±0.001	0.985±0.004	0.957±0.003	1.549±0.006	0.991±0.007	1.127±0.002
Uterus (mg/g wet tissue)	TC	5.100±0.419	9.512±1.102*	7.196±0.731	5.274±0.858	5.644±0.231	5.848±0.446	5.104±0.269	6.023±0.231	6.208±0.601
	TG	9.588±1.017	9.588±0.977	8.036±1.431	8.432±0.702	9.556±0.476	8.608±1.052	9.586±1.474	9.244±0.677	9.059±0.757
	HDL-C	2.703±0.285	2.661±0.259	3.433±0.528	2.832±0.213	2.748±0.187	3.048±0.347	3.133±0.433	3.046±0.285	3.541±0.771
	LDL-C	0.890±0.001	0.891±0.031	0.915±0.007	1.050±0.004	0.980±0.002	1.008±0.001	0.907±0.003	1.013±0.008	0.896±0.005

Values are expressed as mean±ESM. *$P<0.05$ significantly different compared to control (distilled water).

Table 2
Effects of *F. asperifolia* on the relative weights of uterus, ovary and liver (mg/100 g body weight).

Organs	Week 1			Week 3			Week 6		
	Control	Aqueous extract 100 mg/kg	Aqueous extract 500 mg/kg	Control	Aqueous extract 100 mg/kg	Aqueous extract 500 mg/kg	Control	Aqueous extract 100 mg/kg	Aqueous extract 500 mg/kg
Uterus	0.149±0.067	0.152±0.030	0.167±0.037	0.148±0.113	0.153±0.011	0.145±0.122	0.151±0.100	0.153±0.018	0.149±0.008
Ovary	0.060±0.001	0 .056±0.012	0.071±0.008	0.055±0.005	0.052±0.003	0.054±0.006	0.059±0.001	0.060±0.045	0.045±0.002
Liver	2.617±0.051	3.123±0.106	2.900±0.132	3.345±0.217	3.241±0.199	2.804±0.084	2.879±0.212	3.241±0.153	2.552±0.132

Values are expressed as mean±ESM, n=5.

4. Discussion

The aim of the present study was to determine the effects of the aqueous extract of the dried fruits of *F. asperifolia* on the rat estrous cyclicity. The female estrous cycle is generally considered as the time between periods of estrus (period of sexual receptivity). It comprises the recurring physiologic changes that are induced by reproductive hormones. The estrous cycle consists of four stages, *viz.* proestrus, estrus, metestrus (or diestrus 1), and diestrus (or diestrus 2). Because rats are continuously polyestrus (*i.e.*, cycle constantly throughout the year), diestrus is immediately followed by the proestrus phase of the next cycle. These stages can be recognized by the presence of cornified cells, intermediate cells and/or leukocytes found in the vaginal smear[2,12,13]. The short length (4 to 5 d) of the rat estrus cycle makes this animal ideal for studying products/compounds that may interfere with the reproductive system (estrus cyclicity). Results of the present work showed that acute- (1 week), mid- (3 weeks) or long-term (6 weeks) administration of the aqueous extract of *F. asperifolia* to female rats with regular estrus cycle did not alter, irrespective of the dose, both the normal sequence of changes in the cytological elements of the vaginal smear and the order of appearance of the estrus stages. The transition between the stages was not altered throughout the study. Hence, anoestrus was not observed. It is generally believed that anoestrus, a period of reproductive quiescence between cycles, is permanent in healthy cycling rats and that estrus cyclicity only ceases during pseudopregnancy, pregnancy and lactation. Further, starting the monitoring of vaginal smear at proestrus stage and associated to the duration of the treatment (1, 3 or 6 weeks) probably contribute to explain the variation observed in the frequency of appearance of the estrus cycle stages of animals. Indeed, short treatment (1 week duration) which theoretically corresponds to 1 estrus cycle + 2 additive d obviously exhibited high frequency of proestrus and estrus while mid- (3 weeks=4 estrus cycles + 1 d) and long-term (6 weeks= 8 estrus cycles + 2 d) treatments revealed a constancy in the frequency of the stages irrespective to animal group. This lack of effect of *F. asperifolia* on estrus cycle could support our preliminary findings reporting that an *in vivo* exposure of the uterus to *F. asperifolia* extracts may contribute to the setup of some modifications in the uterine endometrium that transform it from a non receptive to a receptive phase allowing the implantation and development of the blastocyst[10]. The fact that all biochemical parameters (TC, TG, HDL-C and LDL-C) as well as the relative organ weights remained unchanged may also account for the fertility potentials of *F. asperifolia* dried fruits[14].

Overall findings indicate that *F. asperifolia* did not disrupt regular estrus cycle in normal rats. Fertility being strictly regulated by nutrition, it would be of great interest to ascertain the effects of this plant in an experimental disrupted estrus cycle model. Work in progress will therefore permit us to better investigate the effects of *F. asperifolia* in high fat diet-induced rat estrus cycle disruption.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors thank the University of Dschang, Cameroun, for research facilities.

Comments

Background

Ficus asperifolia (Moraceae), growing in western region of Cameroon, is used to reverse some cases of sterility/infertility in women. Ealier studies have shown that this plant, especially its aqueous extract, possesses real pro-implantation, pro-development, uterotrophic and uterotonic-like activities. Alternative treatments with medicinal plants as a source of fertility drugs has been emphasized by many researchers and fertility agents obtained from indigenous medicinal plants would be of immense benefit especially to inhabitants of developing countries since the cost of these drugs would be within their means.

