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In-Vitro Effect of Most Potent Ethyl-Acetate Solvent Fraction of *Curcuma Amada* Rhizomes for the Management of Diabetes Induced Male Reproductive Impairments

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Abstract

Curcuma amada is employed as a medicinal herb in traditional medicinal practices. The aim of this research was to determine if the rhizome of *Curcuma amada* could directly improve the male reproductive system of diabetic rats. To explore whether the ethyl-acetate fraction of *Curcuma amada* rhizomes has a direct antioxidative impact on STZ-induced diabetic animals, the activities of antioxidant enzymes such as catalase and superoxide dismutase, as well as the level of Thiobarbituric acid reactive substances in testicular tissue, were evaluated. The activities of Δ5, 3β- hydroxysteroid dehydrogenase, and 17β- hydroxysteroid dehydrogenase were also evaluated to determine the direct impact of the ethyl-acetate fraction on the androgenic domain. When the testes of diabetic rats were directly exposed to the ethyl-acetate fraction of the plant, it did not lead to any significant recovery in the said enzyme activities. Following the direct exposure of the ethyl-acetate fraction of *Curcuma amada* rhizomes to the testes of type 1 diabetic rats, said parameters associated with oxidative stress, exhibited a positive improvement towards control. This improvement could be ascribed to the antioxidative phytomolecules present in the ethyl-acetate fraction of the plant, which effectively suppressed testicular oxidative stress by scavenging free radicals in the target tissue.

Keywords: In-vitro, Diabetes, Herbal Medicine, Male infertility, *Curcuma amada*

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Introduction

Diabetes mellitus (DM) stands as a prevalent chronic metabolic disorder characterized by elevated levels of glucose in blood and this condition arises from insulin deficiency, stemming from either autoimmune destruction of pancreatic β-cells known as Type 1 DM or reduced insulin sensitivity in body tissues known as Type 2 DM **(Mukhtar et al., 2019)**. Oxidative stress denotes a state of imbalance between free radical generation and antioxidants activities within biological systems, resulting in cellular damage. Excessive oxidative stress has been associated with the complications of type 2 DM. Research has confirmed that type 2 DM negatively impacts the reproductive function of males **(ADA, 2014)**. Around 90% of individuals with diabetes encounter disruptions in sexual function, which may manifest as decreased libido, erectile dysfunction, and finally infertility **(Corona et al., 2014).** Pharmaceutical industries have increasingly focused on developing drugs from natural resources, with herbal medicines garnering significant attention. These remedies are widely regarded as both effective and safe, often boasting fewer side effects when compared to their chemical counterparts. Different phyto-compounds also known as secondary metabolites found in natural resources, including alkaloids, tannin, phenolics, flavonoids, and terpenoids, exhibit notable antioxidant properties and have demonstrated efficacy in treating a range of ailments such as atherosclerosis, cardiovascular disease, coronary heart disease, neurological disorders, cancer, and diabetes **(Ghosh et al., 2015)**.

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The rhizome of *Curcuma amada* is frequently utilized in the preparation of sauce, pickles, chutney, and candy. It contains bioactive compounds such as curcuminoids, alkaloids, terpenoids, phenolics, and flavonoids, which possess the ability to quench reactive oxygen species (ROS) **(Policegoudra et al., 2011).** These bioactive compounds in *Curcuma amada* rhizome possess antioxidant capabilities, effectively scavenging reactive oxygen species (ROS) and reactive nitrogen species (RNS), converting them into non-radical products and thereby preventing the generation of free radicals. An *in vivo* study demonstrated that the ethyl-acetate fraction of *Curcuma amada* rhizomes, administered at a dose of 2.5 mg per 100 g body weight, effectively reduced testicular oxidative stress in diabetic rats **(Sarkar et al., 2022).** The study aimed to investigate whether *Curcuma amada* rhizome has a direct therapeutic effect on the male reproductive system of diabetic rats by correcting androgenic and antioxidant profiles.

