

Evaluating the Effects of Administration of the Ethanolic Leaf Extracts of *Jatropha curcas* and Ascorbic Acid on the Male Reproductive Functions in Alloxan-Induced Diabetic Rats

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ABSTRACT

Introduction: The focus of this study is to compare the androgenic, antioxidant, and antidiabetic properties of ethanolic leaf extract of *Jatropha curcas* when co-administered with ascorbic acid to when administered separately in alloxan-induced diabetic rats.

Method: 48 adult male Wistar rats were divided into 8 groups. Group I: Normal control rats, Group II, Group III, Group IV were normal rats fed with *Jatropha curcas* (500mg/kg/d), Ascorbic acid (250mg/kg/d), and *Jatropha curcas*+ascorbic acid respectively. Diabetic rats were untreated in Group V, while diabetic rats in Groups VI, VII, and VIII were given *Jatropha curcas* (500mg/kg/day), ascorbic acid alone (250mg/kg/d), and *Jatropha curcas*+ascorbic acid, respectively. They were treated for 28 days, and the body weights and fasting blood glucose were checked weekly. The level of testicular antioxidant enzymes, sperm quality, and reproductive hormone profile were determined.

Results: There was a significant reduction in serum glucose levels, increased levels of testicular antioxidant enzymes, increased serum levels of testosterone, and improved sperm quality in groups separately treated with *Jatropha curcas* and ascorbic acid compared to the diabetic untreated group. There was significant reduction in sperm quality of groups treated with co-administration of *Jatropha curcas* and ascorbic acid compared to when used separately.

Conclusion: The findings demonstrate that ethanolic leaf extracts of *Jatropha curcas* could trigger profertility properties in male diabetic rats, due to their potent hypoglycemic, antioxidant, and androgenic effects. However, co-administration of *Jatropha* and ascorbic acid could be deleterious to male reproductive functions.

Key words: Diabetes mellitus, Male infertility, *Jatropha curcas*, Ascorbic acid

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INTRODUCTION

Diabetes mellitus is a group of metabolic diseases marked by persistent hyperglycemia caused by deficiencies in insulin secretion, insulin action, or both [1]. Carbohydrate, lipid, and protein metabolism are all disrupted in DM [2]. Diabetes is currently one of the most rapidly growing public health issues. 547 million

adults worldwide had diabetes in 2021 and has caused 6.7 million annual deaths worldwide in 2021, with long-term human, social, and financial consequences [1,3].

Diabetes mellitus has been demonstrated to have deleterious impacts on reproductive functions (male and female), resulting in higher rates of male infertility [4,5]. As the prevalence of diabetes rises, so will the prevalence of infertility among males of reproductive age [6]. When we look at fertility statistics in modern nations, we can see that the rising prevalence of diabetes is linked to lower birth rates and lower fertility [7]. Several clinical and experimental studies have discovered different levels of male reproductive deterioration in diabetics, including disruption of the hypothalamic-pituitary-gonadal axis, testicular and epididymal oxidative stress, reduction in the number of spermatogonia, reduction in the number

of Leydig and Sertoli cells, abnormal testicular energy metabolism, and altered sperm parameters (decreased sperm concentration, motility, abnormal morphology) [8-10].

Because of the expensive cost and negative side effects of anti-diabetic medicines, researchers are looking for medicinal plants that have effective hypoglycaemic, antioxidant, and male reproductive capabilities. Medicinal plants offer anti-hyperglycemic and antioxidant properties and are important in the development of novel medications because they contain bioactive substances (phytochemicals) with a diverse set of biological activities [11].

Jatropha curcas (purging nut or physic nut) is a drought-tolerant shrub or tree from the Euphorbiaceae family that is grown in Africa, India, Southeast Asia, Central and South America [12]. It is known as Lapalapa in Yoruba, Bi ni da zugu in Hausa, Gyedan in Tiv, and Ochigbede in Idoma, and is widely grown throughout Nigeria. It can withstand high levels of aridity and thrive in harsh environments such as drought, limited nutrient availability, and salinity, allowing it to be grown in deserts [13]. According to recent research, the *Jatropha curcas* plant contains hypoglycemic and antioxidant characteristics, and has the ability to reverse diabetes-related tissue oxidative damage [14,15]. However, there is scarcity of information about this plant's androgenic properties.

