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Original Research Article

Ameliorative Effect of Pigeon Pea Leaves and Ginger Extract on Oxidative Stress Condition in Kidney and Liver of Experimental Diabetic Rats

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ABSTRACT

Pigeon pea (*Cajanus cajan*) leaves and ginger (*Zingiber officinale*) are known to have strong antioxidant activity both *in vitro* and *in vivo*, but information on the effectiveness of these plants in reducing oxidative stress in diabetes mellitus (DM) is limited. Therefore, this study aimed to analyze the oxidative stress condition in kidney and liver of experimental DM rats treated with the extract of pigeon pea leaves and ginger. The extract was analyzed for polyphenol profile using Acquity Ultra Performance Liquid Chromatography instrument (UPLC) equipped with tandem micro mass. *In vivo* experiment was conducted with 25 male *Sprague Dawley* rats, randomly allocated into five groups: negative control (NC), DM rats as positive control (PC), DM treated with glibenclamide (G), and two DM groups treated with different doses of pigeon pea leaves and ginger extract (A and B). DM condition was achieved using 110 mg/kg BW alloxan-induction and all treatments were conducted for 28 days. The oxidative stress marker, malondialdehyde (MDA), and antioxidant-superoxide dismutase (SOD) were analyzed using a spectrophotometer and Cu, Zn-SOD with the immunohistochemical technique. In addition, the blood glucose level was analyzed. The results showed that pigeon pea leaves and ginger extract increased SOD activity and Cu,Zn-SOD content but reduced MDA levels in kidney and liver of DM rats. The treatment also lowered blood glucose levels, hence, it was concluded that pigeon pea leaves and ginger extract ameliorated oxidative stress in kidney and liver of DM rats.

Keywords: kidney; liver; malondialdehyde, pigeon pea, superoxide dismutase

Introduction

Diabetes mellitus (DM) is a metabolic disorder known to affect the processing of carbohydrates, proteins, and fats, leading to high blood glucose levels (hyperglycemia). In DM sufferers, hyperglycemia is caused by a lack of insulin secretion or decreased sensitivity.[1] This condition leads to elevated production of free radicals (ROS, RNS, RCS) through several pathways including increased glycolysis, activation of the sorbitol or polyol pathway, advanced glycation end products (AGEs) formation, autooxidation of glucose, glycation of non-enzymatic protein, hexosamine pathway, NADPH oxidase, lipid oxidation, and others.[2,3]

The high production of free radicals leads to oxidative stress, and prolonged expression can worsen the patient's condition, potentially resulting in complications, namely microvascular issues including neuropathy, cataracts, and retinopathy, as well as macrovascular problems in the form of stroke and heart failure.[4] Therefore, it is important to ameliorate oxidative stress conditions in DM patients.

Previous reports stated that methanolic and 96% ethanolic extract of pigeon pea (*Cajanus cajan*) leaves demonstrated hypoglycemic activity.[5,6] Furthermore, aqueous and methanolic extracts of ginger (*Zingiber officinale*) were reported to show antioxidant and hypoglycemic activities.[7,8]

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In a previous study, ethanolic extract of pigeon pea leaves with ginger var. amarum showed antioxidant, alpha-glucosidase inhibitory, and hypoglycemic activities.[6] A repeated 28-day oral toxicity study demonstrated that there were no toxic effects detected in rats administered with pigeon pea leaves and ginger extract.[9] Using oral glucose tolerant test (OGTT), the combination of ethanolic pigeon pea leaves and ginger extract caused a greater hypoglycemic effect compared to single extract administration.[6] However, no studies combined pigeon pea leaves and ginger extract to treat oxidative stress in DM rats. This study aimed to analyze the oxidative stress condition in kidney and liver of DM rats administered with a combination of pigeon pea leaves and ginger extract using malondialdehyde (MDA) and antioxidant-superoxide dismutase (SOD) as the main biomarker. The level of blood glucose and the polyphenol profile were also analyzed to support the mechanism of action for the combined extract.

