Potency of mangosteen (Garcinia mangostana L.) pericarp on seminiferous tubules testes streptozotocin-induced diabetic rats

by Cicilia Novi Primiani

Submission date: 02-Nov-2019 07:58AM (UTC+0700)
Submission ID: 1205290775
File name: 8._JOURNAL_OF_PHYSIC_CICILIA_UMI.pdf (557.06K)
Word count: 3808
Character count: 19266

Journal of Physics: Conference Series

Potency of mangosteen (*Garcinia mangostana* L.) pericarp on seminiferous tubules testes streptozotocin-induced diabetic rats

IOP Conf. Series: Journal of Physics: Conf. Series **1217** (2019) 012165 doi:10.1088/1742-6596/1217/1/012165

Potency of mangosteen (*Garcinia mangostana* L.) pericarp on seminiferous tubules testes streptozotocin-induced diabetic rats

C N Primiani[1], U Lestari[2]
[1]Faculty of Teacher Training and Education, PGRI Madiun University, Indonesia
Jl. Setiabudi No. 85 Madiun 63118, Indonesia
[2]Biology Department, Faculty of Science, State University of Malang, Indonesia
Jl. Semarang No.5 Malang, Indonesia
E-mail: primiani@unipma.ac.id

Abstract. The diversity of compounds in mangosteen peel (*Garciniamangostana L.*) is suspected to have hypoglycemic activity. The objective of this study to analyze the blood glucose level and seminiferous testicular tubules tissue structure of streptozotocin-induced diabetic rats. The experiment animals were twelve male *wistar* strain white rats (*Rattus norvegicus*), 2-3 months old, weighing 120-150 g, put into four different groups i.e. (1) negative control treatment (P_0), (2) streptozotocin (STZ) induced intraperitoneal as much as 80 mg/kg in 0.1 M buffer citrate with positive control pH 4.5 (P_1), (3) 83.3 mg/kg mangosteen pericarp powder (P_2), and (4) 0.09 mg/kg glibenclamide (P_3) for 36 days. The analysis of the blood glucose levels and seminiferous testicular tubules tissue structure using HE staining, identification with the optilab microscope. The study found that diabetic tubules testes structure experienced degeneration, the spermatogenic cells were not much identified, lumen tubules testes diameter 703,25 µm (P_1), spermatogonia cells were identified, the spermatogenesis developed, lumen tubules testes diameter 570,53 µm (P_2). The mangosteen solution and glibenclamide could lower the blood glucose level and improve the spermatogenesis of the diabetic rats. The mangosteen pericarp solution did not damage the renal tissue structure, unlike the glibenclamide. It was concluded that the mangosteen solution has the potential as natural anti-diabetic substance.

1. Introduction

The biodiversity of plants can potentially be utilized as medicinal herbs. It has widely been known that people commonly use all parts of the plant for different types of treatments. The major principle of treatments using herbs, in this sense, is making use all parts of the plant such as the leaves, flowers, fruits, seeds, and roots in fresh or usually called simplicia. Additionally, making use of the plant as a whole result in a much better effect due to the complex compounds contained in different parts of the plants interacting with one another.

Concerning this, one of the plants which can grow well in tropical countries is mangosteen (*Garcinia mangostana*). Mangosteen is a tropical fruit which can directly be consumed in its fresh form or processed into commercial products for various purposes such as food supplements, cosmetics, and pharmaceutical products. This fruit has been widely used as traditional medicine in many countries like India, Myanmar, Thailand, Malaysia, Taiwan, The Philippines, Indonesia, and Sri-Lanka [1,2].

