Adverse Effect of Rheumatoid Arthritis on Male Wistar Rat's Fertility: Protective Role of Costus Extract

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Abstract

Rheumatoid arthritis (RA) is an autoimmune systemic complaint. *Costus speciosus*, an ornamental plant, which traditionally used in urinary diseases, rheumatism, jaundice and dropsy. The purpose of the study is to assess the protective effect of Costus on rheumatoid arthritis induced male rat in fertility. Thirty male adult Wistar rats (190-200 g) were sectioned into 6 groups. They were subdivided into 3 groups; group 1 was control received Distilled water, groups II and III received two various doses of Costus extract (200 and 400 mg/kg, respectively) for 60 days. Another 3 groups were subjected to RA induction via Freund's adjuvant. First group of RA induced rats was given Distilled water. Other two groups were given orally (200 and 400 mg/kg dosage of extract, respectively) from the 2nd day of RA induction for 60 days. Sex organ relative weight, sperm concentration assay, testicular histopathology and immunohistochemistry of androgen receptors, TNF α and Bax protein were determined. Rheumatoid arthritis caused significant decrease in relative weight of sex organs which relatively improved by doses of Costus (200, 400 mg/kg). In addition, RA caused a significant reduction in sperm count which was improved via Costus (200, 400 mg/kg). RA induction caused testicular degeneration with marked depletion of spermatozoa, which markedly improved with costus treatment as shown in histopathological sections. Rheumatoid arthritis caused a reduction in %IHC of androgen receptors and increase of %IHC of both TNF α and Bax protein. Costus (200, 400 mg/kg) significantly improved %IHC of androgen receptors and significantly decreased %IHC of both TNF α and Bax protein, in RA induced groups. We can concluded that *Costus speciosus* had potentially useful role in improving fertility disorders caused by RA.

Introduction

Infertility and gonadal dysfunction with chronic rheumatic disorder are multifactorial (**Østensen, 2004**~~{Østensen, 2004 #54}~~**and Silva and Brunner, 2007**). The unhealthy conditions, as malnutrition, bad drug use, alcohol, obesity, tobacco, in addition to female and male genital illness (**Balen and Rutherford, 2007**) may decrease fertility (**Østensen, 2004**~~{Østensen, 2004 #54}~~**and Silva and Brunner, 2007**). In addition, hypothalamic–pituitary–adrenal axis dysfunction (**Suehiro et al., 2008 and Medeiros et al., 2009**); autoimmune disorders with release of anti-endometrial, anti–corpus luteum (**Silva and Brunner, 2007**), or anti-sperm antibodies (**Soares et al., 2007and Suehiro et al., 2008**), high activity of disease or chronic renal disorder and immunosuppressive medications (**Latta et al., 2001 and Østensen et al., 2006**) can initiate reduction of fertility in patients with rheumatic sicknesses. In the healthy people, the reproductive ability also decreases with age in both sexes (**Balen and Rutherford, 2007**).

Rheumatoid arthritis (RA) is a systemic disorder characterized by joint swelling with pain function loss, joint damage and permanent deformity if left without treatment (**Tripathy et al., 2009**). The spread of RA is reliable globally influencing about 0.5-1.0% of the population. It commonly occurs in individuals between 25 and 55 years of age (**Meera et al., 2008**). Although the exact cause is unknown but various hypotheses suggested that it is caused by the integration of genetic tendency and exposure to environmental factors like viruses (**Babushetty and Sultanpur, 2012**). The RA has several adverse effects on the body systems including heart and blood vessels, lungs, kidneys, liver and skin.

Tumor necrosis factor α (TNF α) is a crucial cytokine in acute inflammation and play a vital role in the reproductive physiology of men (**Sharkey, 1998 and Harada, 2001**). TNF α showed a wide diversity of biologic actions which may impair the reproductive functions, like stimulation of the immune-cascade and chemotaxis of neutrophils, cytolytic and cytostatic effect on tumor cells, initiation of fibroblastic progression, prostaglandin and collagenase synthesis, and possible efficacy on sperm movement and function (**Hill et al., 1989**).