Materials and Methods

Chemicals and materials

The chemicals used include alloxan (Sigma-Aldrich, USA), Cu,Zn-SOD antibody (Sigma-Aldrich, S2147), Starr Trek Universal Detection System (Biocare, USA), Accu-Check strips, and glucometer (Roche, Germany), xylol (Merck, Germany), paraffin (Thermo Scientific, USA), MDA reagent, and SOD reagent.

The materials used in this study comprised pigeon pea leaves sourced from Lombok City, West Nusa Tenggara, Indonesia (-8.66090 LS, 116.25066 BT; 166 mdpl), while Ginger var. amarum was collected from Solo City, Central Java (-7.93832 LS, 111.24429 BT; 624 mdpl), in July 2022. These plant specimens were identified and subsequently stored at the Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia. The assigned voucher specimen numbers were BMK0515072022 for pigeon pea and BMK0388012018 for ginger.

Extraction of pigeon pea leaves and ginger

Pigeon pea leaves and ginger rhizome were dried in the oven at 45°C for 2 days and one night followed by grinding and filtering separately at a size of 80 mesh. Extraction of powdered two samples was conducted using 96% ethanol and maceration methods, separately, as described by Wresdiyati *et al.*[6,10] Subsequently, the two types of extract were evaporated separately to obtain dry samples.

Identification of Polyphenol Components with UPLC-QTOF-MS/MS

This study identified polyphenols with antidiabetic effectivity, including flavonoids and phenolic acids. The Analysis methods, as described by Alfarisi *et al.*[11], use an Acquity Ultra Performance Liquid Chromatography instrument (UPLC) equipped with a tandem micro mass (MS/MS). Data from UPLC-QTOF-MS/MS (Waters, USA) was then processed into Mass-Lynx V.4.1 application. Detection of spectral fragments (flavonoids and phenolic acids) matched with database spectra available in the Human Metabolome Database (http://www.hmbd.ca) and Mass Bank (http://www.massbank.eu).

Animal Management and Experimental Design

This study used 8-week-old male *Sprague-Dawley* rats obtained from the National Agency for Drug and Food Control of Indonesia. Adaptation was conducted for 7 days by feeding with standard feed,[12] alongside vitamins, antibiotics, anti-parasite, and drinking water *ad libitum.* In addition, standard conditions were maintained at 22-24°C, 50-63% humidity, and a 12h bright light-dark cycle. A total of 25 male rats were allocated into five groups: negative control/normal rats (NC), positive control (PC), DM rats given glibenclamide (G), DM treated with pigeon pea leaves at 300 mg/kg BW and ginger at 60 mg/kg BW extracts (A), as well as DM rats given pigeon pea leaves at 300 mg/kg BW and ginger at 125 mg/kg BW extracts orally (B). DM condition was achieved by alloxan induction (110 mg/kg BW).[13]

The treatment was conducted for 28 days and feed consumption was measured every day. The level of blood glucose was measured every 4 days using a glucometer and Accu-Check strips. Bodyweight was also assessed every 4 days. Experimental procedures and care for rats followed the Ethical Approval Letter from the Animal Ethic Committee School of Veterinary Medicine and Biomedical Science, IPB University, number 040/KEH/SKE/XI/2022.

Sampling kidney and liver organs

Rats were anesthetized with ketamine (70 mg/kg BW) and xylazine (10 mg/kg BW), intraperitoneally. Kidney and liver tissues were collected and homogenated separately to collect lysate, which was subsequently analyzed for the level of MDA and SOD activity using a spectrophotometer according to the method of Alfarisi *et al.*[14]

Another part of kidney and liver tissues were fixated using Bouin solution, then the tissues were processed with paraffin standard method. The content of cooper, zinc-superoxide dismutase (Cu,Zn-SOD) was immunohistochemically analyzed using Cu,Zn-SOD-monoclonal antibody (Sigma-Aldrich, US, S2147). The positive reaction in the renal tubule cells and hepatocytes was indicated with a brown color.[13]

Data analysis

The data on blood glucose level and body weight were qualitatively analyzed, while evaluation of Cu,Zn-SOD content in kidney and liver used a light microscope (Olympus, CH20 Japan). The micrograph of Cu,Zn-SOD content was documented with a camera microscope (Olympus, CX31, Japan, and CCD10 USB Camera). The positive reaction cells in different levels of Cu,Zn-SOD were graded to be four classes namely strong (+++), moderate (++), and weak positive reaction (+), as well as negative reaction (-). The number of positive and negative reactions of renal tubule cells and hepatocytes were counted with the ImageJ 1.54 (National Institute of Health, USA 2024) software. The total food consumption, MDA, and SOD level, as well as the cell number of renal tubules and hepatocytes across various antioxidant content levels of Cu,Zn-SOD were analyzed using the software SPSS 22 (IBM SPSS Statistics 22; 2013) with the one-way Analysis of variance (ANOVA). Duncan's analysis was subsequently used when there was a significant difference.

