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Studies on the Use of *Aloe vera* Extract as a Contraceptive in Female Rats

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ABSTRACT

Background: The study on the natural herbal contraception has become one of the main interests of modern contraceptive studies. Herbs have been used by women since the beginning of time in an attempt to control their fertility. The development of new fertility regulating drugs derived from medicinal plants is an attractive proposition, *Aloe Vera* is a durable plant belonging to Sousanian family. **Aim of the work:** This work was assessed to evaluate the probable contraceptive effect of the aqueous extract of *Aloe vera* plant and its effect on the some vital organs in the female albino rats. **Materials and Methods:** This study was performed on twenty female albino rats with an average 120-140g body weight. The animals were divided into two groups (5 /cage); **Group I** (Control untreated group) and **Group II** (*Aloe vera* group that supplied orally with 7 mg/kg body wt/day of the plant extract for 30 ± 2 days).**Results** A prolonged proestrus and estrus phases of the estrous cycle were observed in the *Aloe vera* group .The mean serum level of estrogen (estradiol) was significantly increased in the *Aloe vera* group as compared to the control group ($P<0.01$) while non significant difference was found for serum level of progesterone and the tumor markers, CA15-3 and CA-125.The results also showed a marked decline ($p<0.01$) in levels of the serum calcium, creatinine, urea, total proteins, albumin, globulin and ratio of TC/HDL accompanied with a marked elevation ($p<0.01$) in the serum phosphorus, total lipid, TC, TG, HDL and LDL levels in the *Aloe vera* group in comparison with those of control group. However, levels of uric acid, AST, ALT, GGT, VLDL and ratios of LDL/HDL (risk factors) and A/G were approximately as that of the control group.

Conclusion: It could be concluded that *Aloe vera* can be used as a contraceptive drug that can increase the estrogen level due to its phytoestrogen components such as beta sitosterol and without deleterious effects on the other vital organ (liver and kidney), however it's use is to be restricted with women suffering from low ca++level as well as osteoporosis.

Keywords: *Aloe vera*, fertility, contraceptive, tumor markers, kidney function, liver function

INTRODUCTION

One of the important concerns of today is the problem of overpopulation. Family planning has been prompted through the use of several synthetic steroidal contraceptives, but the fear of cancer and cardiovascular disease overshadows its continuous use among

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women[1-5]. Therefore, a great attention has now been focused on indigenous plants for their possible contraceptive effect. The goal of herbal remedy is to enable the body to readjust excess levels of hormones, bring them to 'normal' and establish normal physiological function[6,7].

Fertility regulation using plants or plant preparation has been reported in the ancient literature of indigenous system of medicine[8]. Several plants products inhibit male & female fertility and may be developed into contraception[9,10].

One of the most important, world-famous herbs is *Aloe*. *Aloe vera*, derived from an Arabic word "Alloeh" means shinning bitter substance and *vera* means "true". It is a succulent cactus like perennial plant originated from arid climates of North Africa[11]. *Aloe vera* is belonging to liliopsida class, liliales order, and liliaceae genus, with over 275 species worldwide[12,13]. The fame that *Aloe vera* has acquired over a few years has been due to its pharmacological benefits. Aloe gel or juice has been known for its local actions such as wound healing, burns and skin infections[14]. Anti-bacterial, anti-inflammatory and anti-cancer activities of *Aloe vera* have also been observed and attributed to glycoprotein and polysaccharides[15-17].

Studies also reveal use of aqueous *Aloe vera* extract by women of western regions of Cameron to treat infertility[18]. A study on the effect of *Aloe vera* on pregnant rats' ovaries showed that this plant causes increased vasculo-genesis around the secondary follicles. Results also showed that *Aloe vera* has similar effect to estrogen and follicle-stimulating hormone[19].

However, there is a limited information available on the effect of *Aloe vera* plants on the reproductive system, and also considering different compositions of this plant including Anthraquinones and Phytoestrogens such as Beta-cytosterol and camposterol, it is possible that these compounds could affect sex hormones[20].