Apoptosis is considered one of the vital cell death mechanisms with necrosis. Apoptosis is controlled by different genes and molecules that all play a pivotal role in stimulation of apoptosis such as Bax proteins. Induction of Bax proteins stimulate the secretion of cytochrome c and other apoptogenic factors leading to apoptosome formation, which then triggers caspase-9 with caspase-3 and 7 (**Aitken et al., 2011**). Sperm DNA damage activated by apoptosis has been demonstrated in different mammals (**Dogan et al., 2012**). The balance between germ cells and sertoli cells in the testes during spermatogenesis is achieved by apoptosis and an imbalance in this process was denoted to cause infertility in males (**Aitken et al., 2011**). Bax proteins have been founded in RA (**Sioud and Mellbye, 1998 and Kobayashi et al., 2000**). These Bax proteins can form hetero and homodimers, in addition the relative plenty of pro-apoptotic and anti-apoptotic molecules are vital in adjustment of apoptosis and cell cycle in RA (**Hilbers et al., 2003**).

Deceased fertility is not uncommon amongst patients with rheumatic disorder (**Gupta et al., 2010 and Clowse et al., 2012**). Drug treatments may considered the major cause for gonadal impairment (**Freire et al., 2006**). The reproductive possibility of male patients is declined by the disease directly in the testis or by immunosuppressive drugs. The assessment of male patients must depend on perfect case history, semen analysis, full physical checkup, and sex hormone examination (**Tiseo et al., 2016**).

Researchers are looking for the traditional medicine for finding extended acting anti-inflammatory medications having little side effects (**Ekambaram et al., 2010 and Patil et al., 2010**). Plant-derived drugs still a vital resource, especially in developing countries, for severe diseases therapy. It has been recorded that 60-90% of RA patients who already utilized alternative and complementary therapy; the majority used traditional Chinese medication (**Zhao et al., 2013**). In India, there are more than 2500 plants species which are used nowadays as herbal medicine (**Sudha and Mathanghi, 2012**).

Costus speciosus Koen. (Keu, Crape ginger), is considered an Indian ornamental herb, has been utilized in traditional therapy for a long period. This herb of Costaceae (Zingiberaceae) family is well-known as keukand (Hindi) and diversified Crepe Ginger (English) (**Srivastava et al., 2011**). The plant have anti-inflammatory, laxative, anti-arthritic and anti-fungal actions. It also utilized in bronchial asthma and gout rheumatism (**Khare, 2007**). *Costus* have a useful anti-arthritic efficacy as it shows a good results in monitoring inflammation in adjuvant induced arthritic rats. The medication is a hopeful anti-arthritic drug from plant extract in the medication of inflammation (**Srivastava et al., 2012**).

The current study directed to explore the effect of *costus* as anti-arthritic treatment on male rat fertility assessed by sex organs weight, sperm count, sperm abnormalities as well as viability. Also testicular

androgen receptors, TNF α and Bax protein were assessed in the present study by immunohistochemistry.

Materials And Methods

Preparation of plant extract:

Aerial parts of the *Costus speciosus* Koen plant were purchased from local market then subjected to morphological identification by Plant Taxonomy Department in Faculty of Sciences, Suez Canal University. The plant was extracted according to **Srivastava et al., (2012)** in which the parts were dried, coarsely powdered and used for the extraction procedure. The plant coarse powder was extracted by soxhlet apparatus with the solvents in increasing manner of polarity starting with petroleum ether, ethyl acetate, chloroform, and methanol. By means of rotary evaporator, the extracts were condensed under decreased pressure after that it was dried in open air. The dried extract was suspended in distilled water (vehicle) and utilized as anti-arthritic drug.

Animals and housing:

The whole experimental procedures and protocols of this study were reviewed by the Ethical research Committee of Faculty of Veterinary Medicine, Suez Canal University, Egypt. Thirty adult male albino Wistar rats were used in the current study and their weight were between (190-200 g). Animals were kept in a perfect environment of 12 hr /12 hr light-dark cycle and in a room temperature between (22- 25°C) in lab animal house in Faculty of Sciences, Suez Canal University. Rats received food and water *ad libitum*. Animals were gathered and kept in stainless steel cages of 20 cm high and 860 cm² foot. They were maintained for 2 weeks for acclimation to animal house circumstances.