Results and Discussion

Polyphenol profile of pigeon pea leaves and ginger var. amarum extract

The Chromatograms (UPLC-QTOF-MS/MS) in base peak intensity (BPI) mode of 96% ethanolic pigeon pea leaves and ginger extract are shown in Figure 1. The polyphenol profile with alpha-glucosidase inhibitors and antidiabetic compounds are presented in Tables 1 and 2. Based on the results, the polyphenol profile in 96% ethanolic pigeon pea leaves extract was composed of 11 flavonoids and two phenolic acids (Table 1). Meanwhile, the 96% ethanolic ginger var. amarum extract was composed of 12 flavonoids and five phenolic acids (Table 2).

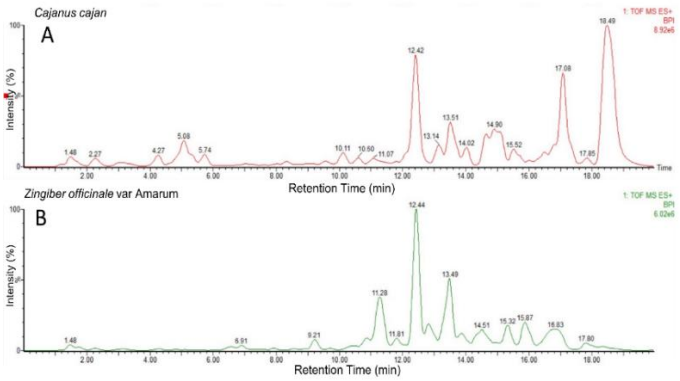

Figure 1: Chromatograms (LCMS) in base peak intensity (BPI) mode pigeon pea leaves (A) and ginger var Amarum (B) extract at low energy (4 volts) with positive electrospray ionization.

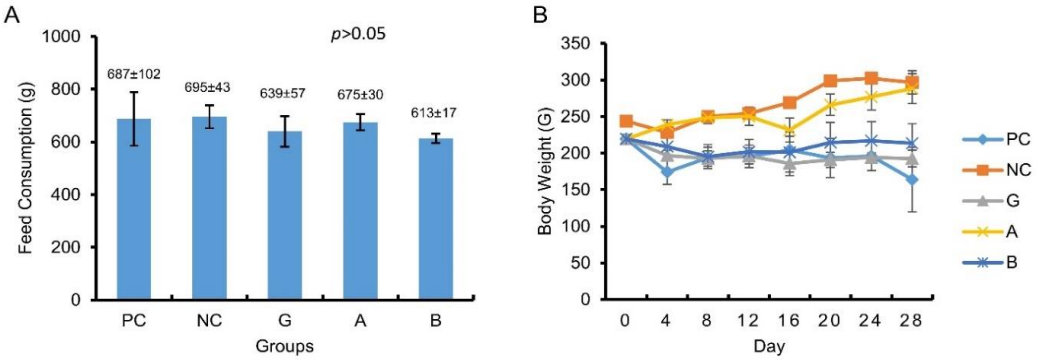

Figure 2: Feed consumption (A) and the body weight growth (B) of DM rats. There is no significant difference (P>0.05) in feed consumption among the treated groups. A group showed highly increased body weight compared to other treatment groups. NC=negative control, PC=positive control, G=DM+glibenclamide, A= DM+*C. cajan* leaves (300 mg/kg BW) and ginger (60 mg/kg BW) extract, B=DM+ *C. cajan* (300 mg/kg BW) and ginger (125 mg/kg BW) extracts.