Keeping in view the clinical importance of *Aloe vera*, the present study is conducted to evaluate: a) the effectiveness of *A. vera* as a contraceptive and its probable effect on estrogen and progesterone levels and, b) its safety margin of it's use by measuring some breast and ovarian cancer tumor markers. c) Also, to determine the effects of of *A. vera* supplements on some liver and kidney parameters in female rat sera.

MATERIALS AND METHODS

Preparation of Aloe Vera extract

Five grams (5 g) of *Aloe vera* were grinded, completed to 100 ml water and rinsed for 12 hours. Then, filtered with muslin cloth and stored in a refrigerator at 4°C till use. The extract was prepared every two days.

Animals and treatment

A total of 20 female rats of Sprague Dawely strain, with an average body weight 120 ± 20g

and about 10-12 weeks old were purchased from Theodore Bilharz Research Institute, Giza, Egypt. Animals were kept at animal house under control conditions (12 hour light/dark cycle, the temperature was 23±3 ℃, and compressed food and water was available *ad-libitum*). The rats were divided into two groups as follows:

Control group: The rats in this group were given food and water *ad-libitum*.

Experimental group: Rats in the experiment group received orally daily 7 mg/kg body weight of *Aloe vera* extract for 30 ±2 days.

Finally, The phases of estrous cycle were determined by daily examination of vaginal smear test and those rats that had reached the stage of diestrus were anesthetized using diethyl ether and blood samples were taken from retro-orbital vein and centrifuged at 3000 rpm for 15 min. Blood sera were quickly extracted and then conserved at -20°C till being used for further biochemical analysis.

Assessment of biochemical parameters

Total protein (TP) [21] and albumin[22] concentration were determined and globulin concentrations were calculated by difference (TP— albumin). Serum total lipids (TL)[23],triglycerides(TG)[24], total cholesterol[24]and HDL-cholesterol content[24] were determined. LDL-C was calculated using the **Friedwald's** formula[25].VLDL was calculated using the **Friedewald's** equation.

Friedewald's equation: **LDL (mg/dl) = TC-{HDL + [TG/5]}.**

VLDL = TG/5

Also, aspartate amino transferase(AST) and alanine amino transferase (ALT) activities were measured [26].In particular, Gammaglutamyl transferase (γGT) was also assayed [27]. Serum urea and creatinine concentrations were determined[28, 29], while serum uric acid was determined by uricase enzymatic colorimetric method [30]. However, serum calcium and phosphorus ions were determined using the Flame photometry method[31].

Collected serums were stored at −20C° and were analyzed simultaneously. CA-125 and CA 15-3levels were measured using the electrochemiluminescence immunoassay technique.

Statistical analysis

The results were expressed as Mean ± SEM of the mean. The data were analyzed by one way analysis of variance (ANOVA) and were performed using the Statistical Package (SPSS) program, version 20.

RESULTS

Analysis of the effect of aqueous *Aloe vera* extract on female rats showed a prolonged proestrus and estrus phases of the estrous cycle compared with the control. The analysis also showed a highly significant increase in the

estrogen hormone and statistically significant reduction in the progesterone hormone concentrations in the experimental group as compared to the control group($P<0.01$)(Fig.1). Moreover, in the *Aloe vera* treated group, serum concentration of CA15-3 and CA-125 was approximately equal to those of the control group(Fig.2).

The present study also showed a highly significant increase in the serum phosphorus level in the treated group after administration of the *Aloe vera* extract as compared to the control rats ($P< 0.01$) (Fig.3). However, There was a significant decrease in the calcium level in the *Aloe vera* treated rats as compared to the control($P< 0.01$)(Fig.3).