Mangosteen, especially the pericarp (fruit peel), contains xanthone and its derivatives active compounds [2]. Xanthone is a kind of polyphenol compound with a tricyclic aromatic ring. This compound has numerous biological activities in the human body [3]. The activities are antioxidant, analgesic, anti-proliferative, anti-inflammatory, anti-carcinogenic, and anti-microbial activities [2,4–6].
Some published studies found that the mangosteen pericarp can be used to reduce blood glucose levels [1,7–9]. Regarding this, it is known that hyperglycemia manifestations can occur in various body organs such as the eyes, teeth, heart, skin, reproductive organs, and some other organs. Hyperglycemia causes a decrease in male fertility, with decreased function of Leydig cells and seminiferous tubules testes. Therefore, the research objective of this study is to analyze the blood glucose levels and seminiferous testicular tubules tissue structure of streptozotocin-induced diabetic rats.

2. Materials and methods

2.1 Materials

The mangosteen fruit used in this study was purchased in a traditional market in Madiun, Indonesia. The pericarp was firstly separated from the skin, washed, and cut into small pieces were dried using an oven at 60 - 70°C for two days. After they were dry, the pieces were smoothed using a blender to make a homogeneous powder with ± 0.65 mm in size. The compounds used were streptozotocin (STZ) and glibenclamide (Sigma, Aldrich, USA).

2.2 Streptozotocin solution

100 mg of STZ was dissolved in 3 ml of citrate buffer with pH 4.5 which consisted of 0.1 M sodium citrate solution and 0.1 M sodium chloride solution [10,11]. It was then put on a vortex until it was homogeneous and stock STZ solution was produced. The stock STZ solution was then stored at 4° C and injected at a dose of 40 mg/kg volume, and the takes were adjusted with the rats' weight.

2.3 Experimental animals

The experimental animals used in this research were male rats (*Rattus norvegicus*), 2-3 months old, 150 – 200 g weight, obtained from a rat farm in Blitar, Indonesia. The rats were put into four treatment groups with each group consisting of six rats: Group 1 (P_0) was the control group; Group 2 (P_1) was the STZ-induced diabetic rats group; Group 3 (P_2) was the diabetic rats treatment group being given mangosteen pericarp powder as much as 83.3 mg/kg; and Group 4 (P_3) the diabetic rats treatment group being given 0.09 mg/kg glibenclamide. The rats were put inside maintenance cages with a temperature between 25 - 27° C, 50 – 60% humidity. The rats were fed with milk A pellet every morning for 36 days.

2.4 Streptozotocin intraperitoneal injection

The STZ injection was completed using intraperitoneal injection method: the disposable needle was placed at 45° angle, and the Streptozotocin solution was slowly injected.

2.5 Measuring the blood glucose levels

Cutting the tip of the rats' tail was completed using surgical scissors. Some blood from the tip of the tail was then dropped for a Blood Glucose Test. The measurement of glucose levels was conducted three times: before the STZ injection, 24 hours after the STZ induction, and 72 hours after the STZ induction.

2.6 Experimental design

The rats were treated according to their respective groups. P_0 control group was given aquadest; P_1treatment group was given a single dose of STZ; and P_2 treatment group was given a single dose of STZ and the measurement of glucose level after 72 hours of STZ induction reached a minimum of 250 mg/dl [12] followed by a treatment of 83.3 mg/kg mangosteen pericarp powder for 36 days; P_3 treatment group was

also given a single dose of STZ and the measurement of glucose level after 72 hours of STZ induction reached a minimum of 250 mg/dl followed by the administration of 0.09 mg/kg glibenclamide for 36 days.

2.7 Organ collection and staining

All the experimental rats were dissected on the 37th day, and their testes organs were examined. The organs then experienced fixations in 4% PFA solution. The histology slides were then conducted in compliance with a suggested procedure[13–15], and the kind of staining used was Hematoxylin-Eosin.

2.8 Data analysis

The measurement of blood glucose levels was completed using blood glucose tests. Changes of the tissue structures and size of the lumen tubules testes were observed using an optilab microscope. The test results of blood glucose tests and lumen tubules testes measurements were statistically analyzed using ANOVA using SPSS ver. 19 software program.

3. Findings

3.1. Tubules testes tissue structure

The analyses of the tubules testes tissue structure changes can be seen in Figure 1.

