The potentiality as an effective antifertility agent is still fragmentary. However, scientific study of this plant in relation with hepatoprotective, radioprotective, antiulcer, anti-inflammatory, hyperlipidemia, renal anticarcinogenic, antidiuretic, antitussive, hypoglycemic, hypertension, gastric ulcer, wound healing, etc. Bark of F. racemosa has been recommended for the treatment of diarrhea, diabetes, hypertension, gastric ulcer, wound healing, etc. The above results revealed the potential, reversible female antifertility effect of alcoholic extract of F. racemosa bark.

Conclusion: The above results revealed the potential, reversible female antifertility effect of alcoholic extract of F. racemosa bark. Therefore, carried out to evaluate the claimed antifertility effect of F. racemosa bark using different aspects of reproductive physiology in Wistar rats.

INTRODUCTION
This century search for antifertility agents is continued to tackle the problem of the population explosion that may lead too economic and health impact on the family in particular and the society in general, especially in developing countries like Ethiopia where the population growth is very high (Ministry of Health, 2003). Fertility control is an issue of global and national public health concern. There is a global need to support individuals in family planning due to the increasing growth rate of the world’s population with its negative impact on the environment, economic growth, and poverty reduction in underdeveloped countries. About 90% of the world’s contraceptive users are women. Although considerable progress has been made in the development of highly effective, acceptable, and reversible methods of contraception in females, progress and possibilities on males are still slow and limited. Aware of this responsibility, health organizations and pharmaceutical companies continue to financially support or actively pursue research toward new contraceptive approaches [1]. Current methods of contraception result in an unacceptable rate of unintended pregnancies and many side effects also.

Ficus racemosa Linnaeus, Family: Moraceae (commonly known in all over India as udumbara, gular), is a perennial herb that is grown in most part of India and is used in the traditional system of Indian medicine against hypertension, gastric ulcer, wound healing, etc. Bark of F. racemosa showed a wide range of pharmacological actions hypoglycemic, hyperlipidemia, renal anticarcinogenic, antidiuretic, antitussive, hepatoprotective, radioprotective, antilucre, anti-inflammatory, antihypertensive, and antifungal, β-sitosterol, glucose, and maltol, the active constituent [2]. However, scientific study of this plant in relation with the potentiality as an effective antifertility agent is still fragmentary.
by distilling the solvent at low temperature. They were then weighed, and percentages of different extractive values were calculated with respect to air-dried substance.

**Phytochemical screening**
Identification of the chemical constituents was carried out on the powdered bark of *F. racemosa*, and the extract was concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents [6].

**Acute oral toxicity**
The acute oral toxicity studies were carried out as per the guidelines of Organization for Economic Co-operation and Development-423, Ministry of Social Justice and Empowerment, Government of India.

**Animals**
Antifertility test was performed on adult female Wistar rats weighing between 180 and 200 g and mice. They were housed in polypropylene cages and fed with standard chow diet and water ad libium. The Institutional Ethical Committee for animal cares and use approved all experimental procedures. The animals were exposed to alternate cycle of 12 hrs of darkness and light each. Before each test, the animals were fasted for at least 12 hrs. The experimental protocols were subjected to the securitization of the Institutional Animal Ethics Committee and were cleared by the same (1587/PO/Re/S/11/CPCSEA).

**Antifertility activity**
Antifertility activity of plant extracts was evaluated with the help of reproductive outcome, anti-implantation, abortifacient, estrogenic, and antiestrogenic study was also performed, which further supported by the hormonal analysis [7-9].

**Reproductive outcome study**
Three groups of mature female rats (five rat/group) were selected for received extracts for 8 days, and control group received vehicle for the same period. All the experimental rats were then allowed to mate with mature fertile male rat and the treatment continued for 21 days. The number of litters was determined after the completion of one gestation period in all-experimental groups. The litters were allowed to grow and the growth of litters produced from the extract-administered group was compared with those of control group. The reversibility of antifertility effect of the extracts was also studied in the treated groups. For this study, the extracts were administered continuously for 21 days, and then, the extract was withdrawn. After 21 days of extract withdrawal, animals were allowed to mate with male rat. The number of litters was determined after the completion of one gestation period (Salhad et al., 1997).

**Anti-implantation study**
With the help of estrous cycle studies, proven fertile female Wistar rats, weighing between 150 and 200 g, were selected and left overnight with male of proven fertile in the ratio of 3:1 (Jain et al. 2012). The extracts were administered orally to separated group rats at the dose level of 500 mg/kg from day 1 to day 7 of pregnancy. Control animal received the vehicle (carboxymethyl cellulose [CMC], 0.5%). The animals were then laparotomized on day 10 of the pregnancy under excess dose of thiopentone sodium and uteri were examined to determine the number of implantation sites (Salhad et al., 1997).