Aloe vera treated rats showed insignificant decrease ($p< 0.01$) in serum ALT, AST and γGT activities as compared to the values of the control group (Fig.4).Similarly, there was an insignificant decrease in the uric acid level in the treated rats when compared to the control ($P< 0.01$)(Fig.5). However, the decrease in the creatinine and urea levels in the *Aloe vera* extract treated rats were significant($P<0.01$) (Fig.5).

On day 30, *Aloe vera* treated group exhibited a significant increase in their total serum lipids, total cholesterol and triglycerides levels as compared to those of the control group(Fig.6). The *Aloe vera* treated rats exhibited a significant increase in their serum HDL and LDL levels when compared to the control rats (Fig.6). However, The VLDL and LDL/HDL ratio in the treated group were approximately the same as that of the control group. A significant reduction in the TC/ HDL level was observed when the rats received *Aloe vera* treatment, indicating that the *Aloe vera* preparations are able to reduce atherogenic LDL cholesterol levels. Furthermore, the protective effects of *Aloe vera* were more pronounced on the atherogenic LDL cholesterol levels than on the HDL cholesterol levels in the treated rats (Fig.6). In addition, *Aloe vera* treated rats showed a marked decline ($p<0.05$) in the level of serum total proteins, albumin and globulin in comparison with those of control rats (Fig.7). The values of A/G ratio showed non-significant changes in the control and the experimental group (Fig.7).

DISCUSSION

Family planning has been prompted through several methods of contraception, but due to adverse effects produced by synthetic steroidal contraceptives attention has now been focused on indigenous plants for their possible contraceptive effect. *A. vera* is a very versatile plant that has many different uses[6]. Only few scientific studies on *A. vera* are demonstrated its effect on sex hormones[17-20]. **Fahim, Wang**[17] concluded that it may be useful as a contraceptive, especially in preventing the transmission of HIV.

The present work showed a significant increase in the duration of proestrus and estrus phases of the estrous cycle in the *Aloe vera*

treated group compared to control (data not shown). This observation is suggestive of antifertility effects as reported by **Marcondes *et al.***[32] and **Okoko *et al.***[33]. Also, there was a highly significant increase in estrogen level in the *Aloe vera* group compared to control group (Fig.1) and that trends in plasma estrogen concentrations confirm to findings previously reported by **Telefo *et al.***[34] who suggested that *Aloe vera* plant extract contains compound that increase ovarian steroidogenesis and serum estrogen concentration. Also, since *Aloe vera* plant extract has similar effects to follicle-stimulating hormones effects on the ovaries [35], hence, it functions in a similar manner, and causes an increase in growth and development of follicles and consequently, an increase in estrogen secretion from follicular cells. However, estrogen concentration significantly increased in the experimental group that received *Aloe vera* extract, while occurred a significant reduction in the level of progesterone (Fig.1). This is consistent with findings of **Telefo *et al.***[18]. Other workers have reported non-significant difference in progesterone levels when compared to the control group[20]. Higher estrogen levels could participate in reducing progesterone levels, since under certain circumstances estrogen enhances conversion of progesterone to reduced products[36,37].

To evaluate whether the use of *Aloe vera* has a pre-carcinogenic potential, measurement of CA-153 and CA-125 expressions were done. The CA15-3 is regarded as one of the most reliable tumor markers used in diagnosis and monitoring of breast cancer development[38,39] and CA-125 is the most extensively studied tumor marker used in screening for ovarian cancer[40]. The observed non alteration in serum levels of CA-153 and CA-125 in *Aloe vera* treated rats as compared with the corresponding control group could be an indication that the extract might not have a pre-carcinogenic potential.

Specifically, this study showed that there was a significant decrease in serum calcium level in the treated group associated with the increase in the estrogen level (Fig.3). This is in consonance with previous study of **Ghoneim**[41], which reported the decrease in the calcium level during contraceptive therapy. Also, Several studies have shown that the use of contraceptives are associated with fluctuation of serum electrolytes[42,43]. Since it is a known that estrogen, like aldosterone, cause fluid retention. **Akhigbe *et al.***[31] suggested that these changes seen in serum level of electrolytes could be as a result of the reabsorption of sodium from the renal tubules.