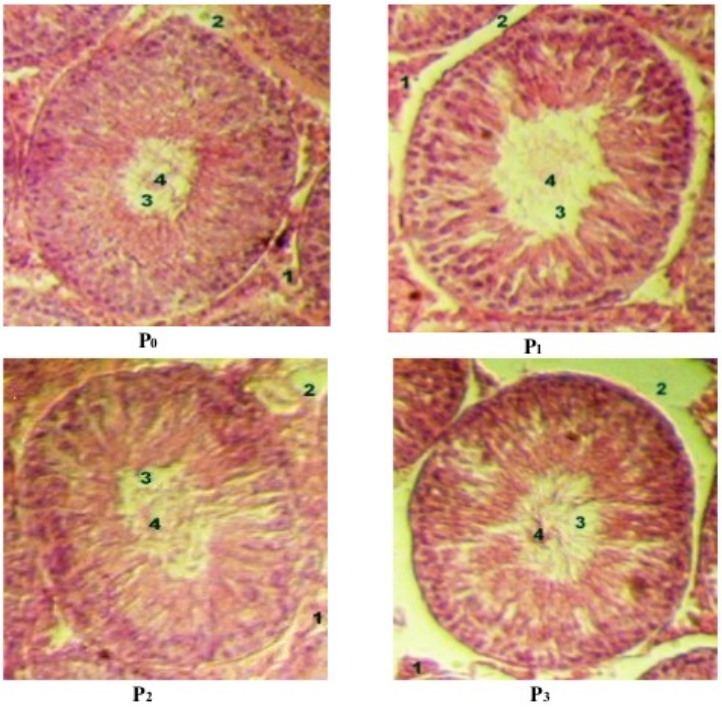

Figure 1. Tubules testes tissue structure of the white rats, staining HE, 400x
1. Leydig cell; 2. interstitial; 3. lumen; 4. spermatozoon

P_0 The spermatogonia cell structure suits the development levels starting from the spermatogonia, spermatocyte, spermatid, and spermatozoa

P_1 The tubules testes structure experienced degeneration, the spermatogenic cells were not much identified, the spermatid cells were not identified, and the spermatozoa cells were identified in a tiny amount, there was fatty degeneration, and the lumen tubules structure was widened

P_2 The spermatogonia cells were identified, the spermatogenesis developed from the basal membrane towards the lumen tubules, and the

P_3 The spermatogonia cells were identified, the spermatogenesis developed, fatty vacuoles were identified, and the lumen tubules contained spermatozoa

3.2. Blood glucose level and lumen tubules testes diameter

Table 1 below shows the glucose level and lumen tubules testes diameter on day 37 of rats from the control group, the STZ-induced treatment group, and the STZ-induced and 83.3 mg/kg mangosteen pericarp powder administered treatment group, as well as the STZ-induced and 0.09 mg/kg glibenclamide administered treatment group.

Table 1. The blood glucose levels and lumen tubules testes diameter measurement results

Experimental group	Blood glucose level (mg/dl)	Lumen tubules testes diameter (µm)
Normal control (P_0)	72.98	471.59
Diabetic control (STZ induction) (P_1)	141.46*	703.08
STZ + serbuk pericarp manggis 83,3 mg/kg (P_2)	111.44**	570.25
STZ + glibenklamid 0,09 mg/kg (P_3)	98.16**	552.53

* significantly different ($p < 0.05$) when compared to the normal control
** significantly different ($p < 0.05$) when compared diabetic control (STZ)
ANOVA analysis, followed LSD ($p < 0.05$)

The analyses of blood glucose level and testes lumen tubules diameter of the STZ induced diabetic rats using the mangosteen pericarp powder treatment, and glibenclamide showed significant differences ($p < 0.05$) when being compared to the control group. Mangosteen pericarp powder treatment affects decreasing blood glucose levels and increasing the process of spermatogenesis in the seminiferous tubules testes. This is indicated a decrease in glucose levels and a decrease in the diameter of the seminiferous tubules testes.