Rats were fed on a standard commercial food pellets (El Damassy Company, Cairo, Egypt). The diet consisted of: yellow corn 56.1%– soybean meal 27.3% – corn gluten 15.3% – dicalcium phosphate 0.13% – limestone powder 0.49% – salt 0.1% - vitamin & mineral mix 0.25% - DL-Methionine 0.17%- L-Lysine HCL 0.1% . According to (**Helrich, 1990**).

Rats were divided into 6 groups, 5 animals for each. The first 3 groups were control, subdivided into 3 subgroups: group 1 was the control group, was given the vehicle (distilled water), groups II and III received two various dosages of costus extract (200 and 400 mg/kg, respectively) for 60 days. The other three groups were subjected to RA induction **Freund's adjuvant**. Rats were injected a dose of 0.1 ml of Freund's Complete Adjuvant (FCA) purchased from Sigma-Aldrich, USA) in the planter area of the left hind paw. After 2 days, FCA exhibited soft tissue puffiness around the ankle joints as a marker for arthritis and then divided into: Group IV of RA induced rats were given vehicle. Groups V and VI were given two various dosages of extract (200 and 400 mg/kg, respectively) per Os via gavage for 60 days.

Relative weights of sex organs:

By the end of the study, rats were euthanized by tetra hydro furan and organs were dissected. Testis, seminal gland, tail of the epididymis and prostate gland were dissected and weighed. The latter relative weights of organ (organ weight/body weight X 100) were determined for each rat in control and treated groups, according to **(Elgawish and Abdelrazek, 2014)**.

Sperm count, viability and abnormalities:

The constituents of epididymis was collected by cutting of the cuda epididymis utilizing surgical blades then squeezed in a clean sterile glass. This constituents was diluted 5 times by 2.9% sodium citrate dihydrate solution and completely mixed to assess the total sperm count **(Bearden and Fuquay, 1980)**. Sperm count was calculated according to the formula: number of sperm counted x dilution factor/volume x 1000. One drop of the mixture was smeared on a glass slide, after then it stained by Eosin Nigrosin stain to measure the viability and sperm abnormalities by means of the principles of **Okamura et al. (2005)**.

Histopathology

Samples from testis were obtained from all control and experimental groups. They were immediately put in 10% formalin saline to be fixed. The latter samples were then organized utilizing standard measures for Hematoxylin and Eosin stain as designated by **(Bancroft et al., 1996)**.

Immunohistochemistry:

It was managed according to **Abdelrazek et al., (2016)**. The formalin fixed and paraffin fixed testis were cut into 5µm sections and mounted on a positive charged slides for androgen receptors, TNF α and Bax protein. Sections were dewaxed, rehydrated and autoclaved at 120°C for 10 min. in 10 Mm citrate buffer (pH 6). After washing with phosphate buffer saline (PBS), endogenous peroxidase was blocked using 0.3% H₂O₂ in methanol for 15 min. Slides were washed in PBS again and blocking was performed by adding blocking buffer and incubated for 30 min. at room temperature. Primary monoclonal and polyclonal antibodies for androgen receptors (Cat. No. MA1-150, Thermo Fisher Scientific Co., USA), TNF α (Cat. No. PRC3014, Thermo Fisher Scientific Co., USA) and Bax (Cat. No. MABC 1597, Sigma- Aldrich, USA) were added after dilution by PBS (2µg/mL for androgen receptors, 1:50 for both TNF α and Bax protein). The slides were washed 3 times for 3 min. each with PBS. Biotinylated polyvalent secondary antibody (Cat. No. 32230, Thermo Scientific Co., UK) was applied to tissue sections and co-incubated for 30 min. The slides were washed three times for 3 min. each with wash buffer. The reaction was visualized by adding Metal Enhanced DAB Substrate Working Solution to the tissue and incubated 10 min. The slides washed two times for 3 min. each with wash buffer. Counterstaining was performed by adding suitable amount of hematoxylin stain to the slide to cover the entire tissue surface **(Bancroft and Cook, 1994)**. For quantitative analysis, the intensity of immunoreactive parts was used as a criterion of cellular activity. Measurement was done using an image analyzer (Image J program). The total field and immunohistochemical (IHC) stained areas were calculated and the percentage of IHC stained area calculated as follow: %IHC stained area = IHC stained area/Total area X 100.

Statistical analysis:

Statistical analyses were fulfilled by ANOVA via utilizing statistical software package, SPSS; version 20. Results were shown as mean $\pm$ SE and the $P < 0.05$ were expressed as statistically significant.