**Abortifacient study**
Female rats at 1<sup>st</sup> day of pregnancy were divided into three groups, consisting of (5-6) animals in each group. The animals were laparotomized under light ether anesthesia and semi-sterile conditions on 10<sup>th</sup> day of pregnancy. Both horns of the uterus were observed for resorption compared with the initial number of implantation observed on 10<sup>th</sup> day of pregnancy (Khanna and Chaudhury, 1968).

**Estrogenic and antiestrogenic study**
 Colony bred immature ovariectomized female rats (21-23 days) weighing between 25 and 30 g were used. They were divided into experimental and control groups, consisting of six animals each group. The extracts were suspended in 0.5% CMC and administered orally for 7 days at the dose level of 500 mg/kg body weight. Ethinyl estradiol (Unicure Remedies Pvt. Ltd., Baroda, India) in olive oil 1 µg/rat/day was injected subcutaneously for 7 days in another group to induce estrous. CMC 0.5% was administered orally to the control animals. The extract at the dose level of 500 mg/kg was also administered orally along with ethinyl estradiol in olive oil at 1 µg/rat/day subcutaneously to different groups of rat for the same period (Sharma, 2003).

On the 8<sup>th</sup> day of the experiment, all the animals were sacrificed by decapitation under light ether anesthesia, and the uteri were dissected out, surrounding tissues removed, blotted on filter paper, and weighed quickly on balance sensitive to 0.0001 g. A portion of the uterine tissues and adrenal glands from the control and treated animals were fixed in Bouin’s fluid for 24 hrs, dehydrated in alcohol and then embedded in paraffin. The paraffin blocks were sectioned at 6 mm intervals and stained with hematoxylin-eosin for histological examinations (Pal, 1990).

**Hormonal analysis**
Hormonal analysis was determined by Merck kit method (Merck cat# 15891) using microlab-300 IX Merck apparatus (Autoanalyzer). Blood (2 ml) was drawn by retro-orbital puncture and was immediately transferred into ethylenediaminetetraacetic acid coated vacutainer. The samples were mixed gently and were left for more than half an hour at room temperature, and finally centrifuged at 3000 rpm for 15 minutes. Serum was separated and assayed for follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol, prolactin, and 17-OH progesterone using enzyme-linked immunnoassay technique (Elisa reader [BIORAD 680 Microplate Reader]) [10,11].

**Statistical data**
All values are expressed as mean±standard error of the mean. Means were statistically analyzed by one-way analysis of variance, and values of p<0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**

**Physicochemical parameters**
Physicochemical parameters of *F. racemosa* bark were determined. In physicochemical parameter, total ash is approximately seven times and four times more than acid-insoluble ash and water-soluble ash, respectively. Ethanol soluble extractive is approximately two times higher than water-soluble extractive. Moisture content was <7.6 % and pH was 6.8 were shown in Table 1.

**Preliminary phytochemical investigation**
A number of phytoconstituents from natural sources have been proved efficacy to prevent the pregnancy. Many scientific reports were published for the antifertility activity of flavonoids (Hiremath and...
et al., 2000), glycosides, alkaloids, and steroid (Sadik et al., 2001). Phytochemical investigation of F. racemosa, alkaloid, glycoside, tannin, and steroid were present in the alcoholic extract (Table 2). Whereas, glycoside, steroid, and tannin were present in petroleum ether extract and terpene, flavonoids, glycoside, and tannins were present in the aqueous extract (WE). The successive solvent extraction with petroleum ether, alcohol, and aqueous water gave 2.5%, 5.8%, and 4.0%, respectively, practical yield.

Acute oral toxicity
Acute toxicity studies were carried out to evaluate toxicity and to determine the minimum lethal dose of the drug extracts, using Wistar rats. No clinical signs were evident in any animal during the treatment period (clinical observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern, tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma). No mortality as well as any clinical sign of toxicity has been observed at a dose level of 2000 mg/kg indicating that all the extracts come under category 5, and hence, LD50 cut-off was found to be 2000 mg/kg body weight. Hence, one-fifth of this dose, i.e., up to 500 mg/kg body weight, was used for antifertility investigation. Hematological and biochemical parameters were also performed before and after treatment and no significant changes were observed [12-16].

Reproductive outcome study
Table 3 shows the effect of different extracts on the fertility of female rats. The control rats showed good number of litters. Treatment of animal with different extracts resulted a significant (p<0.05, p<0.01). A significant antifertility activity of 56.5% and 40.3% was exhibited by alcoholic F. racemosa extract (AFR) and aqueous F. racemosa extract (WFR), respectively.

