

Ameliorative Properties of Crude Diosgenin from *Costus speciosus* and Taurine on Testicular Disorders in Alloxan-Induced Diabetic Mice

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ABSTRACT

Diosgenin and taurine are two bioactive chemicals that get high attention in recent decades because it is claimed as an antioxidant, antidiabetic, and pro fertility. The study aimed to investigate the effect of the two substances when used in combination against diabetes-related infertility in male mice. By using completely randomized design, 35 Swiss albino male mice divided into 7 groups (n=5). Group 1 treated with distilled water as normal control. Group 2 receive only alloxan as diabetic control. Group 3 only fed diosgenin 20% as diosgenin control. Group 4 treated with alloxan and diosgenin 20%. Group 5 receive alloxan and diosgenin 30%. Group 6 treated with alloxan and taurine. Group 7 treated with alloxan, diosgenin 20% and taurine. Treatments were given intraperitoneally once daily for 14 days. The parameters assessed including blood glucose levels, body and testis weight, spermatogenic cells counts, sperm counts as well as sperm motility and viability. The results showed crude diosgenin extract from *C. speciosus* in normal mice lowers the number of spermatogonia, spermatocytes and spermatids cells in addition to decrease sperm motility. In alloxan-induced mice, diosgenin ameliorates the blood glucose levels but not effective in normalization of the testicular parameters of the animals. Taurine, on the contrary, was effective both to cope with the blood parameters and testicular disorders in alloxan-induced mice. Application of diosgenin combined with taurine in alloxan-induced mice, at the end of treatment, most effective in lowering blood glucose levels compared with other treatment but shows little contribution in normalization testicular parameters. Thus, it can be concluded that crude diosgenin extract from *C. speciosus* even though combined with taurine has little effect on testicular disorders in alloxan-induced diabetic mice in comparison to the application of taurine alone.

Keywords: Diosgenin, *Costus speciosus*, Pacing, Diabetes, Taurine, Alloxan.

INTRODUCTION

ostus speciosus is the scientific name of crepe ginger, which in Indonesia is called *pacing*, is one among the genus *Costus* that widely cultivated as ornamental plant in the country. In the Indonesian folk medicine system this plant commonly used to treat insect bite, skin diseases, wounds, dysentery, diarrhoea, fever, backache and sore eyes¹. In other Asian regions, crepe ginger

traditionally applied to treat headache, pneumonia, rheumatism, dropsy, urinary diseases, jaundice, feve and dysentery².

Pharmacognosy and pharmacological studies on this plant have widely carried out, among the results are as follows. The plant has been found to possess antibacterial, antifungal, anticholineesterase, antioxidant, antihyperglycemic, antiinflammatory, analgesic,

antipyretic, antidiuretic, larvicidal, antistress and estrogenic activities. Crepe ginger has properties including bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant, tonic, improve digestion, and stimulant³.

Crepe ginger currently getting higher reputation, due to some studies have showed indications of anti-diabetic effects of this plant. In alloxan-induced diabetic rats, for example, crepe ginger plant extract possesses anti-hyperglycemic, antihyperlipemic and antioxidative effects⁴ and shows significant reduction in blood glucose, glycosylated haemoglobin, blood urea, serum uric acid, serum creatinine, triglycerides, total cholesterol, phospholipids, LDL, VLDL, and increase in liver glycogen, insulin and lactate dehydrogenase⁵. In streptozotocin-induced diabetic rats, for another example, *C. speciosus* extract 400 and 600 mg/kg body weight induced a decrease in blood glucose and an increase in serum insulin level, glucokinase, aldolase, pyruvate kinase, succinate dehydrogenase, and glycogen synthase activities⁶. Other claim about the medical benefits of this plant is its effect on the sex-related parameters, among others, can decrease the fertility of women⁷.

Among the bioactive components of *C. speciosus* suggested to play a key role in the antidiabetic activity as well as steroid hormone attributes is diosgenin. Diosgenin can be used as industrial raw materials of steroid drugs⁸. very useful compound to control hypercholesterolemia by both improving the lipid profile and modulating oxidative stress⁹. In addition, diosgenin isolated from *C. speciosus* possess anticancer and apoptotic effects on cell proliferation¹⁰. Much earlier, it has been known that in rats fed cholesterol diosgenin decreases the elevated levels of cholesterol in serum LDL and increases cholesterol in serum HDL¹¹.

The other biochemicals that are well known, which has been marketed widely, which may also be associated with diabetes and fertility treatment is taurine. Clinically, taurine has been used in the treatment of a wide variety of illnesses, including cardiovascular, epilepsy and other

seizure disorders, macular degeneration, Alzheimer's disease, hepatic disorders, and cystic fibrosis¹². The functional significance of taurine including cytoprotective, cell development, nutrition and survival¹³.