Results

Organ relative weights

Table (1) showed that relative weight of testis, induced RA showed a significant ($P < 0.05$) decrease in relative testicular weight compared to control. Treatment of RA via (200, 400 mg/kg) significantly ($P < 0.05$) increase relative testicular weight when compared to RA groups, but the dose level hasn't effect, there was no significant change between dose 200 and 400 mg/kg but does not return to normal levels. The same results regarding the relative weight of tail of epididymis. Treatment of RA with costus (200, 400 mg/kg) was significantly ($P < 0.05$) increase the relative weight of tail of epididymis and returned to the normal levels. Concerning relative weight of seminal vesicle, no significant change was observed among all groups. Whereas in prostate gland, there was a significant ($P < 0.05$) decline in relative prostate gland weight in induced RA group compared to control one. No change was observed in relative weight of prostate gland after treatment of induced RA with (200, 400 mg/kg).

Sperm count, viability and abnormalities:

In RA induced rats, the count of sperm cell significantly ($P < 0.05$) decreased compared to other groups. Treatment of RA with (200, 400 mg/kg) significantly increased sperm cell count than RA group but not returned to the control group value. The sperm viability % significantly ($P < 0.05$) decreased in RA induced group more than the control. Treatment of RA rats with (200, 400 mg/kg) significantly ($P < 0.05$) increased sperm viability % than RA group but not returned to the control value, whereas there was a dose dependent increase. Regarding the sperm abnormalities, there was a significant ($P < 0.05$) rise in sperm abnormalities in RA induced rats than control. Treatment of RA with (200, 400 mg/kg) significantly ($P < 0.05$) increase sperm abnormalities than RA non-treated group but not returned to the control value (*Table 2*).

Histopathology of testis:

The testis of control group consisted of convoluted seminiferous tubules in a stroma with leydig cells. Each seminiferous tubule showed normal cell association, which lined with a stratified epithelium of spermatogenic cells besides sertoli cells and showed the different phases of spermatogenesis, primary and secondary spermatocytes with formation of spermatids then finally spermatozoa. Between the tubules the interstitial cells of leydig and interstitial connective tissue were seen. Histopathological changes in seminiferous tubules of costus (200, 400 mg/kg) showed normal tissue architecture with formation of abundant number of spermatozoa. RA induced group showed sloughing and disrupted cell arrangement, moderate number of tubules were lined with moderate to few number of spermatogenic

cells and large number of spermatids exhibited degeneration and necrosis and vacuolation. Interstitial space was enlarged due to edema, congestion of blood vessels and tubular atrophy in the seminiferous tubules. Most of the tubules showed thickening and hyalinization of their basement membranes, hyperplasia of sertoli cells and lined with few number of spermatogenic cells that were characterized by degeneration. Vacuolization and necrotic changes with sloughing coagulation of spermatids at center of tubules were also observed. Treatment with Costus (200, 400 mg/kg) showed mild to moderate degeneration of germ cells in seminiferous tubules, mature spermatozoa could be observed in lumen of moderate to large number of tubules (*Figure 1*).

Immunohistochemistry:

Concerning the results of androgen receptors, it was denoted a significant decrease ($P<0.05$) in the %IHC of androgen receptors in the testicular tissue of RA induced rats related to control groups. Treatment with costus (200, 400 mg/kg) exhibited a significant rise ($P<0.05$) in the %IHC of androgen receptors than RA group to a level analogous to that of the testis of control rats. Both TNF α and Bax exhibited significantly ($P<0.05$) elevated %IHC in RA induced group compared with the control. Treatment with costus (200, 400 mg/kg) exhibited a significant reduction ($P<0.05$) in the %IHC of TNF α to a level than RA non treated group, The %IHC of Bax in RA induced rats treated with Costus (200, 400 mg/kg) revealed dosage dependent reduction ($P<0.05$) than RA non- treated group that followed dose dependent manner (*Table 3*).

