Print ISSN 0975-4261
Online ISSN 0975-6892

Volume 13 • Number 3 • September 2021

Medicinal Plants

International Journal of Phytomedicines and Related Industries

A Publication of
National Medicinal Plants Board
New Delhi

In collaboration with

Society for Conservation and Resource Development of Medicinal Plants
www.medplantsociety.com

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Medicinal Plants International Journal of Phytomedicines and Related Industries

Publisher: Society For Conservation And Resource Development Of Medicinal Plants

Print ISSN: 0975-4261

Online ISSN: 0975-6892

Number of issues per year: 4

Print frequency: Quarterly

Month(s) of publication: March, June, September and December

Description:

Society for Conservation and Resource Development of Medicinal Plants (SMP) has been functioning with following aims and objectives of:

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Indexed/Abstracted - UGC CARE(Group II), SCOPUS, EMBASE, Index Copernicus International(2016, ICV- 106.50), NAAS Rating for 2017 - 5.12, MIAR(ICDS - 7.4), Indian Citation Index, Scientific Journal Impact Factor(SJIF - 3.484), InfoBase Index (IB Factor - 3.1), Indian Science Abstract, MAPA, Google Scholar, CAB Abstract, J-Gate, EBSCO Discovery, Summon(ProQuest), Primo and Primo Central, Agricola, Indian Science, Cite Factor, DRJI, ISRA-JIF, I2OR, ESJI, EZB and IIJIF.

Subscription Rates (USD) for the year : 350.00 2022

Medicinal Plants

An International Journal of Phytomedicines and Related Industries
Published by : Society for Conservation and Resource Development of Medicinal Plants (SMP) New Delhi

NAAS rating (2021) : 5.14
UGC-CARE Approved

Indexed in: SCOPUS, CNKI Scholar, EBSCO Discovery, Summon Proquest, Primo and Primo Central, J-Gate, ICI, NAAS 2021 (5.14), Elsevier, Index Copernicus (2017) (ICV - 106.50 (2016), EMBASE, CABI, INFOBASE INDEX (IB Factor 2017 - 3.1), Indian Science, Cite Factor, I2OR, ESJI, DRJI, ISRA-JIF, EZB, IIJIF, MIAR (MIAR (ICDS - 3.5)

Print ISSN 0975-4261
Online ISSN 0975-6892

Volume 13 | Number 3 | September 2021

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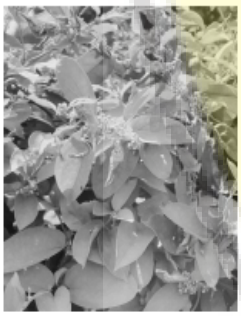

Gymnema sylvestre, a lactiferous perennial climber belonging to family Apocynaceae, is commonly known as gurmar or madhunashini (literal meaning—sweet destroyer) in India. Since ancient times, *G. sylvestre* has been used to treat diabetes and currently it is one of the major botanicals being used for control of diabetes. Leaves of this plant have also been used to treat the patients suffering from the honey urine disorder, treatment of bronchial asthma, cough, leprosy, skin diseases, wounds etc. in the Indian Systems of Medicine.

(Photo courtesy: Dr. Satyanshu Kumar, ICAR-DMAPR, Anand)
(Industrial Crops and Products, 2015, 77:901-909)

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Print ISSN 0975-4261
Online ISSN 0975-6892

Volume 13 | Number 3 | September 2021

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Medicinal Plants
Vol. 13 (3), September 2021, 493-498

doi : 10.5958/0975-6892.2021.00057.5

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Research Article

Effects of *Passiflora edulis* var. flavicarpa on liver functional parameters in alloxan induced diabetic mice

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Received: April 07, 2021; Accepted: July 30, 2021