Methods

Experimental design

Eighteen tested fertile male albino rats of Wistar strain were purchased from Saha Enterprise (West Bengal, India), is a CPCSEA certified vendor. Body weight of each experimental animal was 110-120 g and age between 120-180 days. Just fifteen days before starting the experiment all the animals were allow for acclimatization as per previous experiments. During this period rats were provided with food known as rat chaw and water *ad libitum*.. The whole experimental schedule along with animal handling all were conducted as per CPCSEA rules **(Chatterjee et al., 2013)**.

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Type 1 diabetes induction: Among eighteen normoglycemic animal, twelve animals were allowed for diabetes induction. Diabetes was induced as per previous experiments. Animal more than 240 mg/dl FBG level after 3 days of diabetes induction were considered as diabetic model animal for this experiment **(Ali et al., 2009)**.

Sample collection

Out of the eighteen rats, twelve rats were made diabetic through STZ. After 7 days of diabetes induction, all the STZ induced diabetic animals and non diabetic animals were allowed to sacrifice through euthanasia. To check and confirmed the testicular status of all the experimental rats following parameters were assessed by *in vitro* study.

Treatment protocol

The treatment schedule of *in vitro* study was performed as follows:

Vehicle treated control: Testis of each normoglycemic fertile experimental rat was collected in the test tube. To that test tube, Kreb's ringer buffer solution (10 ml) was added in O_2 (95%) and CO_2 (5%) gaseous mixture. Lastly 0.5 ml of distilled water was added to that mixture containing test tube. Incubate all the test tube at 37°C for 2 hours. After that testicular tissue (testis) were transferred into 1 ml of sodium phosphate buffer solution before assay.

Vehicle treated diabetic: Testis was collected from each diabetic rat and placed in Kreb's ringer buffer solution (10 ml) along with O_2 (95%) and CO_2 (5%) gaseous mixture containing test tube. Then distilled water (0.5 ml) was added to all the test tubes and allow for incubation at 37°C for 2 hours. Just before the test, testes were transferred into

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sodium phosphate buffer (1 ml) as previous experiment.

Diabetic + ethyl acetate fraction treated: Testis of each diabetic rat was collected and kept them into the test tubes where the ethyl-acetate solvent fraction at the effective dose i.e., 2.5 mg/ 0.5 ml distilled water was added. Kreb's ringer buffer solution (10 ml) along with O_2 (95%) and CO_2 (5%) gaseous mixture also added to that test tube. After 2 hour incubation at 37ºC testes were placed separately in sodium phosphate buffer (1 ml) containing test tubes. Finally the testes were used for the different assay.

Sensor and test protocol

To investigate the ethyl acetate fraction of *Curcuma amada* rhizomes has any direct antioxidative effect on STZ induced diabetic animal, antioxidant enzymes such as catalase and superoxide dismutase activities along with TBARS level in testicular tissue were assessed **(Beers and Sizer, 1952; Marklund and Marklund, 1974; Ohkawa et al., 1979)**. The Δ^5, 3β-HSD and 17β-HSD (**(Talalay, 1962; Jarabak et al., 1962)** activities were also assessed to indentify the direct effect of ethyl acetate fraction on androgenic domain.

Results

Androgenic domain

The Δ^5, 3β-HSD and 17β-HSD activities in testis were suppressed about 54.89 % ($p < 0.05$) and 66.90 % ($p < 0.05$) in testicular tissue of VTD rat as compared with VTC. Ethyl acetate fraction of potent extract i.e., hydro-methanol (60:40) of *Curcuma amada* rhizomes direct exposure to STZ induced diabetic rat showed negligible improvement

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like 5.55 % ($p > 0.05$) in Δ^5, 3β-HSD and 8.86 % ($p > 0.05$) 17β-HSD activities with respect to VTD and there was no significant difference ($p > 0.05$) in level of the said sensors between fraction treated and VTD groups **(Figure 1)**.