Table 1: Polyphenol profile of 96% ethanolic pigeon pea (*Cajanus cajan*) leaves extract

No	Bioactive Compounds	Other name	Molecular Formula	Compounds	Retention Time (min)	Observed (M+H)+	Daughter Ion	Reference
1	Flavonoid	Chalcones	$C_{15}H_{14}O_5$	Phloretin	2.667	275.0888	169.0519, 107.0484	MassBank
2	Flavonoid	Chalcones	$C_{21}H_{24}O_{10}$	Phloridzin	1.541	437.1406	419.1275, 275.0886	HMBD
3	Flavonoid	Flavanones	$C_{16}H_{14}O_6$	Hesperetin	10.02	303.0883	304.0925, 305.0968	MassBank
4	Flavonoid	Flavanones	$C_{15}H_{12}O_5$	Naringenin	5.169	273.0772	147.0442, 194.9356	MassBank
5	Flavonoid	Flavanones	$C_{15}H_{12}O_6$	Eriodictyol	8.06	289.0685	163.0412, 153.017	MassBank
6	Flavonoid	Flavones	$C_{20}H_{20}O_7$	Tangeretin	9.317	373.1272	343.1205, 345.0953	HMBD
7	Flavonoid	Flavones	$C_{27}H_{30}O_{14}$	Rhoifolin	5.563	579.1714	580.1744, 581.178	MassBank
8	Flavonoid	Isoflavanoid	$C_{21}H_{20}O_9$	Daidzin	5.303	417.1174	255.066, 256.0689	MassBank
9	Flavonoid	Hydroxybenzoic acids	$C_{14}H_6O_8$	Ellagic acid	1.541	303.0114	275.0167, 257.0053	HMBD
10	Flavonoid	Hydroxybenzoic acids	$C_7H_6O_5$	Gallic acid	1.583	171.0265	154.0203, 143.0365	HMBD
11	Flavonoid	Hydroxycinnamic acids	$C_{18}H_{16}O_8$	Rosmarinic acid	9.451	361.0895	343.0812, 331.0813	HMBD
12	Phenolic Acid	Hydroxycinnamic acids	$C_{11}H_{12}O_5$	Sinapic acid	7.672	225.075	209.0433, 208.0497	MassBank
13	Phenolic Acid	Hydroxycinnamic acids	$C_{22}H_{18}O_{12}$	Chicoric acid	6.596	475.0847	457.0749, 439.0668	HMBD

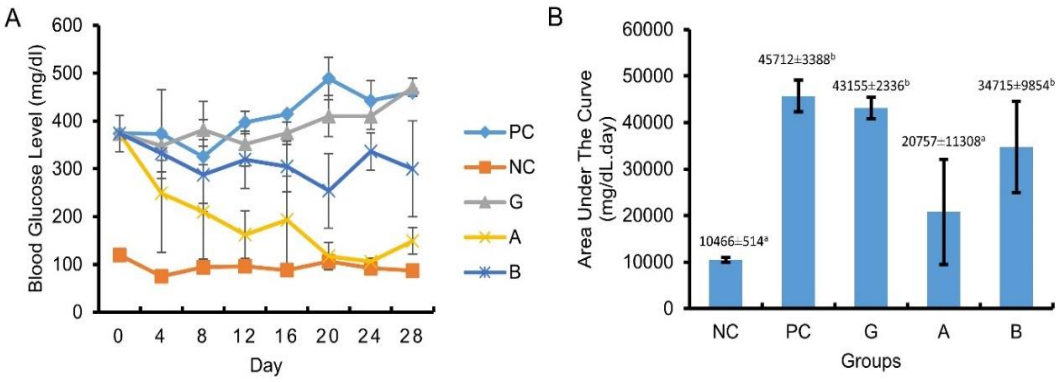

Figure 3: Blood glucose levels (A) and the area under the curve (AUC) (B) of DM rats. A group showed highly decreased blood glucose levels compared to other treatment groups. A group showed the significantly smallest AUC ($P<0.05$) among other DM treatment groups and was not significantly different ($P<0.05$) from NC group. NC=negative control, PC=positive control, G=DM+glibenclamide, A= DM+*C. cajan* leaves (300 mg/kg BW) and ginger (60 mg/kg BW) extract, B=DM+ *C. cajan* (300 mg/kg BW) and ginger (125 mg/kg BW) extracts. Bar=50 μm.