Experiments in animal models, taurine has been reported to increase the hormone levels of testosterone and luteinizing hormone in rats of different ages, and increase the numbers and motility of spermatozoa in aged rats^{14, 15}. In addition, it also has been reported previously that administration of taurine exerts a beneficial effect on testicular ischemia-reperfusion injury in rats¹⁶.

Because phytochemical of *C. speciosus* have been shown to have anti-diabetic properties and is also thought to have an influence on the sex related parameters, while taurine has a pro-fertility properties, then infertility disorder related to diabetes could be cured by the extract of this plant combined with taurine. To test this hypothesis, in this study mice were induced with alloxan in order to make the animals suffer from diabetes, and then treated with crude diosgenin extract from *C. speciosus*, taurine and the combination of both substances.

METHOD

Plant material and extraction

The rhizome samples of *Costus speciosus* plants were collected from forests near Kalianda, the capital of South Lampung district, Lampung province, Indonesia. Taxonomic identification of the plant was carried out by botanist at the Laboratory of Botany of Lampung University, Indonesia. After being washed with water, the rhizomes air-dried and cut into small pieces. Upon dry these pieces were ground into powder. The rhizome powder then used for extraction by reflux method using 80% ethanol for 45 minutes with three times replication. The ethanolic extract evaporated using a rotary evaporator until a concentrated extract was obtained. The concentrated ethanolic extract then mixed with benzene to remove excess lipids. Next, the extract diluted with water, a concentrated hydrochloric acid were added until a 4N solution formed. To hydrolyze saponin from saponin, this acidic solution was refluxed for 4 hours. The results

of hydrolysis were cooled and the precipitate sapogenin filtered. Sapogenin then rinsed with 50% ethanol and refluxed with the benzene-methanol (3:1) solvent. The precipitated compound was the diosgenin which were then used in the experiment. The baseline dosage of the crude diosgenin extract used in this study was 200 mg/kg b.w., adopted from Revalthy *et al.*⁵.

Taurine

Taurine powder used in the study was manufactured by NOW Foods, USA. The daily dosage of the powder which were applied for each mouse was 1,656 mg/kg body weight. Such dosage was based on the *no observed adverse effect level* (NOAEL) on rats issued by Intertek¹⁷.

Table 1: Changes of blood glucose levels of mice after alloxan induction and application of crude diosgenin extract (CD) and taurine

Treatments	Blood glucose levels (mg/dL)			
	Day-1	Day-4	Day-7	Day-14
Normal	150±24.48 ^a	155.6±44.2 ^{cd}	158±41.46 ^b	153.5±52 ^b
Alloxan	125.2±24.69 ^{ab}	351.2±192.7 ^a	320.75±186.02 ^a	309.75±194.76 ^a
CD 20%	122±15.77 ^{ab}	124.5±19.8 ^d	152.75±48.45 ^{bc}	130.5±35.83 ^{bc}
Alloxan+CD 20%	140.4±25.63 ^a	329.8±165.8 ^{ab}	143.25±54.51 ^{bc}	133.25±35.83 ^{bc}
Alloxan+CD 30%	132±30.31 ^a	301.2±151.7 ^{abc}	119.75±15.71 ^{bcd}	127±36.31 ^{bc}
Alloxan+Taurine	98±23.58 ^b	190.8±51.6 ^{bcd}	125±44.83 ^{bcd}	110±22.01 ^{bcd}
A+CD20%+Taurine	123.25±30.43 ^{ab}	177.8±15.8 ^{bcd}	119±21.79 ^{bcd}	96.75±12.31 ^d
F-value	2.06	2.77	3.36	3.4
P-value	0.001	0.002	0.017	0.016

Data are presented as mean±SD; A=alloxan; values in the same column that shared the same superscript are not different at $\alpha=0.05$ (LSD test)

Table 2: Body weight and testicular weight of mice after alloxan induction and application of crude diosgenin extract (CD) and taurine

Treatments	Body weight (g)	Testicular weight (g)
Normal	38.07±4.527	0.294±0.0378 ^a
Alloxan	28.12±12.754	0.1325±0.1081 ^c
CD 20%	37.12±5.434	0.2275±0.0287 ^{ab}
Alloxan+CD 20%	39.96±4.758	0.205±0.0592 ^{bc}
Alloxan+CD 30%	38.49±5.371	0.2575±0.0126 ^{ab}
Alloxan+Taurine	39.44±7.364	0.242±0.0517 ^{ab}
A+CD20%+Taurine	31.24±8.158	0.2325±0.015 ^{ab}
F-value	0.79	3.79
P-value	0.59	0.001

Data are presented as mean±SD; A=alloxan; values in the same column that shared the same superscript are not different at $\alpha=0.05$ (LSD test)

Alloxan and diabetes induction

Diabetes was induced by injection intraperitoneally of alloxan monohydrate, a Sigma-Aldrich product, purchased from The Sawittoku Chemical Laboratories Ltd., Makassar, Indonesia. The dosage applied in the study was 150 mg/kg body weight, the moderate dose of alloxan adopted from Etuk¹⁸.