Serum concentrations of creatinine and urea could be used as indicators of kidney functions[44,45]. The effect of *Aloe vera* extract on renal function was investigated by evaluating the serum concentrations of urea, uric acid and creatinine. The current study revealed that the use of *Aloe vera* extract induced a significant decrease in the serum urea

and creatinine levels that is in consonance with the study of **Bolkent *et al.***[46]. This findings is disagree with that found by **Saka *et al.***[47]who suggested that the use of aloe causing impaired renal function evident by an increase in serum creatinine concentration. This study also recorded insignificant reduction in the uric acid levels that are accordance with that found by **Bolkent *et al.***[46]. Many authors such **Chatterjee *et al.***[48]found that nephroprotective effect of *Aloe vera* extract could be due to the inherent antioxidant and free-radical-scavenging principle(s) contained in the extract that produced significant protection on renal function by the significant reduction in serum creatinine, urea and uric acid concentrations.

ALT, AST and γGT are liver enzymes that can serve as markers of hepatocellular injury[49]. The observed non alteration in serum levels of AST, ALT and γGT in *Aloe vera* rats as compared with the corresponding control group is an indication that the extract might not have altered liver function in the rats. Moreover, significant reduction in the serum total protein, albumin and globulin of the *Aloe vera* treated animals accompanied with slight non-significant elevation in the A/G ratio were also observed. These findings are in agreement with those of **Adesokan *et al.***[50]who reported that administration of aqueous extract of *Aloe vera* did not have any adverse effect on the liver and kidney functions in rats showing that the use of *Aloe vera* extract is quite safe and exerts untoxic effects, on functions of vital organs. **Kinosian *et al*.**[51]study showed that the changes in TC/HDL and LDL/HDL ratios were better predictors of coronary heart disease than the changes in LDL level. The present investigation, revealed marked decrease in the TC/HDL ratio and insignificant decrease in LDL/HDL ratio in *Aloe vera* treated group when compared with the control group. However, an elevation in total lipids, cholesterol and triglycerides which are well known as risk factors for cardiovascular diseases were also recorded[52].A well-known consequence of most common hormone replacement therapy is an increase in plasma triglycerides that occurs through estrogen-induced inhibition of hepatic triglyceride lipase[53].

In conclusion, the results of the present study suggest the possibility of using continuous administration of *Aloe vera* extract as a new and effective contraceptive without deleterious effects on liver and kidney function. However additional toxicological studies have be undertaken done before final and/ or recommendation of usage *Aloe vera* as contraceptive, especially for women with low Ca^{++} level or suffer from osteoporosis.

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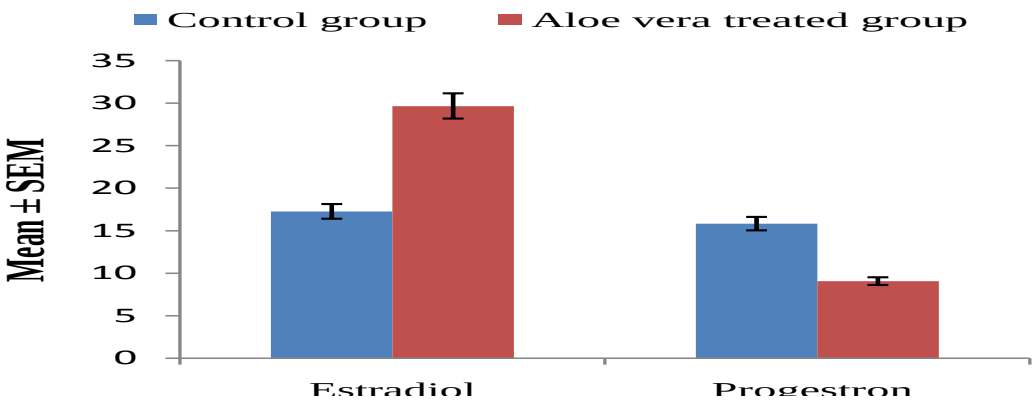

Fig.1: Levels of Estradiol and progesterone in the control and *Aloe vera* groups.