Two general classes of polyphenols are flavonoids and phenolic acids, which were both identified in the extract. Flavonoids and phenolic acids play a crucial role in ameliorating oxidative stress in DM rats. In this context, phloridzin, eriodictyol, daidzin, and chicoric acid stimulate glucose uptake by activating Akt and AMPK signaling pathways.[15–18] Ellagic acid, syringic acid, and gallic acid reduced the damage to the pancreatic organ, inhibited beta-cell apoptosis, and stimulated the regeneration of β-cells in DM rats.[19,20] Furthermore, phloridzin, sinapic acid, naringin, naringenin, tangeretin, rhoifolin, as well as ellagic, chicoric, and gallic acid were reported to stimulate insulin production, improve sensitivity, and demonstrate insulinotropic effects.[15,19,21–24] Tangeretin controls glucose metabolism and enhances glycolytic enzymes in the hepatic tissues, while apigenin increases glycogen content in the muscle and liver.[23–25]

Total feed consumption and body weight

The combination of ethanolic pigeon pea leaves and ginger extract treatments on DM rats showed that among the treatment groups, there were no significant differences ($P\geq0.05$) in the total feed consumption (Figure 2A). However, NC and A treatment groups showed the highest growth in body weight compared to others (Figure 2B). G (DM+glibenclamide) group had relatively constant growth, B group showed a slight increase, but not optimal, and PC (DM) group experienced a decrease in the growing body weight.

In DM rats where blood glucose cannot enter the cells, the body resorts to other sources of energy, such as protein and fat, through gluconeogenesis. This process continuously impacts rapid body weight loss,[15] as shown in PC group.

Blood glucose level and the area under the curve (AUC)

PC and G treatment groups showed the highest blood glucose levels and were above the normal range in hyperglycemia (>200 mg/dL) than the other groups (Figure 3A). The glucose level in B group slightly decreased and was still above the normal range. Blood glucose levels in A group decreased from the initial state of DM condition to the range of normal levels. These results implied that the combination of pigeon pea leaves at 300 mg/kg BW and ginger extract at 60 mg/kg BW had the best hypoglycemic effect. Furthermore, AUC of blood glucose levels in A group was significantly smaller ($P\leq0.05$) compared to the other DM treatment groups (PC, G, and B) almost similar to NC group (Figure 3B).

DM in rats was induced using Alloxan, a compound known for selective toxicity to pancreatic beta cells, resulting in the loss of cellular integrity, and apoptosis.[26] Consequently, PC group experienced limited insulin secretion, leading to impaired glucose entry into the cells. This condition led to elevated blood glucose levels in DM group, followed by increased ROS, RNS, and RCS production through several pathways, as reported by Yaribeygi *et al*.[2]

Table 2: Polyphenol profile of 96% ethanolic ginger (*Zingiber officinale* var. Amarum) extract