ABSTRACT

Uncontrolled diabetes mellitus type 2 can lead to severe impairment to organs such as the heart, blood vessels, eyes, kidneys, and nerves which caused complications such as cardiovascular disease. Passion fruit (*Passiflora edulis*) is rich in phytochemicals, including flavonoids, triterpenoids, and carotenoids. The fruit of this plant has many pharmacological activities. One of the varieties was *P. edulis* var. *flavicarpa* showed antioxidant activity in vitro and antidiabetic *in vivo* in its leaves and stems. The benefits of this fruit in liver disorders associated with diabetes have not been inspected. This research was planned to study the effects of *P. edulis* var. *flavicarpa* on liver functional parameters and oxidative stress level in alloxan-induced diabetic mice. Six groups of Balb-C strain male mice consist of normal group, diabetic group, metformin group, and yellow passion fruit group with dose 40, 50, and 60 mL/kg were treated for 14 days. The diabetic effect was induced by alloxan on all groups except normal. On the 1st and 15th day of the treatment, the aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, and triglyceride levels were measured. The malondialdehyde (MDA) level as oxidative stress parameter was measured only on the 15th day. This study showed that the *P. edulis* var. *flavicarpa* decreased the AST, ALT, total cholesterol, and triglyceride levels. The MDA level of the *P. edulis* var. *flavicarpa* treatment groups were significantly different from the diabetic group. The best activity was achieved at dose of 60 mL/kg. Our results suggests the *P. edulis* var. *flavicarpa* fruit improved liver functional and oxidative stress parameters in alloxan-induced diabetic mice.

Keywords: Passion fruit, ALT, AST, cholesterol, diabetes mellitus, triglyceride, oxidative stress

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia and impaired carbohydrate, protein, and fat metabolism (DiPiro *et al.*, 2016). It will lead to severe impairment to organs such as the heart, blood vessels, eyes, kidneys, and nerves, and caused complications such as cardiovascular disease (WHO, 2016). The International Diabetes Federation (IDF) data for 2019 stated that around 463 million people worldwide are diagnosed with diabetes and are expected to become 700 million in 2045. Meanwhile, in South East Asia, as of 2019, people with

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diabetes are estimated around 163 million and projected to reach 153 million by 2045 (International Diabetes Federation, 2019).

Type 2 diabetes is the most common diabetes caused by inadequate insulin production or insulin resistance (WHO, 2016). Insulin resistance encourages triglycerides (TGs) production in the liver, thus increase the serum lipid level and forming non-alcoholic fatty liver disease (NAFLD) (Matsuzaka, 2020). The elevated level of these lipids causes diabetic dyslipidemia, which can be identified by increasing very-low-density lipoprotein cholesterol (VLDL) and small dense low-density lipoprotein (LDL) cholesterol, and low concentrations of high-density lipoprotein (HDL) cholesterol (Katz and Barrett, 2019). Dyslipidemia with increased TGs and non-HDL-C levels with a declined HDL-C level is a cardiovascular disease risk factor in type 2 diabetes patients (Matsuzaka, 2020). Non-alcoholic fatty liver disease can develop into severe liver fibrosis and progress to the stage of liver cirrhosis. The damage of the liver can be detected through enzyme liver function tests, such as aspartate transaminase (AST) and alanine transaminase (ALT) levels in the blood (Singh *et al.*, 2014).

Passion fruit (*P. edulis*) has a wide range of biological activities such as antioxidant, hepatoprotective, anti-cholesterol, antihypertensive, antitumor, and antidiabetic (He *et al.*, 2020; Zas and John, 2016) There are several varieties of *Passiflora edulis*, namely *P. edulis* Sims, *P. edulis* f. *edulis*, *P. edulis* f. *flavicarpa* O. Deg., *P. edulis* var. *kerii* (Spreng.) Mast., *P. edulis* var. *pomifera* (M. Roem.) Mast., *P. edulis* var. *rubricaulis* (Jacq.) Mast., and *P. edulis* var. *verrucifera* (Lindl.) Mast (He *et al.*, 2020). There are more than 100 phytochemicals that has been identified from this fruit including flavonoids, triterpenoids, and carotenoids. *P edulis* also contain several nutrients such as carbohydrates, lipids, carboxylic acids, polyphenols, volatile compounds, protein and amino acids, vitamins, and minerals (dos Reis *et al.*, 2018; He *et al.*, 2020).

Several studies showed the benefit of *P. edulis* in diabetes mellitus. Barbalho *et al.* (2011) suggested *P. edulis* had beneficial effects for dyslipidemia and hyperglycemia in diabetic Wistar rat offspring. Kandandapani *et al.* (2015) showed the antidiabetic and antioxidant potential of *P edulis* against streptozotocin-induced diabetes. Extract of *Passiflora edulis* var *flavicarpa* (PEF) leaves and stems revealed its biologic activity as an antioxidant, antihyperglycemic, and antihyperlipidemic (Panchanathan and Rajendran, 2015). The potential effect of PEF as hepatoprotector in diabetes have not been investigated. Meanwhile, the complications of diabetes mellitus can lead to liver damage. Thus, the aim of this study was to examine the effects of PEF on liver functional parameters and oxidative stress level in alloxan-induced diabetic mice.