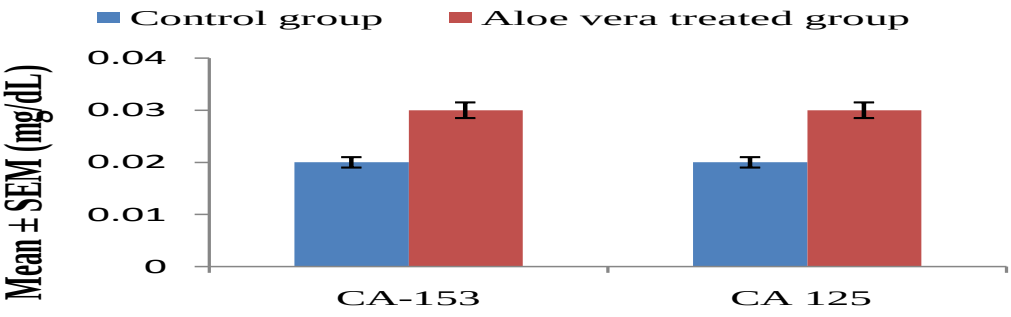

Fig.2: CA 15-3 and CA-125 levels in the control and *Aloe vera* treated groups

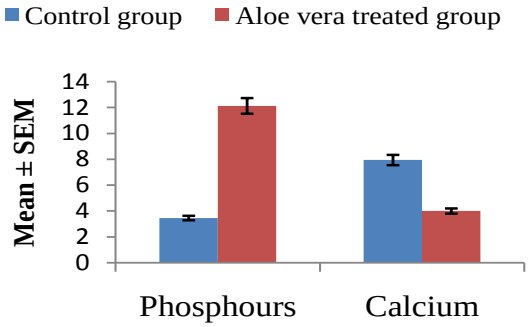

Fig.3: Phosphors and calcium levels in control and treated groups.

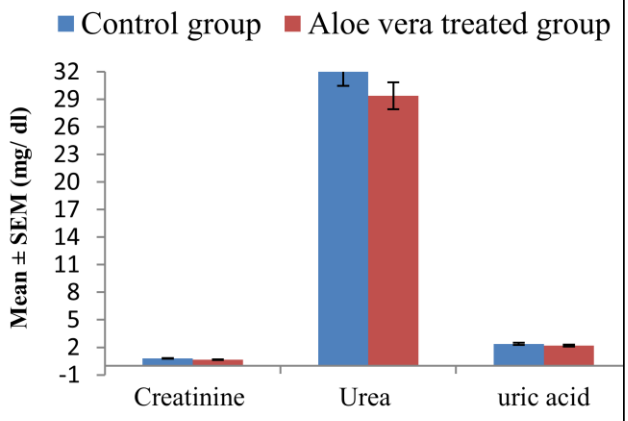

Fig.4: Changes in creatinine, urea and uric acid levels in the control and treated

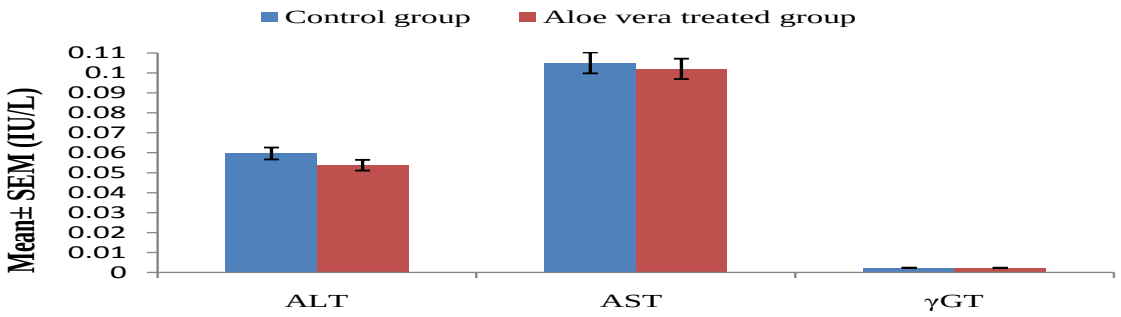

Fig.5: Changes in the ALT, AST and γGT activities in the control and *Aloe vera* treated groups