Animals

Swiss albino male mice (*Mus musculus*) aged 3-4 months, weighing 20-30 grams from the

Veterinary Office of Lampung, were used for the study. All aspects of animal care followed the ethical guidelines and technical requirements approved by the Institutional Research Ethics Committee. Before treatment, mice were allowed to acclimatize for 7 days under standard laboratory condition in the plastic cages with a size of 28 x 30 x 13 cm (22-24 °C, humidity 60-70%, 12 h light : 12 h dark cycle) with free access to standard diet and water *ad libitum*. Prior to any kind of treatment (alloxan induction and/or active substances application) all mice were fasted for 18 hours.

Table 3: Spermatogenic cell counts of mice after alloxan induction and application of crude diosgenin extract (CD) and taurine

Treatments	Spermatogonia (10v /ml)	Spermatocytes (10v /ml)	Spermatids (10v /ml)
Normal	51.8±6.54 ^a	60.2±10.28 ^{ab}	90.8±34.4 ^a
Alloxan	30.2±8.44 ^{de}	20±5.15 ^c	12.4±5.41 ^b
CD 20%	23.2±5.36 ^e	32.2±7.36 ^c	28±8.46 ^b
Alloxan+CD 20%	37.8±6.69 ^{cd}	30.8±2.39 ^c	31.6±11.99 ^b
Alloxan+CD 30%	46.8±6.30 ^{ab}	31±8.57 ^c	32.6±11.37 ^b
Alloxan+Taurine	53.6±6.95 ^a	72±8.94 ^a	106.8±31.53 ^a
A+CD20%+Taurine	40.8±3.56 ^{bc}	47±20.74 ^b	37.2±20.08 ^b
F-value	15.22	15.66	14.96
P-value	0.000	0.000	0.000

Data are presented as mean±SD; A=alloxan; values in the same column that shared the same superscript are not different at $\alpha=0.05$ (LSD test)

Table 4: Sperm quantity and quality in mice after alloxan induction and application of crude diosgenin extract (CD) and taurine.

Treatments	Sperm counts (10 ⁶ /ml)	Sperm motility (%)	Sperm viability (%)
Normal	18.36±3.36 ^{ab}	64.8±4.38 ^a	75.39±7.26 ^a
Alloxan	15.63±8.54 ^{abcd}	11.8±12.81 ^c	36.23±19.25 ^c
CD 20%	12.5±4.74 ^{bcd}	10.94±9.26 ^c	72.29±5.59 ^a
Alloxan+CD 20%	10.5±1.85 ^{cd}	23.18±17.78 ^{bc}	50.4±16.67 ^{bc}
Alloxan+CD 30%	9.57±1.64 ^d	24.92±15.6 ^{bc}	43.06±14.66 ^c
Alloxan+Taurine	20.62±6.50 ^a	75.34±7.17 ^a	54.52±2.98 ^{bc}
A+CD20%+Taurine	17.36±5.31 ^{abc}	35.82±32.8 ^b	62.96±15.81 ^{ab}
F-value	3.21	11.55	5.72
P-value	0.000	0.000	0.001

Data are presented as mean±SD; A=alloxan; values in the same column that shared the same superscript are not different at $\alpha=0.05$ (LSD test)

Experimental design and treatment

Experimental animals, 35 mice were randomly divided into 7 groups (n=5) as follows:
 Group 1: mice that only received diet and water, as normal control;
 Group 2: animals that treated only with alloxan 150mg/kg body weight, as diabetic control;
 Group 3: mice given only crude diosgenin 200 mg/kg body weight, as diosgenin control;
 Group 4: animals treated with alloxan and diosgenin 200 mg/kg body weight;
 Group 5: mice treated with alloxan and diosgenin 300 mg/kg body weight;
 Group 6: animals treated with alloxan and taurine 15.6 mg/g body weight;
 Group 7: mice treated with alloxan, dosgenin 200 mg/kg and taurine 15.6 mg/g body weight.