MATERIALS AND METHODS

Animals

Animals used in this study were 24 male Balb/C strain mice, weighing 20-30 grams and 2-3 months old. The experimental procedure performed on animals had been approved by the Ethical Committee of Faculty of Dentistry Jember University (491/UN25.8/KEPK/DL/2019).

Materials

Alloxan and reagents for Malondialdehyde (MDA) analysis (Trichloroacetic acid (TCA), HCl, and Na- Thiobarbituric acid (TBA) were purchased from Sigma Aldrich. Reagents for blood biochemical analysis (cholesterol total, triglyceride, AST, ALT) was procured from Analyticon Biotechnologies.

Plant material

The yellow passion fruit from Kediri, East Java, Indonesia was collected at the Laboratory of Plants, Jember Polytechnic State, East Java, Indonesia, and taxonomically identified as *Passiflora edulis* var. flavicarpa (11/PL17.3.1.02/LL/2019). The fresh and ripe yellow passion fruit split into two parts, and the fruit flesh was taken out and then filtered. This filtering was aimed to separate the seeds so that fresh PEF juice (PEFJ) is obtained.

Diabetes induction

Mice were injected intra-peritoneal with alloxan at a dose of 210 mg/kg. After three days, the blood levels of the mice were measured and classified as diabetic if their blood glucose levels > 200 mg/dL.

Experimental design

Mice were randomly divided into six groups, with each group consisting of four mice, as listed below:

Group 1: normal group, mice were not induced with alloxan and given water for 14 days.

Group 2: diabetic group, mice were induced with alloxan and given water for 14 days.

Group 3: Metformin group, mice were induced with alloxan and given metformin (110,5 mg/kg) for 14 days.

Group 4, 5, 6: treatment group with PEFJ dose 40, 50, and 60 ml/kg respectively, mice were induced with alloxan and given PEFJ for 14 days.

Blood analysis

Animals were sliced and blood was taken twice during the study. The first sample was taken after the animals were considered diabetics and called pre-test data, and the second one was taken after treatment for 14 days (on the 15th day) and stated as post-test data. The blood samples were centrifuged at 3000 rpm for 10 minutes, and blood plasma was taken. The plasma was used to measure biochemical parameters such as total cholesterol and triglyceride, AST, ALT, and oxidative stress parameter MDA. The examination for blood analysis for total cholesterol, and triglyceride, AST, ALT was operated according to the reagents manufacture's instruction using photometer (Biolyzer 100). The percentage decrease of the biochemical parameters calculated as:

$$\% \text{ decrease} = \frac{\text{Pre test data-post test data}}{\text{Pre test data}} \times 100\%$$

MDA assay

The MDA level was measured with UV-Vis spectrophotometer and only quantified the post-test data. 50 µL serum sample added with 1 mL aquadest, 100 µL TCA 20%, 250 µL HCl 1 N dan 100 µL Na-TBA 1% and then mixed with vortex. The mixture was incubated in a water-bath at 100ºC for 30 minutes and then cooled down in room temperature. The solution was centrifugated at 3500 rpms for 10 minutes, and the supernatant was taken for measurement of its absorbance in UV-Vis spectrophotometer at 532 nm.

RESULT

The results of the effect of PEFJ on liver functional parameters are shown in Table 1 for lipid profile (total cholesterol and triglyceride) and Table 2 for liver enzymes (AST and ALT). The normal group and the diabetic group showed elevated both for total cholesterol and triglyceride levels compared to the normal group (Table 1). Meanwhile, the treatment groups decreased these parameters in all the groups, including the Metformin group and treatment groups with PEFJ after given for 14 days (Table 1).

The hepatoprotective effect showed by enzymatic liver parameters AST and ALT. All the Alloxan-induced groups had high AST and ALT levels in pre-test data. After 14 days of treatment, the treated groups indicated declining levels of both AST and ALT in groups with Metformin and PEFJ in all doses (Table 2).

The percentage decrease in all parameters calculated and listed in Table 3. The dose of 60 ml/kg of PEFJ gave the best result in lowering lipid effect and liver function parameters improvement as compared to the other given dose. But it was not as effective as Metformin groups.