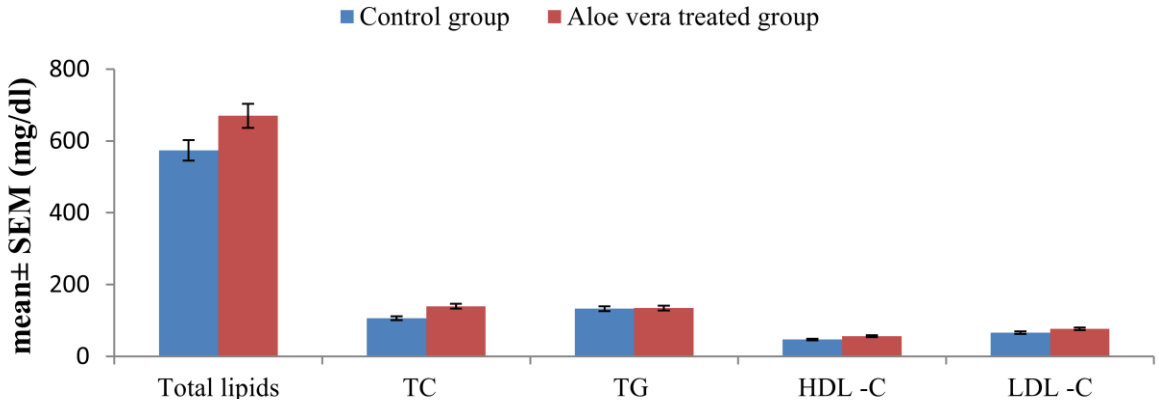

Fig.6: Changes in the levels of total lipid (TL), triglycerides (TG), total Cholesterol (TC), HDL cholesterol (HDL-C) and LDL-cholesterol (LDLC) in the control and *Aloe vera* treated groups.

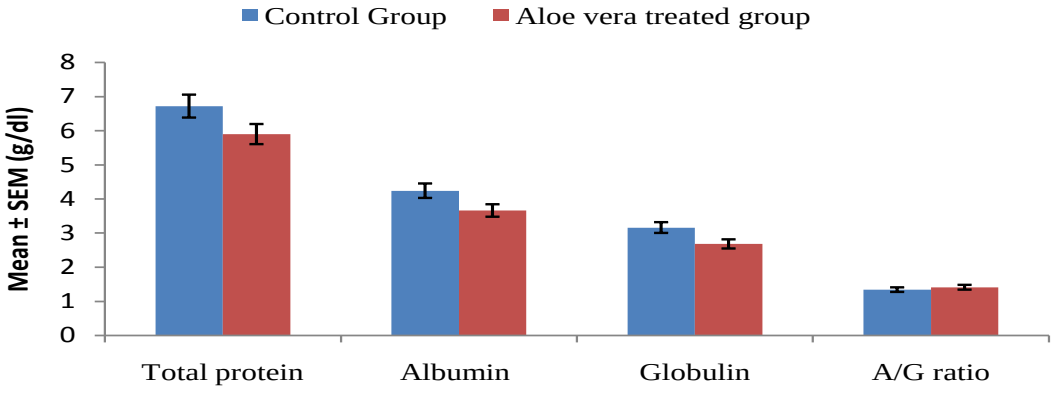

Fig.7: Changes in the levels of serum proteins profile (g/dl) and A/G ratio in the control and *Aloe vera* treated group.