Regarding the results of immuno-staining for androgen receptors, control and costus (200, 400 mg/kg) groups showed normal %IHC of androgen receptors. RA group showed scanty to minimal expression. RA costus (200, 400 mg/kg) treated groups showed dose dependent expression (*Figure 2*). The immuno-staining for both TNF α and Bax protein in the testis showed that control and costus (200, 400 mg/kg) groups had minimal TNF α and Bax protein immune reactive spermatogenic cells. The RA group showed intense positive immune reaction of both spermatids cells and macrophages in interstitial tissue. RA costus (200, 400 mg/kg) showed down regulation of the immune reaction (*Figure 3 & Figure 4 respectively*).

Discussion

Rheumatoid arthritis is an autoimmune disorder. The influence of rheumatic illness on fecundity and reproduction can be notable (**Bazzani et al., 2015**). The present study revealed that RA caused significant decrease in relative weight of sex organs. The treatment of RA rats with costus (200, 400 mg/kg) significantly ameliorated the relative weight of testis but did not reach the control value. Moreover, the latter Costus doses significantly enhanced the relative weight of tail of epididymis. No alteration was detected in relative weight of prostate gland and the seminal vesicle. The histopathology of testis showed that induction of RA caused testicular degeneration with marked depletion of spermatozoa in male Wistar rats, which markedly improved with costus treatment. Rheumatoid arthritis caused scanty to minimal

testicular expression of androgen receptors. Costus (200, 400 mg/kg) treated groups showed dose dependent increase in androgen expression than RA group.

Our results were in approval with that reported via (Gordon et al., 1986) and Shiraishi et al., 2009) who found that RA caused testicular damage and may affect fertility in males. Also, (Silva et al., 2010) showed that reduced fertility was popular in patients with rheumatic disorders.

Moreover, the current study revealed that RA made a significant decline in sperm count which was relatively improved by costus treatment (200, 400 mg/kg). This result disagree with that of (Sari et al., 2016) who demonstrated that the aqueous extract of *Costus speciosus* rhizome reduced both quantity and quality of spermatozoa, which both influenced male fertility. Previous work done on methanolic extract of *Costus lucanuscianus* stem, it was denoted that such extract caused decrease sperm count and increase sperm cell defects. However, no defect was detected in the testicular and epididymal segments of rats in all the treated groups (Kagbo and Obinna, 2017). A study by (Kagbo and Obinna, 2018) showed that methanolic leaf extract of *Costus lucanuscianus* had no significant influence on testosterone levels, testicular and epididymal weights, sperm cell count and characteristics. The testicular and epididymal sections of rats in all the treated groups were not affected. The cause for the variation in the data among studies was accredited to the distribution of the phytochemicals in the stem and leaf parts of the plant.

The study revealed that RA cause marked reduction in the %IHC of androgen receptors. This results in harmony with (Gordon et al., 1988) who found an adverse effect of RA on testosterone production. In addition, Cutolo, (2009) observed that inflammation clearly down regulated androgen production. Such result was confirmed in our study were increased expression of testicular TNF α protein, as inflammatory mediator, was associated with reduced androgen expression.

In this study, *Costus speciosus* extract showed a significant improvement in a dosage dependent manner and increased the %IHC of testicular androgen receptors which markedly decreased in RA treated groups. The existence of alkaloids and flavonoids in Costus extract could ameliorated the oxidative and inflammatory damage of RA on testes. (Srivastava et al., 2012). This amelioration was seemed to be mediated through the reduction of testicular TNF α that promoted androgen receptors expression (Xiong and Hales, 1993) as shown in our study.

TNF α %IHC was detected by several studies in testis and suggested a paracrine mechanism of action in the normal testicular tissue (Lysiak, 2004). The current study showed a remarkable increase in the %IHC of TNF α receptors in the testis of RA induced rats which explained that TNF α is one of the most vital cytokines concerned with the cascade of inflammatory reactions of RA. This significant increase resulted in a marked reduction in basal testosterone release besides cAMP-stimulated testosterone production (Xiong and Hales, 1993). This because of the decline in mRNA as well as protein levels of two cytochrome P450 enzymes essential in testosterone biosynthesis, cholesterol side-chain cleavage

enzyme and 17 α -hydroxylase/C_{17–20} lyase **(Xiong and Hales, 1993)**. Normally, the body blocks excess TNF α naturally, but in rheumatic disease, it still active and creates more inflammation.