3.3. Tubules renal tissue structure

The analyses of the tubules renal tissue structure changes can be seen in Figure 2.

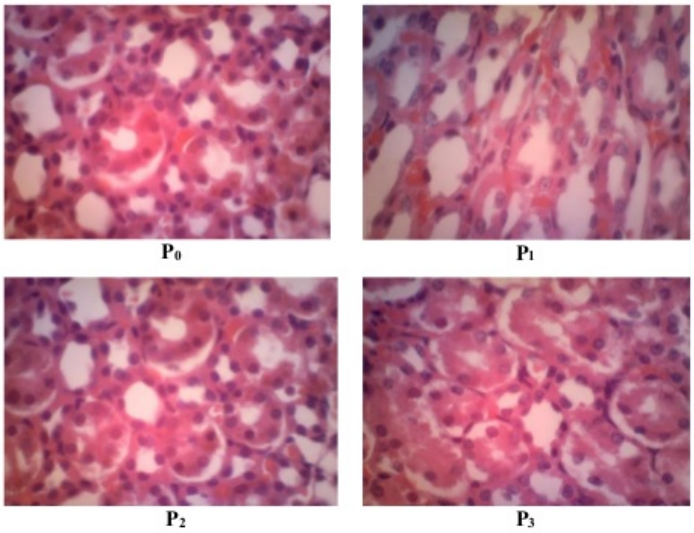

Figure 2. Tubules renal tissue structure of the white rats, staining HE, 100x

P_0 Normal kidneys tubules
P_1 Hemorrhagic renal tubules, fatty vacuoles were identified, necrosis
P_2 Normal renal tubules, necrosis was not found
P_3 There is fatty vacuoles were identified, necrosis

4. Discussion

Changes in the tubules tissue structures (Figure 1) of the STZ-induced diabetic rats were identified: (1) there was fatty degeneration of the testes; (2) the germinal cells were not identified; (3) the lumen tubules was widened because of the compromised spermatogenesis. The provision of 83.3 mg/kg mangosteen pericarp powder later caused the spermatogenesis to take place in such a way that inside the tubules testes the spermatozoa cells were then identified. The provision of 0.09 mg/kg glibenclamide caused normal spermatogenesis, but there were some fatty vacuoles identified. The intraperitoneal provision of a single dose of 80 mg/kg STZ required 56 hours long with 291 mg/dl glucose level on average. The research study results of [16,17] showed that the intraperitoneal provision of 40-65 mg/kg STZ single dose caused hyperglycemia within 72 hours. The number of epithelial and germinal cells in the tubules testes experienced degeneration. The tubules testes of the STZ-induced white rats experienced an improvement in the fatty vacuoles droplets on the Leydig cells and Sertoli cells [18]. The tubules testes structures experienced disruptions and decreases in the number of germinal cells [11].

The research findings reported in [19] showed that diabetes mellitus on male caused damage in the reproduction system resulting in the decrease of androgen synthesis and Leydig cells in seminiferous tubules. Luheinizing Hormone (LH) plays a significant role in the regulation of Leydig cells due to the fact that there is insulin receptor inside Leydig cells [20]. Insulin is an important element in the regulation of reproduction organ functions [21]; insulin can improve the secretion of GnRH from the hypothalamus. The results of a research study conducted by [22] showed that the decrease in the level of insulin on experimental animals could also reduce the secretion of LH. The statement made by [23], testosterone biosynthesis is regulated by the secretion of LH and the steroidogenesis in Leydig cells is regulated by the production of cytokine and growth factor. Leptin is one of the factors directly related to the reproduction function on

insulin resistance [24–26], and leptin receptor is located in Leydig cells which can inhibit human chorionic gonadotropin (HCG) stimulation and testosterone secretion [27].