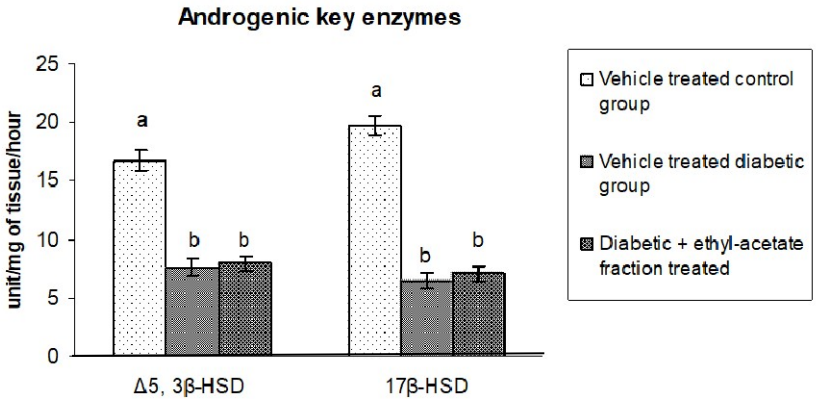

Figure 1: Direct effect of ethyl acetate fraction of *Curcuma amada* rhizomes exposure on the activities of testicular Δ5, 3β HSD and17β-HSD in diabetic rat. Calculated data were represented here as Mean ± SEM (n=6). Analysis of Variance followed by "Multiple-Comparison Student's Two Tail t-test". Different superscripts as a, b varies from each other significantly, ($p < 0.05$).

Antioxidant profile

Compared with VTC animal, testicular catalase and SOD activities were diminished about 53.25 % ($p < 0.05$) and 73.52 % ($p < 0.05$) in VTD animal. Direct ethyl acetate fraction of said extract of *Curcuma amada* rhizomes exposure to type 1 diabetic rat testes showed the increased activities of said antioxidant enzymes such as catalase by 42.17 % ($p < 0.05$) and SOD by 28 % ($p < 0.05$) in favour of VTC animal **(Figure 2 and Figure 3)**.

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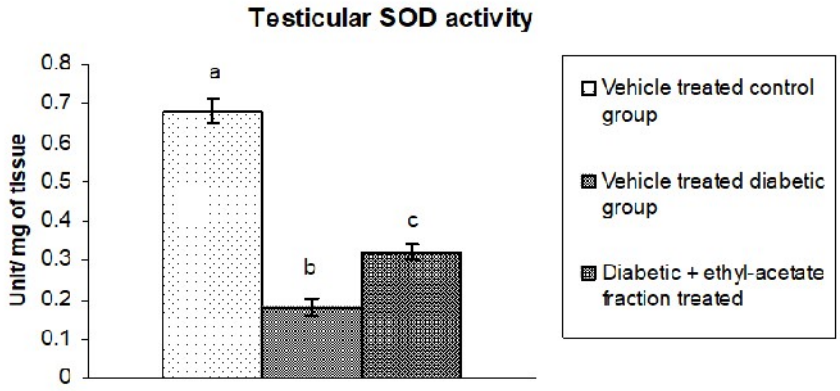

Figure 2: Recovery in testicular catalase activity after direct exposure of ethyl acetate fraction of *Curcuma amada* rhizomes to type 1 diabetic rat. Calculated data were represented here as Mean ± SEM (n=6). Analysis of Variance followed by "Multiple-Comparison Student's Two Tail t-test". Different superscripts as a, b, c varies from each other significantly,($p < 0.05$)

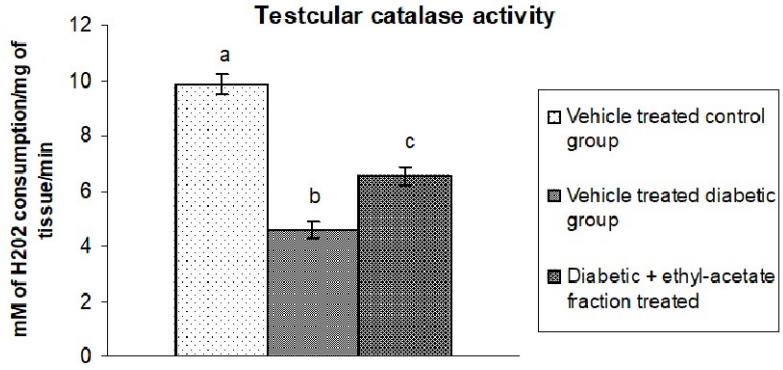

Figure 3: The testicular superoxide dismutase activity after direct exposure of ethyl acetate fraction of *Curcuma amada* rhizomes to testicular tissue of type 1 diabetic rat. Calculated data were represented here as Mean ± SEM (n=6). Analysis of Variance followed by "MultipleComparison Student's Two Tail t-test". Different superscripts as a, b, c varies from each other significantly,($p < 0.05$).

