Tittle of Manuscript: **Effect of *Andrographis paniculata* on Blood Sugar Levels Through Regulation of Alpha-Glucosidase Enzyme Expression: An In Vivo Study**

1.Proofread document received (January 27th, 2022)
-Document from proofreading service
2.Submitted to the journal "Eureka Herba Indonesia (February 5th, 2022)
3.Peer Reviewer results: Revision Required (February 14th, 2022)
4.Revised version received by journal (February 19th, 2022)
5.Paper Accepted for publication (February 22th, 2022)
6.Galley proof (February 24th, 2022)
7.Paper published (February 25th, 2022)

-Document from proofreading service

January 27th, 2022

HM Publisher
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CONFIDENTIAL
January 27th, 2022

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Regards,

Khrishna Murti, PhD
Head of Language Institute-HM Publisher
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Submitted to the journal "Eureka Herba Indonesia (February 5th, 2022)

Effects *of Andrographis paniculata* On Blood Sugar Levels Through Regulation of Alpha Glucosidase Enzyme Expression: In Vivo Study

Rachmat Hidayat[1*], Patricia Wulandari[2]

[1]Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

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Abstract

Diabetes mellitus (DM) is a disease characterized by an increase in blood glucose levels caused by a lack of insulin, either absolute or relative. The enzyme α-glucosidase serves to accelerate the absorption of glucose by the small intestine by catalyzing the hydrolytic division of oligosaccharides into monosaccharides. This study aims to explore the potential of AP extract in regulating blood sugar levels through inhibition of α-glucosidase activity in the intestine. This study is an experimental study of Invivo. A total of 30 White Rats were divided into control groups and treated by being given *andrographis paniculata* extract. Examination of blood sugar levels is carried out by spectrophotometry and examination of the enzyme alpha glucosidase with ELISA. Data analysis was carried out with SPSS software by performing univariate and bivariate analysis. Andrographis paniculata extract is able to lower blood sugar levels and is able to decrease the activity of the enzyme alpha glucosidase as the dose increases. Andrographis paniculata extract is able to lower blood sugar levels through inhibition of the activity of the enzyme alpha glucosidase in the intestine.

Keywords: Diabetes Mellitus; Andrographis paniculata; Alpha glucosidase; Glucose

1. Introduction

Diabetes mellitus (DM) is a disease characterized by an increase in blood glucose levels caused by a lack of insulin, either absolute or relative. Currently, diabetes mellitus is a serious threat to humans and is the 7th leading cause of death in the world and Indonesia is ranked 4th

after the United States, India, and China for the highest number of people with diabetes mellitus in the world. [1] The incidence of DM from year to year will increase. The prevalence of diabetes mellitus in Indonesia in 2013 was 6.9%, while in 2018 it was 8.5%. [2] There is one therapeutic approach that can be used to treat diabetes mellitus, namely by inhibiting enzymes related to glucose absorption in the body, such as the enzyme α-glucosidase. The enzyme α-glucosidase serves to accelerate the absorption of glucose by the small intestine by catalyzing the hydrolytic division of oligosaccharides into monosaccharides, which causes an increase in blood glucose levels in the body after eating. To slow down or delay the absorption of glucose in the intestines that can prevent an increase in post-prandial blood glucose levels, an inhibitor of the enzyme α-glucosidase is needed. [3]

Andrographis paniculata (AP) is one of the most commonly found plants in Indonesia. This plant is known by the name of sambiloto and has a distinctive feature that sambiloto leaves taste bitter. AP has been widely used ethnopharmacologically in overcoming various health disorders, including to treat fever, diarrhea, high blood pressure, and diabetes mellitus. AP is used by the community in overcoming DM by boiling and drinking boiled water. Several studies have shown the potential of AP extract in the regulation of blood sugar levels in vivo. [4-8] This study is the first study that aims to explore the potential of AP extract in regulating blood sugar levels through inhibition of α-glucosidase activity in the intestine.

2. Methods

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A total of 1 kg of wet AP leaves is carried out the cleaning and drying process using an oven at a temperature of 60^{o}C. After the AP leaves are dry, then the process of smoothing the AP leaves is carried out so that AP simplicia powder is obtained. Simplisia AP powder is then carried out an extraction process using the maceration method using a 96% ethanol solvent 1: 10 for 3x24 hours. Next, the process of separating the pulp and maserate is carried out. Maserat is carried out the evaporation process so that it becomes a viscous extract using a rotary evaporator. The DM induction process is carried out by aloxan induction at a dose of 110 mg / kgBW intraperitoneally. If the results of the blood sugar level examination with spectrophotomaterty have shown more than 200 mg / dL, the White Rat has been considered to have DM.