Ascorbic acid is an endogenous antioxidant that shields lipids from oxidative damage caused by non-lipid peroxyl radicals produced in the aqueous phase [16]. Because there are several linkages between diabetes mellitus, oxidative stress, and male factor infertility, its favorable effect on male fertility would be expected [17].

Therefore, the focus of this study is to compare the androgenic, antioxidant and antidiabetic properties of the *Jatrophas curcas* ethanolic leaf extract when co-administered with ascorbic acid to when administered separately in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Jatropha curcas ethanolic leaf extracts preparation

The freshly collected leaves of *Jatropha curcas* gotten from a local farm in Abuja was identified and authenticated at the herbarium unit NIPRD, Abuja with (VSN= NIPRD/H/6736). The ethanolic leaf extract of *Jatropha curcas* (ELEJC) was done in accordance as described by [18]. The leaves were air-dried for four days before being ground up in an industrial blender. 50 g of crude fiber was extracted with ethanol by soaking in 1500 ml of 98% ethanol for 72 hours. The filter paper used to filter the sample was Whatman. A rotary evaporator (IKA RV 10, Staufen, Germany) was used to concentrate the extract. The extract was kept in the fridge until it was time to conduct the experiment.

Phytochemical Analysis of ELEJC

Tannin, alkaloids, flavonoids, saponins, and triterpenoids

were discovered in a 50 percent ethanolic leaf extract of *Jatropha curcas* during preliminary phytochemical screening [19].

Oral acute toxicity test

The LD50 of ELEJC was determined using mice according to [20]. Fifteen fasted male albino mice (25-30 g, 10 weeks old) were divided into five groups A, B, C, D, and E, each with three animals. Group A animals had only distilled water, while groups B, C, D, and E had 5, 50, 300, and 2000 mg/kg body weight of ELEJC in distilled water via orogastric tubes, respectively. To detect changes in autonomic or behavioral responses, the animals were monitored at 2, 6, 24, and 48 hours following ELEJC administration. Mortality was observed for 24 hrs.

Result: The ELEJC was shown to be safe to use in animals and exhibited no mortality at 2000 mg/kg body weight. Dosages of up to 500 mg/kg BW were assumed to be safe and used for the present study.

Ascorbic acid (Vitamin C)

Daily preparation of Ascorbic acid (Nice Chemicals Edappally, India) was done by diluting the appropriate quantity (250mg/kg/day for each rat) in warm water [17] and was administered orally. It was kept in dark containers to avoid exposure to light.

Experimental animals

For the study, healthy adult male albino rats of the Wistar strain weighing between 170 and 200g were procured from the Animal house of National Institute of Pharmaceutical Research and Development (NIPRD) Abuja. The rats were housed in their cages with a 12-hour light-dark cycle and were kept at room temperature (27 2°C) and humidity (55 percent). They were fed with rat chows and water ad-libitum. They had 2 weeks of acclimatization before the experiment. The animal studies were carried out in accordance with the policies specified in the US National Institute of Health's "Guide for the Care and Use of Laboratory Animals" [21]. The experimental protocol was approved by the local Ethics Committee (UAECAU/2021/002).

Experimental induction of diabetes

After two weeks of acclimating to their cages, all animals were fasted overnight and injected intraperitoneal with 150 mg/kg of 5 percent alloxan monohydrate freshly dissolved in normal saline using 2ml disposable needles and syringes (19). They had unrestricted access to food and water subsequently. Determination of Fasting blood glucose (FBG) was done 48 hours after alloxan administration, using capillary samples obtained from tail snips and only animals with FBG above 12mmol/L were utilized in the investigation (15). ON CALL PLUS GLUCOMETER (ACON Laboratories San Diego, USA), a glucose test meter, was used to measure FBG, and the results were compared to the relevant standard values. FBG was done weekly.

Experimental design

A total of 48 male wistar rats were used in the

experiment. The rats were split into eight groups, each with six (6) rats. After induction of diabetes, they were given *Jatropha curcas* ethanolic leaf extract (ELEJC) and ascorbic acid (AA) once daily orally for 28 days.

Group I (NC): Normal control animals were given rat chow and water liberally

Group II (JCE): *Jatropha curcas* only(500mg/kg/day)+rat chow and water

Group III (AA): Ascorbic acid (250mg/kg/day)+rat chow and water.

Group IV(JAA): Co-administration of *Jatropha curcas* (500mg/kg/day) & Ascorbic acid(250mg/kg/day) (DJA)+rat chow and water

Group V (DC): Untreated diabetic rats+rat chow and water.

