ORIGINAL ARTICLE

Sperm DNA Fragmentation Index and Malondialdehyde of Diabetic Rats Treated By *Aloe Vera* Peel Extract

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ABSTRACT

Background: Sperm DNA fragmentation and the presence of free radicals have effects on the male infertility. *Aloe vera* peel is assumed to increase antioxidant enzymes.
Objectives: The aim of this study was to determine the effect of *Aloe vera* peel extract on the DNA fragmentation index (DFI) and Malondialdehyde (MDA) levels of diabetic rats.
Method: The design of this study was posttest only randomized control group design. Eighteen rats were divided into three groups: the control group (A), group-B and C. Two latter groups were given 100 mg and 200 mg/kg of *Aloe vera* peel extract for 28 days respectively. Data were obtained from sperm DNA fragmentation and MDA levels from epididymal fluid and serum.
Results: *Aloe vera* peel extract significantly influenced MDA levels between groups ($p<0.05$). Malondialdehyde epididymal fluid in the control group was higher (9.44±0.39) than group B (3.37±0.38) and group C (3.29±0.31). While MDA serum in the group C (3.07±0.34) was lower than group B (6.06±0.45) and control (9.07 ± 0.45). The mean value of sperm DFI in the control was significantly higher (9.92±3.68) than group B (6.00±1.41) and group C (4.08±1.46), but no significant difference between group B and C ($p<0.05$).
Conclusion: *Aloe vera* peel extract reduced sperm DFI and MDA levels of epididymal fluid and serum
Keywords: Aloe vera, Malondialdehyde, sperm DFI

INTRODUCTION

The diagnosis of male infertility is generally based on conventional semen testing despite many limitations of the examination.[1] Sperm DNA fragmentation has been known as a major factor in male infertility and is associated with fertility disorders, such as embryo quality, increased abortion, birth defects and very low birth rates[2,3] Many factors that can affect DNA fragmentation include alcohol,[4] varicocel,[5] and free radicals[6].

High blood glucose in diabetes can increase the formation of free radicals including those from mitochondria and non mitochondria such as activation of protein kinase C and increased end-use glycation products.[7] Increased lipid peroxidation can increase insulin resistension.[8] The oxidative stress that occurs causes antioxidant levels decreases and significantly increases MDA as a marker of lipid peroxidation.[9,10] Assessment of oxidative stress status can help in male infertility medical treatment with the right antioxidants[11,12].

On the other hand, the presence of free radicals can have an impact on fertility. Several studies have shown that there is an association between hyperglycemia and sexual dysfunction.[13] Sperm is very susceptible to free radicals because there are many lipid membranes of plasma in the form of polyunsaturated fatty acids with more than two carbon double bonds and the cytoplasm has low antioxidant enzyme[14]. Lipid peroxides accumulate in the sperm membrane resulting in loss of motility and oxidative damage to DNA.[15]

Many researchers have proved that *Aloe vera* gel extract has antidiabetic potential by lowering blood glucose levels and increasing antioxidant enzymes.[16,17] However, there was still little information related to the utilization of *Aloe vera* peel. Preliminary research has shown that *Aloe vera* peel extract significantly lowers levels of 8-oxo-2'-deoxyguanosine and improves the total antioxidant status of type 2 diabetic rats[18]. The other researchers have shown that peel extracts are also effective in reducing blood glucose levels in alloxan induced diabetic rats.[19] There is a significant correlation between total phenolic content in peel extract and antioxidant capacity. [20] Peel extract has higher phenol and flavanoid content than *Aloe vera* gel,[21] while antioxidant activity of leather extract is also better than flowers. [22] This study aims to show that *Aloe vera* peel extract has the potential to reduce serum and epididymal fluid MDA levels and sperm DNA fragmentation index.