Alloxan, crude diosgenin extract and taurine were administered intraperitoneally by gavage using a Sonde feeding needle..

Study parameters

Blood glucose

In order to determine plasma glucose levels, blood were collected on day-1, 4, 7 and 7) from the tail vein. The glucose levels were measured using Lab Test Set for Glucose, Nesco® *multicheck*.

Body and estis weight

At the end of treatment, body and testis weight of each animal were recorded.

Spermatogenic parameters

The spermatogenic cells counts were determined by fixing the left testis of each mouse in Bouin's solution. The organs were cut at the thickness of 5µm and stained with hematoxylin and eosin and examined under a light microscope at 400x magnification. Spermatogonia, spermatocytes and spermatids counts were expressed as average values of spermatogenic cells in 10 seminiferous tubules.

Sperm parameters

Immediately after weighed, the epididymis of each animal was dissected and transferred into physiological saline medium and cut to small slices in order to swim out the sperm into the medium. After 10 min of diffusion, the solution was diluted

with formaldehyde fixative. The diluted solution was transferred into each chamber of Neubauer hemocytometer and sperm cells was manually counted under a light microscope. Sperm counts were expressed as the number of sperm (in millions) per ml. Quantitative epididymal sperm motility carried out by placing 10 µl of the sperm suspension on semen analysis chamber. Five microscopic fields were assessed to evaluate sperm motility on at least 200 sperm for each animal and expressed as percent (%) motility. Sperm viability examined using supravital staining made by adding 10 µL of eosin-Y 0,5% into the 10µL of semen suspension. Sperm morphology was assessed from a epididymis filtrate smeared on a clean glass slides by addition of a drop of 1% eosin. Once the object dried, observation done under a light microscope at 400x magnification and the abnormalities of either head or tail were noted.

RESULTS

The effects of alloxan induction, application of crude diosgenin extract and taurine treatment on the blood glucose levels in male mice are tabulated in Table 1. Alloxan induction in normal mice, significantly doubling the blood glucose levels of the animals on day 4 onwards, till to the end of treatment. On the other hand, application of crude diosgenin extract in normal mice tend to maintain the blood sugar at the normal levels or lower until the end of treatment. In alloxan-induced mice, at the end of experiment (day-14), application of diosgenin 20% and 30% or taurine solely significantly lowered the blood glucose levels. In addition, the glucose levels in alloxan-induced mice significantly decreased by treatment of crude diosgenin extract combined with taurine ($P=0.016$).

Table 2 is tabulation the effects of alloxan induction, application of crude diosgenin extract and taurine treatment on body weight and testicular weight. None of the treatments give effect on the body weight of mice ($P=0.59$). Alloxan given to the normal mice significantly decrease testicular weight. However, treatment of crude diosgenin extract in normal mice give no significant effect on the testis weight. In the alloxan-induced mice, except for diosgenin extract 20%, all treatments significantly increase the weight of testes ($P < 0.01$).

Spermatogenic parameters resulting from alloxan induction, application of crude diosgenin extract and taurine treatment are presented in Table 3. In normal mice, both alloxan induction and application of crude diosgenin extract significantly decrease the number of spermatogonia, spermatocytes and spermatids ($P < 0.001$). In alloxan-induced mice, crude diosgenin extract 20% showed no significant effect on each type of spermatogenic cells, but diosgenin 30% significantly reversed the spermatogonia counts but not the number of spermatocytes and spermatids. Taurine application in alloxan-induced mice significantly increase the number of all spermatogenic cells, even exceed the normal levels. When diosgenin given in combination with taurine the number of spermatogonia and spermatocytes increase significantly but showed no effect on spermatids counts ($P < 0.001$).

The depiction of both sperm quantity and quality due to alloxan induction, application of crude diosgenin extract and taurine treatment are presented in Table 4. Alloxan induction significantly decrease sperm motility and viability but not the sperm counts. Application of crude diosgenin extract in normal mice tend to decrease the sperm motility, but give no effect on the sperm counts and viability. In the alloxan-induced mice, diosgenin application showed no effect either on sperm quantity or quality. Taurine applied in alloxan-induced mice give effects on sperm motility and viability but not on the sperm counts. However, crude diosgenin extract when combined with taurine was significantly reverse the motility and viability of sperm in the alloxan-induced mice, though never reach the normal condition.