No	Bioactive Compounds	Other Names	Molecular Formula	Compounds	Retention Time (min)	Observed (M+H)+	Daughter Ion	References
1	Flavonoid	Chalcones	$C_{15}H_{14}O_5$	Phloretin	1.541	275.0919	107.0488, 169.0464	MassBank
2	Flavonoid	Chalcones	$C_{21}H_{24}O_{10}$	Phloridzin	1.541	437.1469	436.2318, 419.1284	HMBD
3	Flavonoid	Flavanones	$C_{27}H_{32}O_{14}$	Naringin	5.036	581.187	237.0763, 435.1396	MassBank
4	Flavonoid	Flavanones	$C_{15}H_{12}O_5$	Naringenin	1.872	273.0752	153.0141, 147.0458	MassBank
5	Flavonoid	Flavanones	$C_{15}H_{12}O_6$	Eriodictyol	1.52	289.0692	153.0246, 163.0401	MassBank
6	Flavonoid	Flavones	$C_{15}H_{10}O_5$	Apigenin	5.387	271.063	272,063	MassBank
7	Flavonoid	Flavones	$C_{20}H_{20}O_7$	Tangeretin	5.697	373.1259	343.1141, 345.1002	HMBD
8	Flavonoid	Flavones	$C_{27}H_{30}O_{14}$	Rhoifolin	5.521	579.1719	580.174, 581.1724	MassBank
9	Flavonoid	Isoflavanoid	$C_{15}H_{10}O_4$	Daidzein	6.379	255.0696	256,069	MassBank
10	Flavonoid	Isoflavanoid	$C_{21}H_{20}O_9$	Daidzin	5.563	417.1198	225.0703, 255.064	MassBank
11	Flavonoid	Isoflavanoid	$C_{21}H_{20}O_{10}$	Genistin	5.831	433.1148	415.0969, 401.0809	HMBD
12	Flavonoid	Isoflavanoid	$C_{16}H_{12}O_5$	Glycitein	4.776	285.0776	255.0615, 259.0587	HMBD
13	Phenolic Acid	Hydroxybenzoic acids	$C_{14}H_6O_8$	Ellagic acid	12.481	303.0129	285.0141, 275.0055	HMBD
14	Phenolic Acid	Hydroxybenzoic acids	$C_9H_{10}O_5$	Syringic acid	11.426	199.0584	183.0258, 169.0506	HMBD
15	Phenolic Acid	Hydroxycinnamic acids	$C_{18}H_{16}O_8$	Rosmarinic acid	1.499	361.0902	343.0901, 325.0746	HMBD
16	Phenolic Acid	Hydroxycinnamic acids	$C_{11}H_{12}O_5$	Sinapic acid	10.836	225.0721	209.0343, 207.0789	MassBank
17	Phenolic Acid	Hydroxycinnamic acids	$C_{22}H_{18}O_{12}$	Chicoric acid	5.831	475.0898	439.0687, 429.0876	HMBD

Table 3: The level of MDA and SOD activity in the kidney and liver of DM rats

Group	MDA Kidney (µg/ml)	MDA Liver (µg/ml)	SOD Kidney (U/ml)	SOD Liver (U/ml)
NC	1.04 ± 0.02^{a}	1.54 ± 0.02^{a}	2063.33 ± 133.33^{d}	2130.00 ± 66.67^{d}
PC	4.18 ± 0.06^{d}	5.87 ± 0.60^{d}	941.11 ± 101.84^{a}	985.56 ± 19.25^{a}
G	3.21 ± 0.57^{c}	4.14 ± 0.15^{c}	1296.67 ± 88.19^{b}	1318.89 ± 83.89^{b}
A	2.45 ± 0.02^{b}	2.99 ± 0.45^{b}	1674.44 ± 88.19^{c}	1752.22 ± 214.30^{c}
B	2.42 ± 0.40^{b}	3.34 ± 0.74^{bc}	1785.56 ± 117.06^{c}	1718.89 ± 183.59^{c}

The different letters of superscript in the same column indicated significant differences ($P<0.01$). A group treated with the combination of pigeon pea leaves and ginger extract showed the best result in ameliorating stress oxidative condition in liver tissues of DM rats. NC=negative control, PC=positive control, G=DM+glibenclamide, A= DM+*C. cajan* leaves (300 mg/kg BW) and ginger (60 mg/kg BW) extract, B=DM+ *C. cajan* (300 mg/kg BW) and ginger (125 mg/kg BW) extracts

DM group given pigeon pea leaves and ginger extract (A and B groups) showed reduced levels of blood glucose and elevated body weight despite consuming the same amount of feed as the other treatment groups. Based on the results, the combination treatment induced hypoglycemic activity in DM rats.

The use of 300 mg/kg BW pigeon pea leaves and 60 mg/kg BW ginger extract (A group) showed a better effect than 300 mg/kg BW and 125 mg/kg BW (B group), respectively. AUC of blood glucose levels in A group was also significantly different from NC group ($P\geq 0.05$) (Figures 3B). The results demonstrated that A group had the optimum dose for hyperglycemia conditions.