Group VI (DJCE): Diabetic animals treated with *Jatropha curcas* only (500mg/kg/day).

Group VII (DAA): Diabetic animals treated with Ascorbic acid (250mg/kg/day).

Group VIII (DJAA): Diabetic animals treated with co-administration of *Jatropha curcas* (500mg/kg/day) & Ascorbic acid (250mg/kg/day)

Sample collection

The rats were weighed, anesthetized with diethyl ether for 5 minutes and decapitated after 28days of ELEJC and ascorbic acid treatment. Na⁺ EDTA bottle was used for sample collection and centrifuged at 3000 rpm for 5 minutes. Each bottle's plasma is transferred into a new plain specimen vial and maintained at -200C until analysis time. Reproductive organs (testes and epididymis) were promptly removed, rinsed in ice-cold physiologic saline solution (0.9 percent w/v), blotted, and weighed using a digital balance (RADWAG, Poland) with a sensitivity of 0,0001 gr. The caudal epididymal contents were used for sperm analysis and the tissue homogenates were prepared from the testis.

Reproductive hormone assays

Using the enzyme-linked immunosorbent assay (ELISA) kits (CALBIOTECH Inc. CA, USA), blood samples were tested for testosterone, LH, and FSH levels, according to the techniques reported by [22].

Preparation of testes homogenate and determination of testicular antioxidant markers

The homogenization of testes tissue was in 0.9 percent sodium chloride (ice cold) at a ratio of 1:5. To obtain the final supernatant, the initial supernatant was centrifuged at 3500rpm for 20 minutes at 40°C. The final supernatant was then utilized to evaluate testicular antioxidant indicators such as:

Catalase (CAT), which was tested using the method described by [23].

Superoxide dismutase activity was measured spectrophotometrically using the method of [24].

Glutathione peroxidase activity were determined spectrophotometrically in-accordance with [25] method.

Malonaldehyde (MDA), lipid peroxidation's breakdown product and thiobarbituric acid reactive substances (TBAR), were determined as described by [26].

Semen analysis/determination of sperm quality

Determination of sperm concentration: The rats' epididymal content were retrieved by cutting the epididymis' tail, tingling it with 2 mL of normal saline, and then teasing each rat's cauda epididymis. To avoid any additional tissue contamination, the suspension was stirred through a steel mesh. Sperm cell concentration was examined microscopically with the aid of hemacytometer according to the method described by [27].

Determination of sperm motility: Motility of spermatozoa was determined according to the methods of [28].

Determination of sperm morphology: Eosin-nigrosin was used to stain the slide to measure the percentage of morphologically abnormal spermatozoa. On each slide, 400 sperm cells (2000 cells per group) were studied, and the total abnormality (which include head and tail) rates of spermatozoa were expressed as a percentage.

Statistical analysis

The findings were presented as mean+standard error of mean (SEM). One-way ANOVA was used to determine the significance of differences between groups, followed by post hoc (LSD) analysis for significant value using the Statistical Package for Social Science (SPSS) version 23.0. if P<0.05, the differences were deemed statistically significant.

RESULTS

Figure 1 shows the effect of ethanolic leaf extract of *Jatropha curcas* and ascorbic acid on the body weights of rats. There is significant reduction in the body weight of diabetic control groups compared to other groups. There is significant reduction in the body weight of rats in groups that received *Jatropha curcas* only after one week of treatment, however, regain their weight back subsequently.

Figure 2 shows the effect of ethanolic leaf extract of *Jatropha curcas* and ascorbic acid on weekly fasting blood glucose levels. There is significant increase in FBG after alloxan induction as seen in DC, DJCE, DAA, and DJAA compared to other groups. There is significant decrease in FBG of DJCE, DAA and DJAA in comparison to DC.

Table 1 shows the effect of ethanolic leaf extract of *Jatropha curcas* and ascorbic acid on glutathione peroxidase, superoxide dismutase, catalase and malonaldehyde. The antioxidant enzymes (CAT, SOD & GSH) increased significantly in all treated groups compared to DC. There is significant increase in MDA level of DC, JAA and DJAA compared to NC. There is significant reduction in MDA

Table 1: Effects of *Jatropha curcas* and ascorbic acid on testicular antioxidant enzymes and malonaldehyde.