It was also found that the litters of the extract treated rats did not show any physical deformity. All litters grew up to the normal adult stage, which indicates that the extracts do not have a teratogenic effect, and the absence of teratogenic effect of extracts at a given dose justifies the safety of the plant. The present observations agree with Salhad et al., (1997), who reported the reversible antifertility effect of Ricinus communis (castor beans) on female rabbits and also supported by Endalk et al., (2005), who reported the same effect of the methanolic root extract of Rumex steudeli on female rats.

After 21 days of the extract-free period, the antifertility effect of the extracts was reversed for all animals. An increase in the number of litters observed in all the post-treatment groups may indicate the reversible antifertility effect of all extracts. These observations correlate the findings of Ganguly et al. (2007) and Gebrie et al. (2005), who reported the reversible antifertility effect with similar observations on the treatment with methanolic extract of Cassampelea pareira leaves in mice and methanolic root extract of R. steudeli in rats, respectively. The animal groups gave 9.06±0.15 litters at an average. This showed that there was no statistically significant change from the control group (10.00±0.03).

Anti-implantation and abortifacient activities
Postcoital antifertility study showed the anti-implantation activity in the treated animals. Treated animals delivered litters, which was significantly less than control (Table 4). The extract treatments with AFR, significantly (p<0.001) reduced the number of litters formation (Table 5). This indicates the abortifacient nature of extracts. An increase in the resorption index (%) by the extract is an indication of failure in the development of the embryo (Dhanawad et al., 2005). Such occurrence of fetal resorption suggests that interruption of pregnancy also occurred after implantation (Elbetlieh, 2000). These observations indicate the pregnancy terminating potential of the extract. Embryonic resorption could be due to modifications of uterine lining function or maternal toxicity which consequently may increase early resorption and late fetal death (Chaves, 1985; Khera, 1987). Hence, the present investigation clearly reveals that the extracts are effective before and after the implantation occurs (Vasudeva and Sharma, 2006).

Both these activities were calculated on the basis of number of implants and number of litters. The mean percentage of anti-implantation and percent resorption (abortifacent) were found to be highest for AFR-41.21% and WFR 28.07, whereas in the case of percent resorption, AFR-32.56% and WFR-20.76%. These results (Tables 4 and 6) indicated that all the extracts inhibited the conversion or development to implants in litters. The decrement in implantation caused by the extracts may be due to estrogenic or antiestrogenic activity as described by Hakiz (1970).

Estrogenic and antiestrogenic study
Antifertility activity of all the extracts was finally evaluated with the help of estrogenic and antiestrogenic activity associated with hormonal level and histological parameters such as uterine weight, diameter of uterus, thickness of endometrium, and height of endometrial epithelium. The stages of estrous cycle and its duration were determined as described by Makonnen et al. (1997). The detailed data have given in Tables 7 and 8. The uterotropic potency, in terms of the weight of uterus, AFR was found to be 84.23% and WFR was found to be 59.11% when compared with standard (ethinyl estradiol). The number of cornified cells in vaginal smears was considerably higher (+ to +++) than that of controls (0 to +) but notably less than that of ethinyl estradiol-treated rats (+++). All the treated rats showed open vagina. Oral administration of alone AFR and WFR at a dose level 500 mg/kg body weight, AFR shows highly significant (p<0.001) change in uterine weight, thickness of endometrial epithelium, and height of endometrial epithelium. The stages of estrous cycle and its duration were determined as described by Makonnen et al. (1997). The detailed data have given in Tables 7 and 8. The uterotropic potency, in terms of the weight of uterus, AFR was found to be 84.23% and WFR was found to be 59.11% when compared with standard (ethinyl estradiol). The number of cornified cells in vaginal smears was considerably higher (+ to +++) than that of controls (0 to +) but notably less than that of ethinyl estradiol-treated rats (+++). All the treated rats showed open vagina. Oral administration of alone AFR and WFR at a dose level 500 mg/kg body weight, AFR shows highly significant (p<0.001) change in uterine weight, thickness of endometrial epithelium, and height of endometrial epithelium. However, along with standard, AFR exhibiting strong estrogenic property, increase in uterine weight, diameter of uterus, thickness of endometrium, and height of endometrial epithelium, was found to be 84.23% and WFR was found to be 59.11% when compared with standard (ethinyl estradiol). The number of cornified cells in vaginal smears was considerably higher (+ to +++) than that of controls (0 to +) but notably less than that of ethinyl estradiol-treated rats (+++). All the treated rats showed open vagina. Oral administration of alone AFR and WFR at a dose level 500 mg/kg body weight, AFR shows highly significant (p<0.001) change in uterine weight, thickness of endometrial epithelium, and height of endometrial epithelium.
when compared with standard. These observations are similar to the finding of Ravichandran et al. (2007) and Vishnukant and Rana (2010) on the effect of hydroalcoholic extract of ailanthus excels (Roxb.) stem bark and Plumbago zeylanica leaves on the uterus of female Wistar rats. These observations revealed that these extracts acted as a competitive antagonist to ethinyl estradiol. Hence, the anti-implantation activity of these extract may be due to their antiestrogenic nature, which antagonise the action of estrogen and cause structural and functional changes in the uterus and finally decreases the implantation [17-21].