The current study revealed a significant increase in the %IHC of Bax protein in the testis of RA induced rats compared to other groups. Bax plays an important role in the mitochondrial apoptotic manner. Under normal circumstances, Bax is mainly cytosolic by continuous retrotranslocation from mitochondria to the cytosol facilitated by BCL2L1/Bcl-xL, which prevents aggregation of toxic Bax secretion at the mitochondrial outer membrane. Under stress situations, a conformational alteration occur to it that causing translocation to the mitochondrion membrane, leading to secretion of cytochrome c then stimulates apoptosis, increases triggering of Caspase-3, and then apoptosis **(Edlich et al., 2011)**.

Costus extract enhanced apoptosis is mainly mitochondria mediated and associated with the failure of the transmembrane potential which leads to the secretion of key apoptogenic molecules like cytochrome from the mitochondria. The antioxidant action of methanol extract of Costus was determined by its greater hydroxyl radical scavenging activity and free radical slaking capability **(Vijayalakshmi and Sarada, 2008)**. Diosgenin is considered the main component separated from *C. speciosus* **(Srivastava et al., 2011)**. Diosgenin has the anti-replicated capacity by powerfully producing ROS and this oxidative stress stimulates apoptosis in cancer cells via triggering of MAPK pathway. The antioxidant phytochemicals comprising diosgenin may release the antiproliferative efficacy to methanol extract of *Costus speciosus* plant **(Nair et al., 2014)**.

Rheumatoid arthritis have adverse effect on fertility in male rats. This reflected on decrease of both sperm count and %IHC of androgen receptors, this reduction accompanied by increase of %IHC of TNF α and Bax protein. **(Bauerova and Bezek, 2000)** demonstrated that the antioxidant characteristics of *Costus speciosus* and its efficacy to stop the COX-2 pathway in the course of the progress of inflammation support the use of the plant extract in rheumatoid arthritis therapy.

Conclusion

From the existing experimental results, it can be concluded that RA have adverse effect on fertility in male and the dosages of 200 mg/kg and 400 mg/kg extract of *Costus speciosus* have potentially beneficial role in improving fertility since it gives good result in monitoring inflammation in induced arthritic model in male rats. *Costus* is a hopeful anti-arthritic drug. However, additional research is needed to clarify the precise mechanism of action of plant in improving fertility.

Declarations

Ethical approval

Ethical approval was gotten from the Ethical research Committee of Faculty of Veterinary Medicine, Suez Canal University, Egypt.

Consent to participate

All authors are knowledgeable and approve the study.

Consent to publish

All authors accept the publication in the journal.

Competing interest

All authors confirm that they have no competing interest.

Data and materials availability

All data applied or investigated during this study are involved in this article.

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Authors Contributions

Samar Kamel performed the data analyses and wrote the manuscript; Hend Tag, Hala Ebeid, Howayda Khaled, Amani Almallah and Mohamed El-Naggar performed the experiment.

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Tables

Table 1: Effect of administration of costus extract on weight of sexual organs in induced Rheumatoid arthritis in male albino rats.

Groups & treatment	Relative weight (g) of sexual organs (Mean $\pm$ SE)			
	Testis	Tail of epididymis	Seminal vesicles	Prostate glands
Control	1.49 $\pm$ 0.04 _c	1.14 $\pm$ 0.01 ^b	0.49 $\pm$ 0.04	0.25 $\pm$ 0.01 ^b
Costus (200 mg)	1.31 $\pm$ 0.05 _c	1.09 $\pm$ 0.01 ^b	0.42 $\pm$ 0.02	0.21 $\pm$ 0.01 ^a
Costus (400mg)	1.35 $\pm$ 0.06 _c	1.11 $\pm$ 0.03 ^b	0.45 $\pm$ 0.02	0.22 $\pm$ 0.01 ^a
Rheumatoid arthritis	0.78 $\pm$ 0.09 _a	0.09 $\pm$ 0.01 ^a	0.42 $\pm$ 0.02	0.21 $\pm$ 0.01 ^a
Rheumatoid arthritis & costus (200 mg)	1.02 $\pm$ 0.04 _b	0.12 $\pm$ 0.04 ^b	0.42 $\pm$ 0.03	0.21 $\pm$ 0.01 ^a
Rheumatoid arthritis & costus (400mg)	1.04 $\pm$ 0.050 _b	0.11 $\pm$ 0.02 ^b	0.43 $\pm$ 0.02	0.22 $\pm$ 0.01 ^a

*Values having different superscripts within the same columns are significantly different at $p \leq 0.05$.