The oxidative stress and antioxidant activity were measured by lipid peroxidation product MDA after 14 days

Table 1: *Pre* and *Post-test* Parameters of Total Cholesterol, and Triglyceride

Groups	Total cholesterol levels (mean ± SD) (mg / dL)		Triglyceride levels (mean± SD) (mg / dL)	
	Pre test	Post test	Pre test	Post test
Normal	81,60±0,52	81,88±0,48	95,59±3,82	96,18±4,03
Diabetic	106,03±1,45	194,48±2,41	143,21±9,99	190,81±8,99
Metformin	185,90±8,16	62,48±1,69	211,55±82,74	101,16±38,79
P. edulis var. flavicarpa juice 40 ml/kg	140,65±0,19	102,90±0,88	189,21±80,27	171,46±72,87
P. edulis var. flavicarpa juice 50 ml/kg	133,86±2,19	91,47±1,24	242,29±6,75	203,83±5,32
P. edulis var. flavicarpa juice 60 ml/kg	127,91±0,83	86,11±1,14	203,08±81,01	105,53±41,65

Table 2: *Pre* dan *Post-test* Parameter of AST and ALT Levels

Groups	AST levels (mean ± SD) (mg / dL)		ALT levels (mean ± SD) (mg/dL)	
	Pre test	Post test	Pre test	Post test
Normal	100,12±34,66	57,45±6,79	20,32±6,43	12,85±1,25
Diabetic	289,52±26,47	342,40±22,73	62,52±12,45	76,83±16,34
Metformin	348,20±91,47	89,55±17,84	70,13±12,49	19,06±3,47
P. edulis var. flavicarpa juice 40 ml/kg	299,97±43,00	176,39±18,89	60,35±9,38	39,59±8,33
P. edulis var. flavicarpa juice 50 ml/kg	285,24±29,71	110,73±8,77	64,83±6,94	28,26±2,31
P. edulis var. flavicarpa juice 60 ml/kg	294,71±46,31	95,33±14,10	68,84±11,02	24,12±4,62

Table 3: The percentage decrease of liver function parameters in Alloxan-induced groups

Treatment	Lipid profile (mean±SD)		Enzymatic liver function (mean±SD)	
	Total cholesterol levels	Triglyceride levels	AST	ALT
Diabetic	-83,43 ± 0,37^{a}	-33,41 ± 3,40^{a}	-18,49 ± 3,61^{a}	-22,67 ± 2,80^{a}
Metformin	66,37 ± 0,82^{b}	52,06 ± 0,99^{b}	73,92 ± 2,42^{b}	72,77 ± 1,56^{b}
P. edulis var. flavicarpa juice 40 ml/kg	26,84 ± 0,56^{c}	9,38 ± 0,91^{c}	40,93 ± 3,82^{c}	34,82 ± 3,58^{c}
P. edulis var. flavicarpa juice 50 ml/kg	31,66 ± 0,37^{d}	15,86 ± 1,16^{d}	61,08 ± 1,91^{d}	56,29 ± 2,14^{d}
P. edulis var. flavicarpa juice 60 ml/kg	32,68 ± 0,47^{e}	48,00 ±1,06^{e}	67,60 ± 1,24^{e}	65,03 ± 2,63^{e}

Data are shown in mean ± SD with n = 4. The same superscript letters indicate that there was no significant difference between treatments based on the LSD test ($p < 0.05$).

of treatment. The diabetic group gave the highest MDA levels compared to other groups (Figure 1). The treatment with PEFJ dose 60 mg/kg had no significant effect than Metformin groups. This result indicated PEFJ gave a strong antioxidant effect in diabetic mice.

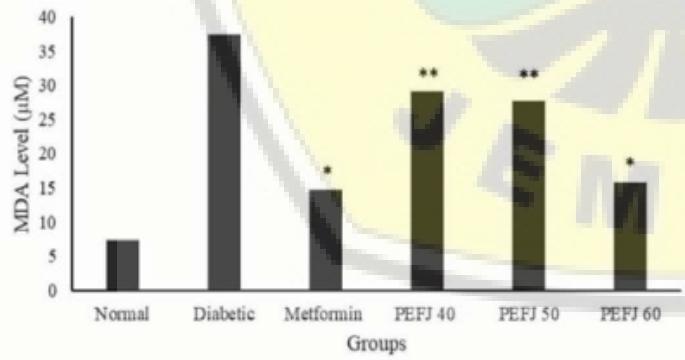

Figure 1: Effect of groups treatment on MDA plasma level (µM) after 14 days. The results are expressed as mean ± SEM
PEFJ: *P. edulis* var. flavicarpa juice. PEFJ 40: dose 40 ml/kg, PEFJ 50: dose 50 ml/kg, PEFJ 60: dose 60 ml/kg. The same superscript indicated that there was no significant difference between treatments based on the LSD test ($p < 0.05$).