Hormonal analysis

Sex hormones were assayed based on their roles in maintaining pregnancy since a failing pregnancy could be correlated to the levels of these hormones in the body fluids (Yakubu and Bukoye, 2009). The reduction in the concentration of FSH is an indication of disturbance of estrus cycle and ovulation (Ganguly et al., 2007). LH is required for continued development and normal function of corpora lutea. The significant reduction in the level of serum LH could be associated with the physiological process of luteolysis preceding parturition (Yakubu and Bukoye, 2009). It could be attributed to pregnancy failure resulting from a luteal phase that is not being maintained. The reduced level of hormone may also be due to inactivation of luteinization of ovarian follicles, which could be responsible for the reduction in the concentration of serum progesterone in this study [14,22-28]. An elevated level of progesterone during pregnancy plays a key role in maintaining the conditions and is an

Table 4: Effect of extracts on anti-implantation activity

| Treatment (dose) | Anti-implantation activity |   |   |
|------------------|----------------------------|---|---|
|                  | Number of implants         | Number of litters | Mean % anti-implantation |
| Control          | 7.23±0.52                  | 7.20±0.65         | Nil                     |
| AFR              | 4.25±0.68                  | 4.20±0.05         | 41.21<sup>a</sup>       |
| WFR              | 5.20±0.29                  | 5.10±0.42         | 28.07<sup>b</sup>       |

Values are expressed as mean±SD. p values a=p<0.05, b=p<0.01 when compared with normal control.

Table 5: Hormonal levels in various groups of animals

| Treatment 500 mg/kg | LH                 | FSH           | Prolactin  | 17β estradiol | 17-OH progesterone |
|--------------------|--------------------|---------------|------------|---------------|-------------------|
| Control            | 6.25±2.42          | 8.64±5.20     | 40.25±6.10 | 745.12±45.40  | 14.54±1.10        |
| AFR                | 6.05±2.70<sup>a</sup> | 7.98±5.20     | 43.12±4.11 | 730±10.12     | 30.16±1.12        |
| WFR                | 4.92±1.40<sup>a</sup> | 4.58±6.20     | 32.42±7.10 | 535±01.10     | 28.24±2.24        |

n=5, data representation as mean±SD. p values a=p<0.05, b=p<0.01 when compared with normal control. LH: Luteinizing hormone, FSH: Follicle-stimulating hormone

Table 6: Effect of extracts on abortifacient activity

| Treatment (dose) | Abortifacient activity |   |   |
|------------------|------------------------|---|---|
|                  | Number of implants     | Number of litters | Resorption % |
| Control          | 7.32±0.62              | 7.10±0.30         | 3.00         |
| AFR              | 4.98±0.53              | 4.95±0.45         | 32.56<sup>a</sup> |
| WFR              | 6.10±0.10              | 5.80±0.45         | 20.76<sup>a</sup> |

Values are expressed as mean±SD. p values a=p<0.05, b=p<0.01, when compared with normal control.

Table 7: Effect of extracts on estrogenic and antiestrogenic study

| Treatment (dose) | Uterine weight (mg/100 g body weight; mean±SD) | Vaginal cornification |
|------------------|-----------------------------------------------|-----------------------|
| Control          | 7.20±0.65                                     | NIL                   |
| Ethinyl estradiol (1 µg/rat/day) | 335.40±7.56<sup>a</sup>    | +++                   |
| AFR (500 mg/kg) | 278.8±6.25<sup>a</sup>                         | + to ++               |
| WFR (500 mg/kg) | 198.0±5.25<sup>a</sup>                         | ++                    |
| Ethinyl estradiol (1 µg/rat/day)+AFR (500 mg/kg) | 425.23±05.48<sup>a</sup> | +++                   |
| Ethinyl estradiol (1 µg/rat/day)+WFR (500 mg/kg) | 325.12±04.25<sup>b</sup> | +++                   |