Group/Parameters	Malonaldehyde (ng/mL)	Catalase (Ku/L)	Superoxide Dismutase (%N)	Glutathione Peroxidase(ng/mL)
Normal Control (NC)	837.91 ± 39.93	63.81 ± 1.48b	157.49 ± 8.17b	16.79 ± 0.39b
Normal+ <i>Jatropha curcas</i> (JCE)	893.04 ± 70.25	58.38 ± 1.90b	154.64 ± 10.85b	14.01 ± 0.46b
Normal+Ascorbic Acid (AA)	857.73 ± 60.61	60.86 ± 2.01b	156.66 ± 6.38b	14.38 ± 0.87b
Normal+ <i>Jatropha curcas</i> +Ascorbic Acid (JAA)	1174.44 ± 44.76a	50.07 ± 1.89b	124.42 ± 4.80b	14.83 ± 1.15b
Diabetic Control (DC)	1620.51 ± 85.82a	30.68 ± 3.77	48.11 ± 6.82	9.03 ± 0.66
Diabetic+ <i>Jatropha Curcas</i> (DJCE)	699.45 ± 31.04bc	53.71 ± 0.81b	140.10 ± 4.80b	15.34 ± 0.35b
Diabetic+Ascorbic Acid (DAA)	985.90 ± 37.62bc	58.68 ± 1.19b	158.33 ± 3.90b	16.06 ± 0.76b
Diabetic+ <i>Jatropha Curcas</i> +Ascorbic Acid (DJAA)	1469.28 ± 61.10a	57.55 ± 0.74b	164.99 ± 4.10b	17.38 ± 0.65b
a=P<0.001 when compared to NC				
b=P<0.001 when compared to DC				
c=P<0.001 when compared to DJAA				

Table 2: Effects of *Jatropha curcas* and ascorbic acid on the serum testosterone, FSH and LH.

Groups/ Parameters	Testosterone (ngh/mL)	FSH (mIU/mL)	LH (mIU/mL)
Normal Control (NC)	38.21 ± 1.47a	4.21 ± 0.08	8.86 ± 0.37d
Normal+ <i>Jatropha curcas</i> (JCE)	23.74 ± 1.06ab	4.47 ± 0.19	8.81 ± 0.52d
Normal+Ascorbic Acid (AA)	24.08 ± 1.60ab	4.05 ± 0.07	8.11 ± 0.31d
Normal+ <i>Jatropha curcas</i> +Ascorbic Acid (JAA)	29.46 ± 1.00ab	4.31 ± 0.20	15.93 ± 1.05
Diabetic Control (DC)	14.76 ± 0.58b	11.20 ± 0.68	20.14 ± 1.18
Diabetic+ <i>Jatropha curcas</i> (DJCE)	27.57 ± 0.81ab	4.47 ± 0.12a	13.08 ± 0.73ac
Diabetic+Ascorbic Acid (DAA)	26.06 ± 1.27ab	4.95 ± 0.85a	14.25 ± 0.85ac
Diabetic+ <i>Jatropha curcas</i> +Ascorbic Acid (DJAA)	27.22 ± 1.84ab	4.00 ± 0.32a	4.06 ± 0.53a
a=P<0.001 in comparison to diabetic group			
b=P<0.001 in comparison to control group			
c=P<0.001 in comparison to Diabetic+ <i>Jatropha Curcas</i> +Ascorbic acid group			
d=P<0.001 in comparison to Normal+ <i>Jatropha Curcas</i> +Ascorbic acid group			

Table 3: Effects of *Jatropha curcas* and ascorbic acid on sperm quality (concentration, morphology, and motility).