After treatment for 7 days, White Rats were evacuated instestine organs by first anesthesia with the anesthetic agent Biopenthyl® 0.1 ml / 10 grBW. The intestine organs are washed, washed and put into containers for further homogenization. Then the process of checking the activity of the enzyme alpha glucosidease is carried out using the enzyme linked immunosorbent assay (ELISA) examination method.

Data analysis was carried out with the help of SPSS 25 software. Furthermore, a univariate analysis was carried out to present the distribution of data, followed by bivariate analysis with a T test and continued with a poh hoc test (bonferroni). P value is set at 5% or 0.05, where if the p value < 0.05 indicates a statistically discrepancy in the average level between groups.

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Table 1 shows the potential of AP extract in lowering blood sugar levels. The P1, P2 and P3 groups showed that they were able to lower blood sugar levels better than the untreated K1 group. The P1 and P2 treatment groups lowered blood sugar levels not as optimally as K2 which received acarbose . The P3 group was able to lower blood sugar levels more optimally than K2 which received acarbose treatment. The higher the dose of AP extract, the more optimal the ability of AP extract to lower blood sugar levels.

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AP extract contains various secondary metabolites, especially flavonoids. Some studies show that AP extract is rich in flavonoids. [9.10] Other studies have shown that flavonoid compounds (quercetin, kaempferitrin, rutin, and kaempferol) have the ability to inhibit the activity of digestive enzymes in the duodenum, both maltase, sucrose and lactase. [11.12] The ability to inhibit digestive enzymes in the duodenum causes the ability of flavonoids to prevent the breakdown of disaccharides into monosaccharides. Other studies have also shown that flavonoid compounds (quercetin and rutin) have the potential to inhibit the activity of the enzyme alpha glucosidase invitro. [13] The enzyme alpha glucosidase is an enzyme located in the brush border intestine that plays a role in the absorption of glucose in the intestine. [14] The ability of AP extract in reducing the activity of the enzyme alpha glucosidase causes a decrease in glucose absorption activity in the intestine so that it will lower blood sugar levels. [15th]

4. Conclusion

Andrographis paniculata extract is effective in lowering blood sugar levels through inhibition of the activity of the enzyme alpha glucosidase in the intestine in white rats.

5.References

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Submitted to the journal “Eureka Herba Indonesia (February 5th, 2022)

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Submission acknowledgement

Dear author(s),

Rachmat Hidayat*, Patricia Wulandari has submitted the manuscript “Effect of *Andrographis paniculata* on Blood Sugar Levels Through Regulation of Alpha-Glucosidase Enzyme Expression: An In Vivo Study“ to Eureka Herba Indonesia. The paper will be screened by editor and reviewed by peer review.

Cordially,

Prof. Paula Magnano, PhD

Editor HM Publisher

Eureka Herba Indonesia

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Peer Review Results

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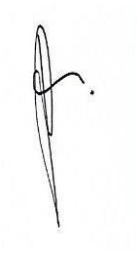

Prof. Paula Magnano, PhD

Editor HM Publisher

(*) Corresponding author

Reviewer 1: Revision required

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5.References →8

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Reviewer Comment:

1→ Title of Manuscripts should be explained independent variable and dependent variable also subject of study.

2→ Keywords should be showed the main words of the study, the authors can use MeSH to develop keywords.

3→ Abstract should be showed the main of background, methods, results and conclusion of study.

- Background abstract should be showed the urgency of study and why the study important, in simple way.
- Conclusion should be wrote in simple way, specific to the main results. Conclusion in abstract should not showed statistic results.

4→Introduction should be showed the urgency of study (epidemiology data), biological plausibility concept, and lack of knowledge in the study.

- Paragraph 1→ need improvement in urgency of study and explain more about epidemiology data. Authors do not only show the data, but try to elaborate and make comparison about the data from year to year.
- Paragraph 2 and 3 need improvement to focus in biological plausibility concept.

5→ Methods should be showed more about how the study develop. Methods should be showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis.

- Methods need to showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis, more specific but not to long.

6→ Results should be showed baseline characteristics subject of study, main results of study. Authors must be focused and try to make results no more table and figure.

7→ Discussion should be explored more biological plausibility, not only showed about statistical results.

8→ Conclusion should more specific and not more showed statistical results

9→ Authors must check the references for make update references. References should no more than 10 years.