The level of MDA and SOD in kidney and liver tissues

Kidney and liver tissues of DM group showed the highest MDA levels ($P<0.05$) and the lowest SOD activity ($P<0.05$) (Table 3), suggesting DM rats experienced oxidative stress in both organs. DM group treated with glibenclamide (G) had better MDA levels and SOD activity compared to PC group. The two treatment groups (A and B), given a combination of pigeon pea leaves and ginger extract at different doses, had significantly lower MDA levels and increased SOD activity ($P<0.05$) compared to PC group. This implied that the combined extracts could increase antioxidant defense in the renal and hepatic organs of DM rats. However, the combination extract of A dose showed more effectiveness in ameliorating stress oxidative than B.

The Cu,Zn-SOD content in kidney and liver tissues

Cu,Zn-SOD profile for kidney and liver tissues across all treatment groups is shown in Figure 4A-B, and Table 4. Based on the results, the content in both organs was significantly lower in DM group compared to other groups (Figure 4A-B). This was indicated by the highest count of cells with negative reactions ($P<0.05$) and the lowest number of cells with strong positive reactions ($P<0.05$). In DM group treated with glibenclamide (G), kidney and liver tissues demonstrated higher levels of the antioxidant Cu,Zn-SOD compared to DM group. This was indicated by the higher number of strong and moderate positive reaction cells ($P<0.05$) in kidney tissues and the higher number of positive reaction cells ($P<0.05$) in liver tissues. In addition, a lower number of negative reaction cells ($P<0.05$) was found in kidney and liver of the glibenclamide group (G) than in PC group.

According to previous studies, DM condition induces oxidative stress with the lowest antioxidant content level of Cu,Zn-SOD in kidney, liver, testis, and pancreatic tissues.[13,27–30] The combination of pigeon pea leaves and ginger extract in both doses (A and B) led to higher Cu,Zn-SOD content in kidney and liver tissues compared to DM group. This was indicated by the higher number of strong positive ($P<0.05$) and the lowest number of negative reaction cells ($P<0.05$) in the treatment groups (A and B) compared to PC group. A group had higher Cu,Zn-SOD content compared to B (Table 4), as shown by a higher number of strong positive ($P<0.05$) and a lower number of negative reaction cells

(P<0.05). The combination extract caused increased antioxidant defense in DM rats, with A group being more effective than B.

Flavonoids and phenolic acids in pigeon pea leaves and ginger var. amarum extract are responsible for several pathways associated with reducing blood glucose levels. This condition reduced free radical production, subsequently lowering the level of MDA and increasing both SOD activity and content of Cu,Zn-SOD in kidney and liver tissues of DM rats. Based on the results, a synergistic effect was found for the combination of pigeon pea leaves and ginger var. amarum extract in ameliorating oxidative stress conditions in kidney and liver tissues of DM rats.

One mechanism of reducing blood glucose levels is through the antioxidant properties of flavonoids and phenolic acids acting as free radical scavengers during oxidative stress. This was demonstrated by increased SOD activity and Cu,Zn-SOD content as well as decreased MDA level. Other mechanisms include several pathways that lead to hypoglycemic action.

Flavonoid compounds and phenolic acids ameliorate oxidative stress conditions by decreasing the level of MDA, as well as increasing SOD activity, and Cu,Zn-SOD content in kidney and liver. These activities reduced free radicals production to levels in the normal range of blood glucose in A and B groups.

Conclusion

In conclusion, this study found that a combination of pigeon pea leaves and ginger var. amarum extract ameliorated oxidative stress conditions in kidney and liver of DM rats. The combination extracts decreased blood glucose level, increased body weight, reduced MDA level, enhanced SOD activity, as well as Cu,Zn-SOD content. The flavonoids and phenolic acids contained in the extracts may play a role in ameliorating oxidative stress conditions through several pathways. This study suggested the potential of combining pigeon pea leaves and ginger var. amarum extract as a therapeutic strategy to alleviate oxidative stress-related complications in DM patients.

Conflict of Interest

The authors declare no conflict of interest.