Data represent $M \pm SE$

Table 2: Effect of administration of costus extract on semen picture in Rheumatoid arthritis induced male albino rats.

Groups & treatment	Sperm cell characteristics (Mean $\pm$ SE)		
	Sperm count (millions)	Sperm abnormality (%)	Sperm Viability (%)
Control	41.2 $\pm$ 3.91 _c	13.67 $\pm$ 1.86 ^a	81.33 $\pm$ 3.38 ^d
Costus treated (200 mg)	48.7 $\pm$ 3.57 _c	13.00 $\pm$ 1.15 ^a	77.33 $\pm$ 2.33 ^d
Costus treated (400 mg)	47.3 $\pm$ 4.33 _c	13.67 $\pm$ 2.33 ^a	81.00 $\pm$ 4.16 ^d
Rheumatoid arthritis (RA)	15.6 $\pm$ 1.08 ^a	36.33 $\pm$ 2.02 ^c	41.33 $\pm$ 1.85 ^a
RA costus (200 mg)	26.0 $\pm$ 1.42 ^b	28.00 $\pm$ 1.52 ^b	52.33 $\pm$ 1.76 ^b
RA costus (400 mg)	32.0 $\pm$ 2.63 ^b	23.33 $\pm$ 1.45 ^b	64.66 $\pm$ 3.48 ^c

*Values having different superscripts within the same columns are significantly different at $p \leq 0.05$.

Data represent $M \pm SE$

Table 3: Effect of administration of costus extract on androgen, TNF α and Bax receptors in Rheumatoid arthritis induced male albino rats:

	Androgen receptors (%IHC)	TNF- α %IHC	Bax %IHC
<i>Control</i>	51.07 $\pm$ 4.91 ^b	18.63 $\pm$ 1.94 ^a	17.90 $\pm$ 2.27 ^a
<i>Costus treated (200 mg)</i>	46.69 $\pm$ 3.66 ^b	22.27 $\pm$ 6.59 ^b	16.38 $\pm$ 2.01 ^a
<i>Costus treated (400 mg)</i>	44.86 $\pm$ 3.78 ^b	15.71 $\pm$ 1.46 ^a	16.59 $\pm$ 1.64 ^a
<i>Rheumatoid arthritis (RA)</i>	23.43 $\pm$ 2.59 ^a	63.35 $\pm$ 2.25 ^c	50.41 $\pm$ 2.65 ^c
<i>RA costus (200 mg)</i>	43.55 $\pm$ 1.97 ^b	26.89 $\pm$ 1.04 ^b	24.95 $\pm$ 1.66 ^b
<i>RA costus (400 mg)</i>	46.73 $\pm$ 1.93 ^b	22.79 $\pm$ 1.29 ^b	22.42 $\pm$ 1.98 ^{ab}

*Values having different superscripts within the same columns are significantly different at $p \leq 0.05$.

Data represent $M \pm SE$

Figures

Figure (1)

Figure 1

Control, costus (200, 400 mg/kg) groups showed normal seminiferous tubules. RA induced group showed different degrees of degeneration in germinal cells with marked depletion of spermatozoa. Treatment with costus (200, 400 mg/kg) showed marked improvement. H&E stain.

Figure (2)

Figure 2

Control, costus (200, 400 mg/kg) groups showed normal %IHC of androgen receptors. RA group showed scanty to minimal expression. RA costus (200, 400 mg/kg) treated groups showed dose dependent expression.

Figure (3)

Figure 3

Immuno-staining for TNF α in the testis (scale bar 100um), control, costus (200, 400 mg/kg) groups showed minimal TNF α immune reactive spermatogenic cells. The RA group showed intense positive immune reaction of both spermatids cells and macrophages in interstitial tissue. RA costus (200, 400 mg/kg) showed down regulation of the immune reaction.

Figure (4)

Figure 4

Immuno-staining for Bax protein in the testis (scale bar 50µm), control, costus (200, 400 mg/kg) groups showed minimal Bax immune reactive spermatogenic cells. The RA group showed intense positive immune reaction of both spermatogenic cells and leydig cells. RA costus (200, 400 mg/kg) showed down regulation of the immune reaction.