DISCUSSION

The results of the study confirms the effectiveness of the use of alloxan—in the sense of the easiest, reliable and the most practicable method, in inducing diabetes in laboratory rodents¹⁸. In this study the alloxan induction manage to maintain blood glucose levels up to 309 mg/dL (153.5mg/dL in normal mice), while in a previous study reported by Shetti *et al.*¹⁹ alloxan induction successfully maintained the blood glucose levels more than 352 mg/dL. In rats, alloxan induction can even increase glucose levels up

459.5 mg/dL on day-14, 479.8 mg/dL on day-21 and 501.8 mg/dL on day-30 (93 – 96 mg/dL in normal rats)²⁰. Alloxan, as noted, establish a redox cycle with the formation of superoxide radicals, which undergo dismutation to hydrogen peroxide (H₂O₂) and more highly reactive hydroxyl radicals, lead to increase in cytosolic calcium concentration ultimately causes rapid destruction of beta cells of pancreatic islets²¹.

The second finding of this study revealed that application of crude diosgenin extract from *C. speciosus* in normal mice tend to maintain blood glucose at the normal levels or lower until the end of treatment. Mechanisms that might account for the decrease in blood glucose after administration the extract is as follows. This study shows that the feeding of the two test diets to diabetic rats results in alterations in the metabolism of glucose with subsequent reduction in plasma glucose concentration. Diosgenin revealed to possess inhibitory properties against crude murine amylase and glucosidase²². The inhibition of the α -glucosidase may have the potential to delay the development of diabetic complications²³. In type 2 diabetic obese KK-Ay mice, diosgenin ameliorate diabetes by promoting adipocyte differentiation and inhibiting inflammation in adipose tissues²⁴.

On the spermatogenic parameters, this study revealed to decrease significantly the number of spermatogonia, spermatocytes and spermatids. This phenomenon seems to be a logical consequence of the diabetic effects of the alloxan itself. Diabetes mellitus severely altered the mechanisms of glucose transport through GLUTs, glucose metabolism, oxidative stress (OS), nuclear and mitochondrial DNA fragmentation²⁵. The fragmentation of DNA and an increase in advanced glycation end products led to deterioration of sperm quality, sperm functions and changes in testicular metabolite levels and spermatogenic gene expression²⁶.

The unexpected findings of this study was that normal mice treated with crude diosgenin extract from *C. speciosus* showed a similar effects as alloxan, led to the decrease in spermatogonia, spermatocytes and spermatids counts. As reported previously, plant extract of *C. speciosus* showing

antidiabetic activity by presenting varying degree of hypoglycemic and antihyperglycemic activities, safe and has no side effects compared to other drugs²⁷.

In alloxan-induced mice, the study found that at the end of the 14 days experimental period all types of treatment was significantly reverse the blood glucose to normal levels or even lower. These data indicate that diosgenin, taurine, or the combination of both can be used to manage diabetes and seem to be related to antioxidant activity possessed by both diosgenin and taurine. Diosgenin extracted from *Tribulus terrestris*, by using a modified TLC method including DPPH assay was reported to show antioxidative properties²⁸. Such antioxidant properties also shown by diosgenin isolated from *Asparagus officinalis*²⁹.

In relation to the parameters of sperm and spermatogenic in alloxan-induced mice, it was taurine instead of diosgenin or the combination of both, that managed to ameliorate the deleterious effects of alloxan even exceed the normal levels. These findings seem to consistent with the conclusion proposed by Mirunalini *et al.*³⁰ that in Hep2 cell line diosgenin shows prooxidant, instead of antioxidant, properties.

In contrast, the results of research actually reinforces the pharmacological and clinical benefits of taurine which has been widely reported. Supplementation of taurine to the semen extender

was suggested to increase the sperm motility, viability, survival and membrane integrity³¹. Due to its antioxidant properties, taurine can even reverse acute effects of Aluminium poisoning in male genital organs³². In male rats fed high cholesterol diets taurine led to increase in Gonadotropin hormones FSH and LH in addition to the testosterone after it has been reduced due to cholesterol and results in testosterone hormone improvement, the sperm viability was improved as well³³.

CONCLUSION

The results of the study can be summarized as follows. The use of crude diosgenin extract from *C. speciosus* in normal mice can negatively affect fertility by lowering the number of spermatogonia, spermatocytes and spermatids cells in addition to decrease sperm motility. In alloxan-induced mice, diosgenin useful in ameliorating the blood glucose levels but not effective in normalization of the testicular disorder status of the animals. Taurine, on the contrary, is effective both to cope with diabetes and testicular disorders in alloxan-induced mice. Application of diosgenin combined with taurine, most effective in lowering blood glucose levels compared with other treatment but shows little contribution in normalization the testicular parameters in alloxan-induced mice. Thus, it can be concluded that diosgenin from *C. speciosus* even though combined with taurine has little effect on testicular disorders in alloxan-induced diabetic mice in comparison to the application of taurine alone.

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