Reviewer 2: Revision required

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[1]Department of Biology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

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A total of 1 kg of wet AP leaves is carried out the cleaning and drying process using an oven at a temperature of 60°C. After the AP leaves are dry, then the process of smoothing the AP leaves is carried out so that AP simplicia powder is obtained. Simplisia AP powder is then carried out an extraction process using the maceration method using a 96% ethanol solvent 1: 10 for 3x24 hours. Next, the process of separating the pulp and maserate is carried out. Maserat is carried out the evaporation process so that it becomes a viscous extract using a rotary evaporator. The DM induction process is carried out by aloxan induction at a dose of 110 mg / kgBW intraperitoneally. If the results of the blood sugar level examination with spectrophotomaterty have shown more than 200 mg / dL, the White Rat has been considered to have DM.

After treatment for 7 days, White Rats were evacuated instestine organs by first anesthesia with the anesthetic agent Biopenthyl® 0.1 ml / 10 grBW. The intestine organs are washed, washed and put into containers for further homogenization. Then the process of checking the activity of the enzyme alpha glucosidease is carried out using the enzyme linked immunosorbent assay (ELISA) examination method.

Data analysis was carried out with the help of SPSS 25 software. Furthermore, a univariate analysis was carried out to present the distribution of data, followed by bivariate analysis with a T test and continued with a poh hoc test (bonferroni). P value is set at 5% or 0.05, where if the p value < 0.05 indicates a statistically discrepancy in the average level between groups.

3. Results and Discussion→6

Table 1 shows the potential of AP extract in lowering blood sugar levels. The P1, P2 and P3 groups showed that they were able to lower blood sugar levels better than the untreated K1 group. The P1 and P2 treatment groups lowered blood sugar levels not as optimally as K2 which received acarbose . The P3 group was able to lower blood sugar levels more optimally than K2 which received acarbose treatment. The higher the dose of AP extract, the more optimal the ability of AP extract to lower blood sugar levels.

Table 1. Comparison of average blood sugar levels between groups

Group	Average Blood Sugar Levels (Mean±SD) mg/dL
K1	453.5±21.2
K2	243.4±11.3
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*Post Hoc Test (Bonferroni) VS K2, p<0.05

Table 2 shows the potential of AP extracts in lowering the activity of the enzyme alpha glucosidase. The P1, P2 and P3 groups showed that they were able to reduce the activity of the enzyme alpha glucosidase better than the K1 group that did not receive treatment. The P1 and P2 treatment groups lowered the activity of the enzyme alpha glucosidase not as optimally as K2 which received acarbose abuse. The P3 group was able to reduce the activity of the enzyme alpha glucosidase more optimally than K2 which received acarbose treatment. The higher the dose of AP extract shows the ability of AP extract in lowering the activity of the enzyme alpha glucosidase the more optimal.

Table 2. Comparison of mean levels of alpha glucosidase between groups

Group	Average Levels of Alpha Glucosidase (Mean±SD) pg/mL
K1	212.5±11.4
K2	77.4±5.3
P1	198.2±12.1*
P2	135.4±11.2*
P3	89.6±6.1*

*Post Hoc Test (Bonferroni) VS K2, p<0.05

AP extract contains various secondary metabolites, especially flavonoids. Some studies show that AP extract is rich in flavonoids. [9.10] Other studies have shown that flavonoid compounds (quercetin, kaempferitrin, rutin, and kaempferol) have the ability to inhibit the activity of digestive enzymes in the duodenum, both maltase, sucrose and lactase. [11.12] The ability to inhibit digestive enzymes in the duodenum causes the ability of flavonoids to prevent the breakdown of disaccharides into monosaccharides. Other studies have also shown that flavonoid compounds (quercetin and rutin) have the potential to inhibit the activity of the enzyme alpha glucosidase invitro. [13] The enzyme alpha glucosidase is an enzyme located in the brush border intestine that plays a role in the absorption of glucose in the intestine. [14] The ability of AP extract in reducing the activity of the enzyme alpha glucosidase causes a decrease in glucose absorption activity in the intestine so that it will lower blood sugar levels. [15th]

4. Conclusion→7

Andrographis paniculata extract is effective in lowering blood sugar levels through inhibition of the activity of the enzyme alpha glucosidase in the intestine in white rats.

5.References→8

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Reviewer Comment:

1→ Title of Manuscripts should be explained independent variable and dependent variable also subject of study.

2→ Keywords should be showed the main words of the study, the authors can use MeSH to develop keywords.

3→ Abstract should be showed the main of background, methods, results and conclusion of study.

- Background abstract should be showed the urgency of study and why the study important, in simple way.
- Conclusion should be wrote in simple way, specific to the main results. Conclusion in abstract should not showed statistic results.