METHODS

Aloe vera peel extract: Fresh, dark green color with a length of about 50 cm *Aloe vera* leaves were selected. The leaves were washed, cut into pieces and sliced longitudinally to separate the thick peel with the center of the gel. The peel was wiped off in a cabinet dryer and extracted with ethanol. After that, it was stored at room temperature for 48 hours then it was filtrated. The filtrate was collected and ethanol was evaporated in a rotary evaporator. Extracts were stored in containers and prepared at all times when it would be given.[23] *Aloe vera* peel extract was administered intragastrically daily in different groups with doses of 0, 100 and 200 mg/kg body weight for 28 days.
Induction of diabetes: The experimental animals were 18 male Wistar albino rats aged over 8 weeks. The experimental period was 5 weeks on which food and water were provided ad libitum. Diabetes was induced by a single intraperitoneal injection with streptozotocin (STZ) (Nacalai Tesque, Inc No: 32238-91) at a dose of 65mg/kg and combined with nicotinamide acid (Nacalai Tesque, Inc No:

24303-84) at dose of 230 mg/kg body weight. After 72 hour time for the development of diabetes, the rats with blood glucose range of above 200 mg/dl were considered as diabetic rats and used for the experiment.[24] The experiments were conducted according to the ethical norms approved by the Commission of Health Research Ethics Faculty of Medicine Diponegoro University/RSUP dr. Kariadi Semarang with the approval number of 75/EC/H/FK-RSDK/X/2017.

Experimental procedure: The animals were divided into 3 groups of six animals in each group as follows: Group-A for control, Group-B receiving *Aloe vera* peel extract of 100 mg/kg and Group-C obtaining 200 mg/kg of *Aloe vera* peel extract. The handling of animals were performed according to the care and use of laboratory animals of Food and Nutrition, Inter-University Food and Nutrition Studies Center (PSPG - PAU), Gadjah Mada University, Yogyakarta. After the last treatment of 28th day, the blood was taken from an orbital vein. The blood samples were centrifuged at 4000 rpm for 15 minutes, then sera were separated and and kept in plastic vials at -20°C until analyses. The sera and epididymal fluid were analyzed for MDA by colorimetric method (BioVision Cat #K739).

TUNEL Assay: The cauda epididymides of the rats were incised and epididymal fluid was centrifuged at 4000 rpm for 15 minutes with phosphate-buffered saline (Gibco^{TM}, Cat A1896702). Spermatozoa with DNA fragmentation in each sample was determined by the the terminal deoxynucleotidyl transferase-mediated (TdT) deoxyuridine triphosphate (dUTP) nick end labeling assay (TUNEL) assay using an in situ cell death detection kit (Roche Diagnostics, Mannheim, Germany) using fluorescent microscopy, in which normal DNA was shown as red and damaged DNA was seen as green (TUNEL positive).[25] For microscopy evaluation, the slides were viewed using an optical and fluorescence ConVocal CARL ZEISS LSM 800. Fluorescein was typically excited by the 488-nm line, and the emission was collected at 530 nm. For each slide, about 200 spermatozoa were evaluated. [26]

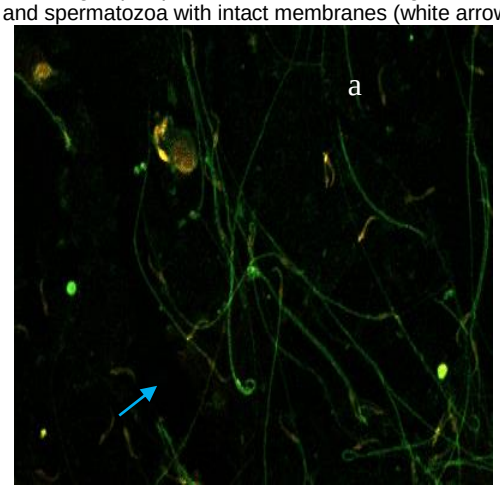
Statistical analysis: The data were analyzed by using one-way Analysis of Variance (ANOVA) followed by Least Significance Different (LSD) and p value of < 0.05 was considered significant. The data were presented as Mean ± S.D.

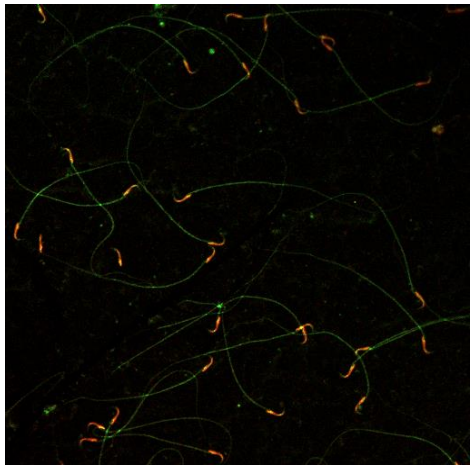
RESULT

The present study showed that STZ-induced diabetes significantly increased MDA level from seminal plasma, serum and DFI ($p < 0.05$). Administration of *Aloe vera* peel extract to the diabetic group resulted in a significant decreased ($p < 0.05$) in epididymal fluid and serum MDA and DFI (Table 1). As it is shown in table 1, the rates of spermatozoa with DNA fragmentation were statistically higher in treatment group B (100 mg/kg) than group C (200 mg/kg) ($p<0.05$).