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Oxidative stress end product

The high level of TBARS about 107.29 % ($p < 0.05$) in testes of VTD animal was noted in respect to VTC rat. This level of oxidative end product i.e., TBARS was reduced by 32.84 % ($p < 0.05$) towards VTC rat after the ethyl acetate fraction of *Curcuma amada* rhizomes direct exposure to diabetic rat testes **(Figure 4)**.

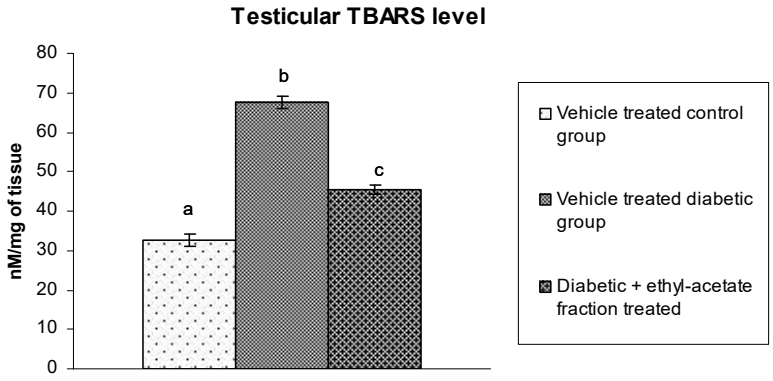

Figure 4: Direct effect of ethyl acetate solvent fraction of *Curcuma amada* rhizomes on TBARS level in testicular tissue of type 1 diabetic rat. Calculated data were represented here as Mean ± SEM (n=6). Analysis of Variance followed by "Multiple-Comparison Student's Two Tail t-test". Different superscripts as a, b, c varies from each other significantly,($p < 0.05$).

Discussion

In the contemporary period, a prevalent health concern is diabetes. *In vivo* studies were conducted which established that *Curcuma amada* rhizomes effectively suppressed the testicular oxidative stress in diabetic rat that improve the streroidogenic enzymes activity by triggering the anti-oxidantive enzymes activities **(Sarkar et al., 2019 & Sarkar et al., 2022)**. This study was performed to find out whether *Curcuma amada* rhizome has

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any direct remedial effect on male reproduction system of diabetic rat by rectifying the androgenic and antioxidant profiles.

Activities of androgenic key enzymes in type 1 diabetic rat were rectified after ethyl acetate solvent fraction treatment at the dose of 2.5 mg / 100 g body weight for 28 days proved by our previous *in vi*vo study **(Sarkar et al, 2022)**. But when the testes of diabetic rat came to direct exposure of ethyl acetate fraction of said plant it did not able to execute any significant recovery in said enzyme activities. This result focused that said plant fraction may unable to exert the enzymes activity at nongenomic level (**Tripathy et al., 2016)**.

The parameters related to oxidative stress such as catalase, SOD and TBARS level were improved in positive way towards VTC after direct ethyl acetate fraction of *Curcma amada* rhizomes exposure to testes of type 1 diabetic rat. This could be attributed to the presence of certain antioxidant compounds in the ethyl acetate fraction of said plant part, which may have mitigated testicular oxidative stress by scavenging free radicals in the specific tissue. Alternatively, it could have occurred through the activation of antioxidant enzymes following the binding of this nutraceutical to the said androgenic enzymes. **(Chatterjee et al., 2012)**.

Conclusion

From that outcome it can be affirmed that the ethyl acetate solvent fraction of *Curcuma amada* rhizomes has the direct effect on male reproductive system due to its high antioxidant capacity that can managed stress induced testicular impairments in diabetic

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Conflict of interest

The authors have stated that they have no conflicts of interest**.**

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