Group/ Parameters	Sperm Concentration (106/ mL)	Sperm Motility (%)	Normal Morphology (%)	Abnormal Morphology (%)	Sperm PH
Normal Control (NC)	212.60 ± 9.99a	82.92 ± 1.20a	93.02 ± 0.82a	6.98 ± 0.82	6.60 ± 0.24
Normal+ <i>Jatropha curcas</i> (JCE)	201.40 ± 4.03ace	78.72 ± 1.38ac	90.60 ± 1.35ac	9.40 ± 1.35	6.60 ± 0.24
Normal+Ascorbic Acid (AA)	180.40 ± 3.17ac	81.64 ± 1.64ac	91.26 ± 1.65ac	8.74 ± 1.65	6.80 ± 0.20
Normal+ <i>Jatropha curcas</i> +Ascorbic Acid (JAA)	32.00 ± 3.51	59.18 ± 1.91	67.00 ± 2.14	33.00 ± 2.14f	6.80 ± 0.12
Diabetic Control (DC)	56.20 ± 3.15	49.58 ± 2.35	61.14 ± 1.33	38.86 ± 1.33f	6.50 ± 0.22
Diabetic+ <i>Jatropha curcas</i> (DJCE)	224.20 ± 3.84abd	77.06 ± 1.50ab	89.90 ± 1.54ab	10.10 ± 1.54	6.40 ± 0.24
Diabetic+Ascorbic Acid (DAA)	174.20 ± 4.93ab	75.66 ± 1.52ab	92.64 ± 1.65ab	7.36 ± 1.65	6.70 ± 0.20
Diabetic+ <i>Jatropha curcas</i> +Ascorbic Acid (DJAA)	30.40 ± 3.93	53.80 ± 1.47	58.44 ± 1.62	41.56 ± 1.62f	7.0 ± 0.00
a=P<0.001 in comparison to diabetic group					
b=P<0.001 in comparison to diabetic+ <i>Jatropha</i> +Ascorbic group					
c=P<0.001 in comparison to Normal+ <i>Jatropha</i> +Ascorbic group					
d=P<0.001 in comparison to diabetic+ascorbic acid					
e=P<0.001 in comparison to normal+ascorbic group					
f=P<0.001 in comparison to normal group					

level of DJCE and DAA compared to DC.

Table 2 shows the effect of ethanolic leaf extract of *Jatropha curcas* and ascorbic acid on serum reproductive hormones (testosterone, FSH and LH). The testosterone decreased significantly in DC when compared to other groups. The testosterone decreased significantly in JCE, AA, DJCE & DJAA when compared to NC. The level LH and FSH of DC increase significantly when compared to other groups. The LH of JAA increased significantly when

compared to JCE & AA. There is significant decrease in LH of DJAA compared to DJCE & DAA.

Table 3 shows the effect of ethanolic leaf extract of *Jatropha curcas* and ascorbic acid on sperm concentration, sperm motility, sperm morphology and sperm PH. The sperm concentration, sperm motility and normal sperm morphology of DC, JAA and DJAA decreased significantly when compared to other groups. The sperm concentration of ascorbic acid treated groups

is significantly reduced compared to *Jatropha curcas* treated groups. There is significant increase in abnormal sperm morphology of DC, JAA and DJAA compared to other groups.

DISCUSSION

The body weight of untreated diabetic rats was significantly lower than that of the normal and treated groups. This is consistent with research that found considerable weight loss in diabetic rats that were not treated [29]. Weight reduction is one of the clinical features of diabetes mellitus and is due to poor glucose uptake because of insulin deficiency or resistant to insulin action. It was also observed that there was significant weight reduction in groups treated with ELEJC only after one week of treatment; however the weight increased subsequently throughout the course of experiment.

The anti-hyperglycemic impact of the ELEJC utilized in this study was significant, as blood glucose levels were corrected within three weeks of treatment. This is consistent with those obtained by [14,15,29]. Medicinal plants with hypoglycemic and antidiabetic properties typically contain significant levels of alkaloids and flavonoids [30]. The presence of high amounts of alkaloids and flavonoids (2.26mg and 3.83mg, respectively) in the ethanolic leaf extract of *Jatropha curcas* may explain its hypoglycemic and antidiabetic effects [31]. When ELEJC and ascorbic acid were given together, there is no significant difference in the hypoglycemic impact compared to when they are given separately. *Jatropha curcas* had a faster hypoglycemic impact than ascorbic acid. [32] reported that ascorbic acid is protective and prevents diabetic end organ damage because of its antioxidant properties.

One of the main causes of diabetes-induced injury has been proposed by [33] as the generation of lipid peroxides (MDA) by free radical derivatives, which leads to tissue damage and the inability of antioxidant defense systems to prevent the development of uncontrolled free radicals. In this present study, the testicular antioxidant enzymes (GPx, SOD, and CAT) decreased significantly in DC compared to groups treated with *Jatropha curcas* and ascorbic acid. Decreased production of antioxidants-glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) may contribute to increased ROS levels in diabetes.