Research frontiers

Studies are being performed in order to determine which are biologically active compounds and pharmacological properties of *F. asperifolia* upon the ovarian function of normally cycling female rats. This plant did not disrupt the cyclicity of normal estrus cycle stages of the rat. Short-term treatment (1 week duration) exhibited high frequency of appearance of proestrus and estrus stages while mid- (3 weeks) and long-term (6 weeks) treatments revealed constancy in the frequency of all stages irrespective to animal groups without changes in reproductive organs.

Related reports

Earlier studies on *F. asperifolia* have shown that aqueous crude extract of this plant possesses real pro-implantation, pro-development, uterotrophic and uterotonic-like activities. Results of the present work showed that acute- (1 week), mid- (3 weeks) or long-term (6 weeks) administration of the aqueous extract of *F. asperifolia* to female rats with regular estrus cycle did not alter both the normal sequence of changes in the cytological elements of the vaginal smear and the order of appearance of the estrus stages.

Innovations and breakthroughs

F. asperifolia extract possesses pro-implantation, pro-development, uterotrophic and uterotonic-like effects without influencing the plasma and organ lipid profile, as well as ovary, uterus and liver growth of animals subjected to acute, mid and chronic ingestion.

Applications

It will be interesting to investigate the effects of *F. asperifolia* in high fat diet-induced rat estrus cycle disruption and if possible to analyse the effects of this plant extract on women cyclicity. Furthermore, data of this study suggest that no dysfunction in ovarian activity could be obtained when a treatment with this plant extract would be selected to improve sexual or reproductive function.

Peer review

This study contributes to the understanding of the local effects of *F. asperifolia* upon the reproductive tract of females. The hypothesis of the authors was ascertained and results provided good evidence to support its traditional use in reproductive medicine of Cameroon folk. Other possibilities of medicinal use of *F. asperifolia* could be potentially analyzed with more precise techniques. The aims of the present study were covered but potential changes in vaginal smears should indicate subtle changes in vaginal cells that commonly indicate major changes in the periphery and induced by the plant fractions such as carotenes and alkaloids. I suggest its publication in the present form.

References

[1] Grégoire A, Allard A, Huamán E, León S, Silva RM, Buff S, et al. Control of the estrous cycle in guinea-pig (*Cavia porcellus*). *Theriogenology* 2012; **78**(4): 842-847.

[2] Byers SL, Wiles MV, Dunn SL, Taft RA. Mouse estrous cycle identification tool and images. *PLoS One* 2012; **7**(4): e35538.

[3] Dicken CL, Israel D, Davis JB, Sun Y, Shu J, Hardin J, et al. Peripubertal Vitamin D3 deficiency delays puberty and disrupts the estrous cycle in adult female mice. *Biol Reprod* 2012; **87**(2): 51.

[4] Lienou LL, Telefo BP, Bale B, Yemele D, Tagne RS, Goka SC, et al. Effect of the aqueous extract of *Senecio biafrae* (Oliv. & Hiern) J. Moore on sexual maturation of immature female rat. *BMC Complement Altern Med* 2012; **12**: 36.

[5] Oyesola TO, Oyesola OA, Okoye CS. Effects of aqueous extract of *Aspilia africana* on reproductive functions of female Wistar rats. *Pak J Biol Sci* 2010; **13**: 126-131.

[6] Ganguly M, Devi N, Mahanta R, Borthakur MK. Effect of *Mimosa pudica* root extract on vaginal estrous and serum hormones for screening of antifertility activity in albino mice. *Contraception* 2007; **76**(6): 482-485.

[7] Musa T, Yakubu, Bimbo B, Bukoye. Abortifacient potentials of aqueous extract of *Bambusa vugaris* leaves in pregnant Dutch rabbits. *Contraception* 2009; **20**: 1-6.

[8] Nkafamiya II, Osemeahon SA, Modibbo UU, Aminu A. Nutritional status of non-conventional leafy vegetables, *Ficus asperifolia* and *Ficus sycomorus*. *Afr J Food Sci* 2010; **4**: 104-108.

[9] Watcho P, Ngadjui E, Nkeng-efouet PA, Nguelefack TB, Kamanyi A. Reproductive effects of *Ficus asperifolia* (Moraceae) in female rats. *Afr Health Sci* 2009; **9**: 49-53.

[10] Watcho P, Ngadjui E, Nkeng-efouet PA, Nguelefack TB, Kamanyi A. Evaluation of *in vitro* uterotonic activities of fruit extracts of *Ficus asperifolia* in rats. *Evid Based Complement Alternat Med* 2009; **21**: 1-7.

[11] De S, Dey YN, Ghosh AK. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae). *Int J Pharm Biol Res* 2010; **5**: 150-157.

[12] Beck KD, Jiao X, Cominski TP, Servatius RJ. Estrus cycle stage modifies the presentation of stress-induced startle suppression in female Sprague-Dawley rats. *Physiol Beh* 2008; **93**: 1019-1023.

[13] Weshood FR. The female rat reproductive cycle: a practical histological guide to staging. *Toxicol Pathol* 2008; **36**: 375-384.

[14] Chandra SJ, Ekambaram SP, Subramanian V. Acute and subacute toxicity studies on the polyherbal antidiabetic formulation Diakyur in experimental model. *Health Sci* 2007; **53**: 245-249.