DISCUSSION

This study demonstrated the effects of *P. edulis* var. flavicarpa on liver functional parameters and oxidative stress level in alloxan-induced diabetic mice. The liver plays an important function in glucose metabolism. Elevated liver enzymes, non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma (HCC), and acute liver failure are the disease associated with liver in type 2 diabetes (Al-Jameil *et al.*, 2014). Insulin resistance in diabetes is connected to fatty liver and the development of NAFLD (Rajeswari *et al.*, 2014). Dyslipidemia is one of the metabolic syndromes caused by diabetes mellitus. Diabetic dyslipidemia is led by the malfunction of lipoprotein lipase (LPL) in the endothelial cells. This condition manifests in elevated lipid profile, oxidative stress markers, and inflammatory mediators in patients with NAFLD and normal liver function tests (Habte *et al.*, 2020; Rajeswari *et al.*, 2014).

Alloxan generated diabetes by caused partial degradation of pancreatic â cells by forming reactive oxygen species (ROS) and superoxide radicals that affect

the quality and quantity of insulin production (Ighodaro *et al.*, 2017). ROS and superoxide radicals were formed due to the reduction reaction of alloxan that causes the depolarization of the pancreatic β cell membrane and increasing level of Ca^{2+}, thus accelerating the pancreatic β cells damage that reduces the insulin production (Rohilla and Ali, 2012). High levels of ROS can cause direct damage to lipid by lipid peroxidation, thus generates secondary lipid peroxidation products, which is Malondialdehyde (MDA) (Ayala *et al.*, 2014).

Passiflora species are rich in pectin, minerals, carotenoids, vitamin C, and flavonoids (Barbalho *et al.*, 2011). *P. edulis* is known to have antioxidant content such as vitamin C and flavonoids (quercetin, kaempferol, and β-carotene) (dos Reis *et al.*, 2018). Flavonoids acted as an antioxidant by modulating oxidative stress because it neutralizes the effect of nitrogen and oxygen species. Quercetin acted as antidiabetic by reducing lipid peroxidation, inhibiting insulin-dependent activation of phosphoinositide 3-kinases (PI3K), and glucose absorption by GLUT2 (Al-Ishaq *at el.*, 2019). Flavonoids have also been proven as an hepatoprotector by giving hepatocytes protection from free radical scavenging activity (Nerdy and Ritarwan, 2019). Flavonoids also had a mechanism to reduce hyperlipidemia by decreasing PPAR-γ (peroxisome proliferative activated receptor), FABP4 (fatty acid-binding protein 4), and CEBP/α (CCAAT enhancer-binding protein alpha), which reduce adipogenesis (Varshney *et al.*, 2019).

Several studies suggest that *P. edulis* species juices give hepatoprotective effect and hypolipidemic activity. The *P. edulis* fruit juice given to mice with ethanol-induced liver injury for 15 days could decrease AST and ALT in the liver, relieve inflammation, and oxidative stress so the liver can be protected from injury (Zhang *et al.*, 2016). Barbalho *et al*. (2011) reported treatment with passion fruit juice for 30 days in diabetic Wistar rat offspring could improve lipid profiles. This study suggested that *P. edulis* may have beneficial effects in the prevention and treatment of hyperglycemia and dyslipidemia (Barbalho *et al.*, 2011).

Our study showed that the *P edulis* var. flavicarpa had benefits in protecting the liver in diabetes by decreasing liver enzymes and in restraining cardiovascular risk in diabetes with dyslipidemia by lowering cholesterol total and triglyceride. The mechanism might involve in the activities was by reducing oxidative stress level. The MDA level of the diabetic mice treated with the *P edulis* var. flavicarpa was lower than the diabetic group. This finding suggested that the antioxidant activity potential of this fruit which can play an important role both in hepatoprotective and anti-dyslipidemia activity.

CONCLUSION

The result of the study concluded that yellow passion fruit had a potential hepatoprotective and anti-dyslipidemia activity against diabetes mellitus. The mechanism might involve the antioxidant potential by its phytochemicals content such as vitamin C and flavonoids and needs further exploration for more clinical uses in human beings.

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