Ascorbic acid and ELEJC has free radical scavenging abilities and could decrease the free radical-induced peroxidation of cellular structures. After 28 days of therapy with both *Jatropha curcas* and ascorbic acid, the testicular antioxidant enzymes (GPx, SOD, and CAT) and MDA activities have significantly improved. This is consistent with previous research that found that ELEJC reduced MDA levels and increased testicular level of GPx, CAT, SOD in diabetic rats, which could be linked to an increase in antioxidant enzyme activities in treated rats, resulting in the lipid peroxidation inactivation [14].

There is a connection between diabetes mellitus and alteration of the hypothalamic–pituitary–gonadal (HPG) axis, changing LH, FSH and testosterone concentrations in males [8-10]. In the present study, there is significant reduction in the serum testosterone level of DC compared to other groups. This may be due decreased Leydig cell function or suppression of the hypothalamic-pituitary-gonadal axis. There is significant increase in the serum LH and FSH levels of DC compared to other groups. This may be a case of hyper gonadotropic hypogonadism due to lack of feedback inhibition to gonadotrophins as a result of decreased testosterone secretion. The findings are consistent with [34], who reported that hyper gonadotropic hypogonadism is the more common form of hypogonadism in men with Type II diabetes mellitus.

The testosterone level decreased significantly in groups treated with ELEJC only and ascorbic acid only; and their co-administration compared to control group. This is the same as [35], who reported that ELEJC decreased the levels of serum testosterone in comparison with control group. Decreased levels of LH and the damage of Leydig cells might account for reduced testosterone production [35].

The testosterone level of diabetic groups treated with *Jatropha curcas* and ascorbic acid increased significantly as compared to diabetic untreated group. This may be due to ELEJC and ascorbic acid free radical scavenging abilities and were able to reverse the initial damage (Leydig cell malfunction) caused by diabetes mellitus. However, there is also no significant difference in the testosterone level of groups treated with co-administration of ELEJC and ascorbic acid compared to those treated with ELEJC only and ascorbic acid only.

Diabetes Mellitus has been demonstrated to negatively alter spermatogenesis and sperm-related parameters [7-10]. The present study also shown similar findings, in which there is significant reduction in the sperm concentration, sperm motility and an increase in abnormal sperm morphology of diabetic untreated groups when compared to groups treated with ELEJC only and ascorbic acid only. The findings showed that ELEJC has androgenic property. This may be due to its antioxidant properties and was able to reverse the testicular oxidative stress, thereby promoting spermatogenesis and other sperm parameters. This is different from what was observed by Airaodion et al., 2020 who reported antifertility propensity of *Jatropha curcas* in male rats.

The ascorbic acid also shown profertility properties in this study. It prevents oxidative stress of sperm by inhibiting H₂O₂, superoxide & hydroxyl radicals; improves sperm motility and boosts sperm concentration [36]. However, the ELEJC has more androgenic property than ascorbic acid as the present study revealed that there was significant increase in the sperm concentration of rats treated with ELEJC only compared to rats treated with ascorbic acid only.

The present study also revealed that co-administration of ELEJC and ascorbic may be anti-androgenic, as the rats in this category had significant decreased in sperm

concentration, reduced sperm motility and an increased in abnormal sperm morphology compared to rats treated with ELEJC only and ascorbic acid only. There may be an antagonistic interaction between ELEJC and ascorbic acid on spermatogenesis, leading to reduced sperm quality in rats treated with their co-administration.

CONCLUSION

Dietary supplementation of ethanolic leaf extract of *Jatropha curcas* and ascorbic acid for 28 days after alloxan treatment resulted in reduced serum glucose levels, increased level of testicular antioxidant enzymes, increased serum level of testosterone, low FSH and LH, and improved sperm concentration, motility, and normal morphology. The androgenic effect is more in *Jatropha curcas* than in ascorbic acid. However, there is significant reduction in sperm quality of groups treated with co-administration of *Jatropha curcas* and ascorbic acid.

RECOMMENDATION

Dietary supplementation with *Jatropha curcas* leaf could be a simple, low-cost, and potential pharmaceutical agent that may prevent diabetic complication of reproductive dysfunction in men.

Jatropha curcas and ascorbic acid should not be taken together due to their anti-fertility effects.

Further research at the molecular level is needed to determine the exact mechanism of action of the *Jatropha curcas* plant in experimental animals.

As this study was done on experimental animal (rats), the outcome may not be the same in human. There is a need to carry-out further studies on the effect of *Jatropha curcas* plant in human subjects.

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