Streptozotocin as a diabetogenic compound in experimental animals is cytotoxic specific to pancreatic beta cells[28,29]. The research conducted by [29] found that STZ was a diabetogenic agent which caused necrosis and apoptosis of pancreatic beta cells so that the insulin production was decreased. Damages in pancreatic beta cells of the STZ-induced experimental animals were used as the model animals with diabetic type I [30]. Streptozotocin is alkylation agent which breaks down the DNA sequence in Langerhans cells in rats. The research findings showed that the toxic effect of STZ was mediated by nitrate oxide (NO) which was produced during the STZ metabolism [31] STZ caused apoptosis of germinal cells caused by oxidative stress [32,33]. Metabolic abnormalities of diabetes mellitus are similar to the diabetes mellitus type I in humans [34].

Used STZ on their experimental animals as the diabetogenic agent causing the decrease in the number of quantity, motility, concentration, and volume of spermatozoa [35–40]. Diabetes mellitus on STZ-induced mice caused the decrease on the quality of sperm and capacity of fertilization [41]. The research findings of [36,42,43] explained that there was an increase in the apoptosis of germinal cells of seminiferous tubules happened in the STZ-induced experimental animals.

Damages in the pancreatic beta cells caused by the induction of STZ resulted in the deficiency of insulin so that the regulation of insulin to pituitary and gonads was affected [44]. The decrease in GnRh secretion is due to the insulin deficiency which causes it to the decline of FSH and LH [35,45,46]. According to [47] the STZ-induced experimental animals experienced the decrease in the function of Leydig cells and testosterone levels due to the damage in the pancreatic beta cells.

The treatment of providing mangosteen pericarp powder did not pass through the extraction process, but the experiments used the whole part of the mangosteen pericarp (crude material). This was based on the principle of using traditional medicine. Xanthone is one of the secondary metabolites contained in the mangosteen pericarp. The compound metabolism of plants inside the body is a complex metabolism. Phytotherapy is a treatment using various combinations of compound constituents [48]. Therefore, it needs a holistic approach or studies towards the herbal medicine [49].

Mangosteen is one of the natural ingredients used as traditional medicine. The chemical compound in mangosteen is very complicated. Each chemical compound can work in synergy and is complimentary for one another in such a way that each compound can provide a good potential to lower the blood sugar level, as well as repair the spermatogenesis of the tubules. The complexity of mangosteen pericarp compounds interacts with one another so that they provide a more effective and efficient potential compared to the glibenclamide as a single compound. Tubules renal tissue structure of the white rat's treatment mangosteen pericarp powder did not experience necrosis compared to glibenclamide treatment rats. The use of a combination of medicinal plants can increase the activity in the body so that it can improve the effectiveness of herbal medicines [50]. The use of plants in medicine is much safer and has a side effect that is lower than the use of synthetic compound [51].

5. Conclusion

Mangosteen pericarp powder was found to be useful to lower the blood glucose level and to increase the process of spermatogenesis in the seminiferous tubules testes. There is a need of analysis of the interaction between mangosteen pericarp complex compounds as well as metabolism analysis of the complex compounds in the body parts so that later mangosteen pericarp can be used as an effective and efficient traditional medicine for diabetes mellitus.

References

[1] Adnyana I K, Abuzaid A S, Iskandar E Y and Kurniati N F 2016 *Int. J. Med. Res. Heal. Sci.* **5** 23
[2] Talbott S M, Morton D A and Templeman J F 2010 *J. Australas. Integr. Med. Assoc. Authors* **16** 10