Values are expressed as mean±SD. p values a=p<0.05, b=p<0.01, c=p<0.001 when compared with normal control. Whereas +: Nucleated epithelial cells, ++: Nucleated epithelial cells and cornified cells, +++: Cornified cells

Table 8: Histological changes in the uterus and endometrium after treatment with extracts

| Treatment (Dose) | Diameter of uterus (µm±SD) | Thickness of endometrium (µm±SD) | Height of endometrial epithelium (µm±SD) |
|------------------|-----------------------------|----------------------------------|------------------------------------------|
| Control          | 30.5±5.25<sup>a</sup>      | 54.14±2.12                       | 17.4±0.25<sup>a</sup>                   |
| Ethinyl estradiol (1 µg/rat/day) | 82.15±6.25<sup>a</sup>    | 24.5±1.15.15<sup>a</sup>         | 45.10±1.48<sup>a</sup>                  |
| AFR (500 mg/kg) | 645.0±6.62<sup>a</sup>      | 274.1±65.42<sup>a</sup>          | 72.40±1.4<sup>a</sup>                   |
| WFR (500 mg/kg) | 327.14±2.14<sup>a</sup>     | 195.0±2.05<sup>a</sup>           | 45.40±2.44<sup>a</sup>                  |
| Ethinyl estradiol (1 µg/rat/day)+AFR (500 mg/kg) | 945.45±6.2<sup>a</sup>    | 274.1±65.42<sup>a</sup>          | 72.40±1.4<sup>a</sup>                   |
| Ethinyl estradiol (1 µg/rat/day)+WFR (500 mg/kg) | 524.48±2.02<sup>a</sup>    | 162.1±7.12<sup>a</sup>           | 34.14±4.25<sup>a</sup>                  |

Values are expressed as mean±SD. p values a=p<0.05, b=p<0.01, c=p<0.001 when compared with normal control.
important factor in the implantation process. Therefore, luteolysis and reduction in the blood levels of progesterone may contribute to abortion and anti-implantation activity of all extracts [29,30]. The findings of the present study were agreed with previous studies which reported the effect of *Inula viscosa* and *Bambusa vulgaris* leaf extract on implantation and abortion in rats and rabbits (Yakubu and Bukanu, 2009). In this study an increase in prolactin level was observed (Table 5); these findings were also supported by Ganguly et al. (2007), who reported that a combination of enhanced prolactin and suppressed LH secretion is due to prolongation of estrus cycle (Ganguly et al., 2007). An imbalance in endogenous estrogen and progesterone levels could be responsible for anti-implantation activity (Dhanwad et al., 2005).

**CONCLUSION**

The present findings inferred that the gathering treated with the most noteworthy convergence of plant concentrate indicated great come about as that of the standard medication and was underpinned by histopathological investigations of the antifertility activity on female Wistar rats. Antifertility activity of plant extracts was evaluated with the help of reproductive outcome, anti-implantation, abortifacient, estrogenic, and antiestrogenic study was also performed, which further supported by the hormonal analysis. It is well known that for implantation exact equilibrium of estrogen and progesterone is essential and any disturbance in the level of these hormones may cause infertility. In our study, it clearly demonstrates that extract of *F. racemosa* bark, the control rats showed good number of litters. Treatment of animal with different extracts resulted a significant (p<0.05, p<0.01). A significant antifertility activity (56.6%) was exhibited by AFR. It was also found that the litters of the extract treated rats did not show any physical deformity. All litters grew up to the normal adult stage, which indicates that the extracts do not have a teratogenic effect, and the absence of teratogenic effect of extracts at a given dose justifies the safety of the plant. After 21 days of the extract-free period, the antifertility effect of the extracts was reversed for all animals. An increase in the number of litters observed in all the post-treatment groups may indicate the reversible antifertility effect of all extracts.

Estrogenic in nature at the dose of 500 mg/kg b.wt. as evident form the along with standard, AFR AE significance increases in the diameter of uterus, height of endometrial epithelium, and thickness of endometrium in extracted animal, whereas, along with standard, WFR WE 500 mg/kg b.wt. showed less antiestrogenic in nature decrease in the diameter of uterus, height of endometrial epithelium, and thickness of endometrium in extracted animal. It is a suitable plant for forming along with standard, AFR AE significance increases in the diameter of uterus, height of endometrial epithelium, and thickness of endometrium in extracted animal. It is a suitable plant for working out and should be experimented for the antifertility program. Further studies on mechanism of antifertility action and isolation of the active components responsible for antifertility effect are in progress.

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