Table 4: The number of renal tubule cells in kidney tissue and hepatocytes in liver of DM rats under 400x magnification per field of view

Group	The number of kidney tubule cells and hepatocytes at different levels of Cu,Zn-SOD content			
	+++	++	+	-
The number of renal tubule cells				
NC	76.7±8.55^{bc}	59.7±19.03^{bc}	38.5±5.21^{b}	16.6±9.32^{a}
PC	17.4±4.01^{a}	33.0±7.62^{a}	68.6±16.96^{c}	74.4±20.77^{c}
G	82.3±18.90^{c}	75.2±23.25^{c}	63.3±12.99^{c}	40.7±10.67^{b}
A	95.7±7.67^{d}	44.8±19.51^{ab}	23.4±7.99^{a}	17.4±3.37^{a}
B	67.1±11.46^{b}	48.7±20.10^{ab}	31.1±9.72^{ab}	54.3±24.49^{b}
The number of hepatocytes				
CN	28.70 ± 3.34^{c}	30.90 ± 4.53^{c}	70.30 ± 15.10^{c}	13.50 ± 4.32^{a}
CP	16.10 ± 5.59^{a}	16.50 ± 4.06^{a}	26.80 ± 6.06^{a}	58.50 ± 8.36^{c}
G	20.90 ± 4.20^{b}	22.80 ± 5.90^{b}	40.90 ± 10.41^{b}	31.30 ± 13.40^{b}
A	36.90 ± 7.58^{d}	29.40 ± 4.74^{c}	101.40 ±11.58^{d}	12.40 ± 2.41^{a}
B	28.60 ± 4.37^{c}	37.50 ± 11.64^{d}	75.30 ± 4.42^{c}	36.10 ± 6.70^{b}

The different letters of superscript in the same column indicated significant differences (P<0.01). A group treated with the combination of pigeon pea leaves and ginger extract showed the best result in ameliorating stress oxidative condition in kidney tissues and liver tissues of DM rats. NC=negative control, PC=positive control, G=DM+glibenclamide, A= DM+*C. cajan* leaves (300 mg/kg BW) and ginger (60 mg/kg BW) extract, B=DM+ *C. cajan* (300 mg/kg BW) and ginger (125 mg/kg BW) extracts.

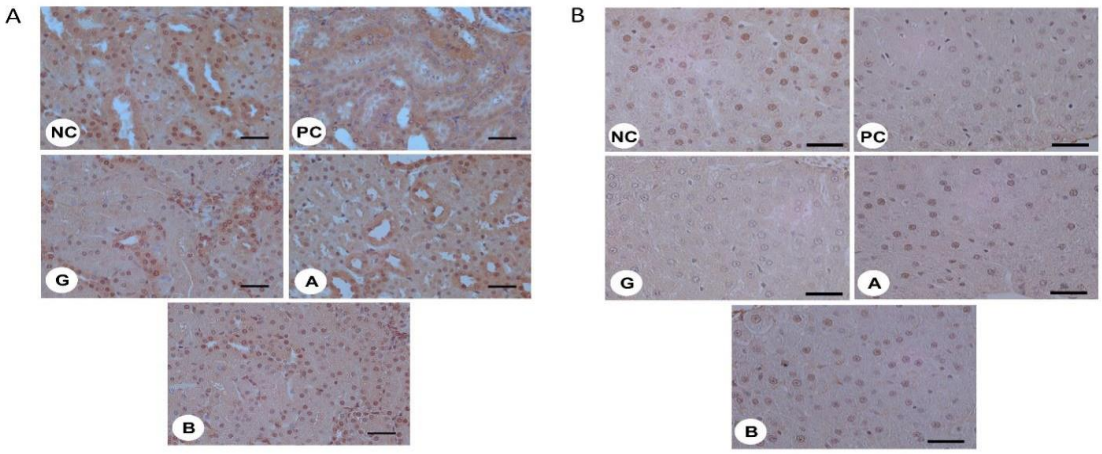

Figure 4: Photomicrograph of kidney (A) and liver tissues (B) of DM rats immunohistochemically stained for Cu,Zn-SOD content. DM group (PC) showed the lowest level of Cu,Zn-SOD content. NC=negative control, PC=positive control, G=DM+glibenclamide, A= DM+*C. cajan* leaves (300 mg/kg BW) and ginger (60 mg/kg BW) extract, B=DM+ *C. cajan* (300 mg/kg BW) and ginger (125 mg/kg BW) extracts. Bar=50 μm.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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