[3] Machmudah S, Shiddiqi Q Y A, Kharisma A D, Widiyastuti, Wahyudiono, Kanda H, Winardi S and Goto M 2015 *J. Adv. Chem. Eng.* **5** 1
[4] Chen L G, Yang L L and Wang C C 2008 *Food Chem. Toxicol.* **46** 688
[5] Cui J, Hu W, Cai Z, Liu Y, Li S, Tao W and Xiang H 2010 *Pharmacol. Biochem. Behav.* **95** 166
[6] Gutierrez-Orozco F and Failla M L 2013 B *Nutrients* **5** 3163
[7] Mazed M A and Mazed S 2011 Nutritional Supplement for the Prevention of Cardovascular Disease, Alzheimers Disease, Dlabetes, and Regulation and Reduction of Blood Sugar and Insulin Resistance 1–29
[8] Nelli G B, K A S and Kilari E K 2013 *Syst. Biol. Reprod. Med.* 1
[9] Kurniawati M, Mahdi C and Aulanni'am A 2014 *J. Pure App. Chem. Res.* **3** 1
[10] Neill J O, Czerwiec A, Agbaje I, Glenn J, Stitt A, Mcclure N and Mallidis C 2010 *Int. J. Androl.* **33** 709
[11] Guneli E, Tugyan K, Ozturk H, Gumustekin M, Cilaker S and Uysal N 2008 *Eur. Surg. Res.* **40** 354
[12] Yang Z, Chen M, Fialkow L B, Ellett J D, Wu R and Nadler J L 2003 *Pancreas* **26** 99
[13] Howard D W, Lewis E J, Keller B J and Cecelia S. Smith 2004 *Histological techniques for marine bivalve mollusks and crustaceans* (Oxford)
[14] Cui L, Zhou Q F, Liao C Y, Fu J J and Jiang G Bin 2009 *Arch. Environ. Contam. Toxicol.* **56** 338
[15] Kumar G l. and Kiernan J A 2010 *Education Guide: Special Stains and H&E* (California: Dako North America)
[16] Gojo A, Utsunomiya K, Taniguchi K, Yokota T, Ishizawa S, Kanazawa Y, Kurata H and Tajima N 2007 *Eur. J. Pharmacol.* **568** 242
[17] Al-Qattan K, Thomson M and Ali M 2008 *E. Spen. Eur. E. J. Clin. Nutr. Metab.* **3** e62
[18] Hassan G and Abdel Moneium T 2011 *Suez Canal Univ Med J* **4** 17
[19] Foglia V, Rosner J, Cattaneo de Peralta Ramos M and Lema B 1969 *Horm. Metab. Res.* **1** 72
[20] Ballester J, Domínguez J, Muñoz M C, Sensat M, Rigau T, Guinovart J J and Rodríguez-Gil J E 2005 *J. Androl.* **26** 706
[21] Bruning J C, Gautham D, Burks D J, Gillete J, Schubert M, Orban P C, Klein R, Krone W, Muller-Wieland D and Khan C R 2000 *Science (80-.).* **289** 2122
[22] Benitez A and Diaz J 1985 *Horm. Metab. Res.* **17** 5
[23] Saez J M 1994 *Endocr. Rev.* **15** 574
[24] Chehab F F, Lim M E and Lu R 1996 *Nat. Genet.* **12** 318
[25] Farooqi I S A F, Jebb S A, Angmack G L, Awrence E L, Heetham C H C, Rentice A N M P, Hughes I A, Camish M A M and Ahilly S T O 2010 *New Engl. J. Med. 100* **341** 879
[26] Chan J L, Heist K, DePaoli A M, Veldhuis J D and Mantzoros C S 2003. *J. Clin. Invest.* **111** 140
[27] Caprio M, Isidori A M, Carta A R, Moretti C, Dufau M L and Fabbri A 1999 *Endocrinology* **140** 4939
[28] Rossini A A, Like A A, Chick W L, Appel M C and Cahill G F 1977 *Proc. Natl. Acad. Sci. U. S. A.* **74** 2485
[29] Gezginci-Oktayoglu S and Bolkent S 2009 *Biochem. Cell Biol.* **87** 641
[30] Steger R W and Rabe M B 1997 *Exp. Biol. Med.* **214** 1
[31] Bellmann K, Wenz A, Radons J, Burkart V, Kleemann R and Kolb H 1995 *J. Clin. Invest.* **95** 2840
[32] Allan D J, Harmon B V. and Roberts S A 1992 *Cell Prolif.* **25** 241
[33] Reiter R J, Tan D X, Osuna C and Gitto E 2000 *J. Biomed. Sci.* **7** 444
[34] Mordes P and Rossini A . 1985 *Animal Model of Diabetes Mellitus, Joslin's Diabetes Mellitus* (Philadelphia: Lea&Ferbiger)
[35] Seethalakshmi L, Menon M and Diamond D 1987 *J. Urol.* **138** 190
[36] Oksanen A 1975 *Horm. Res.* **6** 138
[37] Sexton W J and Jarow J P 1997 *Urology* **49** 508