The TUNEL assay, used to detect damage cells, revealed the presence of a high number of DNA fragmentation in the control group and significantly differed from the treatment group ($P<0.05$) (Figure 1).

Fig. 1: Micrograph showing sperm DNA fragmentation using TUNEL assay of rats spermatozoa. (a) Sample from control group, (b) Treatment group. Spermatozoa with DNA fragmentation (blue arrow) and spermatozoa with intact membranes (white arrow)





Tabel 1. DNA sperm fragmentation index and MDA level albino rats after 4 weeks of *Aloe vera* peel extract administration

Variables	A Control	B (100 mg/kg)	C (200 mg/kg)	Sig
MDA epididymal fluid(nmol/ml)	9.44 ± 0.39	6.28 ± 0.45$^{*\#}$	3.37 ± 0.38$^{*\#}$	P<0.05
MDA serum (nmol/ml)	9.07 ± 0.45	6.06 ± 0.45$^{*\#}$	3.07 ± 0.34$^{*\#}$	P<0.05
DFI (%)	9.92 ± 3.68	6.00 ± 1.4*	4.08 ± 1.46*	P<0.05

Data are presented as mean ± SD. * p <0.05 compared to control, $^{\#}$ p <0.05 between treatments

DISCUSSION

The mechanism of streptozotocin (STZ) induced DM is probably through the generation of reactive oxygen species (ROS). The production of free radicals is engendered by uncontrolled hyperglycemia, which may occur via several routes.[27] Elevated ROS levels, cause a cascade of events leading to lipid peroxidation an increase in the level of free

radicals this was due to the overproduction of ROS in semen, which is associated with reduced sperm fertilizing potential.[28] That spermatozoa can be readily damaged by oxygen species. In addition to this, sperm cells are known to contain high concentration of polyunsaturated fatty acids and their cytoplasm contain low concentration of scavenging enzyme.[3]

Lipid peroxidation of spermatozoa membrane is responsible for causing perturbation of membrane structure and function as transport processes, maintenance of ion, receptor.[29] Besides the evaluation of sperm DNA fragmentation and oxidation, the malondialdehyde (MDA) assay was also used to measure lipid peroxidation in spermatozoa in this study.[30] The finding showed a relationship between sperm DNA fragmentation and the MDA level in diabetic rat. The MDA level was significantly higher in rat diabetic than group with *Aloe vera* peel extract.

The findings of this study revealed that oral administration of *Aloe vera* gel extract have antioxidative effect which is manifested by the significant decrease in serum MDA levels with a significant increase of total antioxidant capacity.[17] *Aloe vera* leaf pulp extract significantly decreased serum glucose, serum levels of MDA and increased serum insulin levels as compared to control diabetic rats.[16]

From literature review, it was found that many herbs and plant products have been shown to have hypoglycemic action. Flavonoids are known to be bioactive antidiabetic principles. Previous reports from our department revealed that extracts have antidiabetic activity and the major constituent responsible was a flavonoid.[31] The *Aloe vera* extract may also act by either directly scavenging the reactive oxygen metabolites or due to the presence of various antioxidant principles like flavonoids.[32]

Antioxidant activity of *Aloe vera* skin was 87.651 %.[33] The best antioxidant activity of the peel extractis in correlation with the content of phenolic and flavonoid compounds. Peel extract had total phenolic content of 7.99±0.26 mg(GA)/g, and flavonoid content of 9.17±0.19 mg(QE)/g while gel extracts had almost three times lower content.[21] The antioxidant action of flavonoids include suppression of ROS formation by inhibition of enzymes, by scavenging free radicals, and regulation of antioxidant defenses. [33] Flavonoids also protect the lipids of the biomembranes which are damaged due to lipid peroxidation.

CONCLUSION

Aloe vera peel extract is rich in potent antioxidant phenol and flavanoid components to reduce lipid peroxide levels such as MDA and decrease sperm DNA damage from free radicals in diabetic rat.

Acknowledgements: This research was supported by the Government of the Republic of Indonesia through the Directorate General of Higher Education (BPPS) and Semarang State University (UNNES).

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