[38] Sainio-Pollanen S, Henriksen K, Parvinen M, Simell O and Pollanen P 1997 *Int. J. Androl.* **20** 243
[39] Gordon Baker H W 1998 *Endocrinol. Metab. Clin. North Am.* **27** 831
[40] Meyer K, Deutscher J, Anil M, Berthold A, Bartsch M and Kiess W 2000 *J. Endocrinol. Invest.* **23** 362
[41] Kim S T and Moley K H 2008 *Reproduction* **136** 313
[42] Cai L, Hales B F and Robaire2 B 1997 *Induction of Apoptosis in the Germ Cells of Adult Male Rats after Exposure to Cyclophosphamide'* vol 56
[43] Cai L, Chen S, Evans T, Deng D X, Mukherjee K and Chakrabarti S 2000 *Urol. Res.* **28** 342
[44] Adashi E Y, Hsueh A J W and Yen S S C 1981 *Endocrinology* **108** 1441
[45] Kirchick H J and Keyes P L 1979 *Endocrinology* **105** 1343
[46] Bestetti G, Locatelli V, Tirone F, Rossi G L and Müller E E 1985 *Endocrinology* **117** 208
[47] La Vignera S, Condorelli R, Vicari E, D'Agata R and Calogero A E 2012 *J. Androl.* **33** 145
[48] Efferth T and Koch E Complex Interactions between Phytochemicals. The Multi-Target Therapeutic Concept of Phytotherapy
[49] Verpoorte R, Choi Y H and Kim H K 2005 *J. Ethnopharmacol.* **100** 53
[50] Rasoanaivo P, Wright C W, Willcox M L and Gilbert B 2011 *Malar. J.* **10** S4
[51] Vickers A, Zollman C and Lee R 2001 Herbal medicine. *West. J. Med.* **175** 125–8

Potency of mangosteen (Garcinia mangostana L.) pericarp on seminiferous tubules testes streptozotocin-induced diabetic rats

ORIGINALITY REPORT

9%	7%	8%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	eprints.uthm.edu.my Internet Source	3%
2	dspace.tul.cz Internet Source	2%
3	www.slideshare.net Internet Source	1%
4	Muhammad Taher, Tg Muhamad Faris Syafiq Tg Zakaria, Deny Susanti, Zainul Amiruddin Zakaria. "Hypoglycaemic activity of ethanolic extract of Garcinia mangostana Linn. in normoglycaemic and streptozotocin-induced diabetic rats", BMC Complementary and Alternative Medicine, 2016 Publication	1%
5	Saber Barsiah, Morteza Behnam-Rassouli, Fahimeh Shahabipour, Sareh Rostami et al. "Evaluation of testis hormonal and histopathological alterations in type I and type II diabetic rats", Journal of Cellular Biochemistry,	1%

2019
Publication

6	Submitted to Universitas Brawijaya Student Paper	<1%
7	drugtargeting.skku.edu Internet Source	<1%
8	gjournals.org Internet Source	<1%
9	Weitao Cong, Dandan Ruan, Yuanhu Xuan, Chao Niu, Youli Tao, Yang Wang, Kungao Zhan, Lu Cai, Litai Jin, Yi Tan. "Cardiac-specific overexpression of catalase prevents diabetes-induced pathological changes by inhibiting NF-κB signaling activation in the heart", Journal of Molecular and Cellular Cardiology, 2015 Publication	<1%
10	openarchive.ki.se Internet Source	<1%
11	89.45.199.148 Internet Source	<1%

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	On		