4→Introduction should be showed the urgency of study (epidemiology data), biological plausibility concept, and lack of knowledge in the study.

- Paragraph 1→ need improvement in urgency of study and explain more about epidemiology data. Authors do not only show the data, but try to elaborate and make comparison about the data from year to year.
- Paragraph 2 and 3 need improvement to focus in biological plausibility concept.

5→ Methods should be showed more about how the study develop. Methods should be showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis.

- Methods need to showed the design of study; population, sample and sample size of study; inclusion criteria; place of study; ethical clearence steatment; independent and dependent variable; data analysis, more specific but not to long.

6→ Results should be showed baseline characteristics subject of study, main results of study. Authors must be focused and try to make results no more table and figure.

7→ Discussion should be explored more biological plausibility, not only showed about statistical results.

8→ Conclusion should more specific and not more showed statistical results

9→ Authors must check the references for make update references. References should no more than 10 years.

Revised version received by journal (February 19th, 2022)

Eureka Herba Indonesia Vol 3 Issue 1 2022

Eureka Herba Indonesia

Journal Homepage: https://eurekabiomedical.com/index.php/EHI

Effect of *Andrographis paniculata* on Blood Sugar Levels Through Regulation of Alpha-Glucosidase Enzyme Expression: An In Vivo Study

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ARTICLE INFO

Keywords:
Diabetes Mellitus
Andrographis paniculata
Alpha-glucosidase
Glucose

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/ehi.v3i1.56

A B S T R A C T

Diabetes mellitus (DM) is a disease characterized by an increase in blood glucose levels caused by insulin deficiency, either absolute or relative. The α-glucosidase enzyme functions to accelerate the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into monosaccharides. This study aims to explore the potential of AP extract in regulating blood sugar levels through inhibition of α-glucosidase activity in the intestine. This study is an in vivo experimental study. A total of 30 \ rats were divided into a control group and treated with *Andrographis paniculata*. Examination of blood sugar levels was carried out by spectrophotometry and examination of alpha-glucosidase enzymes by ELISA. Data analysis was performed using SPSS software with univariate and bivariate analysis. *Andrographis paniculata* extract was able to reduce blood sugar levels and was able to decrease the activity of the alpha-glucosidase enzyme as the dose increased. *Andrographis paniculata* extract is able to reduce blood sugar levels by inhibiting the activity of the alpha-glucosidase enzyme in the intestine.

1. Introduction

Diabetes mellitus (DM) is a disease characterized by an increase in blood glucose levels caused by a lack of insulin, either absolute or relative. Currently, diabetes mellitus is a serious threat to humans and is the 7th leading cause of death in the world, and Indonesia is ranked 4th after the United States, India, and China for the largest number of people with diabetes mellitus in the world.[1] The incidence of DM from year to year will increase. The prevalence of diabetes mellitus in Indonesia in 2013 was 6.9%, while in 2018, it was 8.5%.[2] There is one therapeutic approach that can be used to treat diabetes mellitus, namely, by inhibiting enzymes related to glucose absorption in the body, such as the α-glucosidase enzyme. The enzyme α-glucosidase functions to accelerate the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into monosaccharides, which causes an increase in blood glucose levels in the body after eating. To slow or delay the absorption of glucose in

the intestine that can prevent a postprandial rise in blood glucose levels, α-glucosidase enzyme inhibitors are needed.[3]

Andrographis paniculata (AP) is one of the most common plants in Indonesia. This plant is known as sambiloto and has the characteristic that bitter leaves taste bitter. AP has been widely used ethnopharmacological in overcoming various health problems, among others, to treat fever, diarrhea, high blood pressure, and diabetes mellitus. AP is used by the community in overcoming DM by boiling and drinking boiled water. Several studies demonstrated the potential of AP extracts in the regulation of blood sugar levels in vivo.[4-8] This study is the first study aimed at exploring the potential of AP extract in regulating blood sugar levels through inhibition of α-glucosidase activity in the intestine.

2. Methods

This study is an experimental study with a post-test-only approach with a control group design. A total of 30 rats (*Rattus norvegicus*) Wistar strain was acclimatized for 7 days, then grouped randomly into 5 groups (K1, K2, P1, P2, and P3). Group K1 is a negative control, where the rats were induced by DM and only given intragastric aquadest. Group K2 is a positive control, where the rats were induced by DM and given acarbose 1 mg/kg BW. Groups P1-P3 were the treatment group with 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW intragastrically administered AP extracts for 7 days. This study was approved by the medical and health research ethics committee CMHC-Science & Research Center, Palembang, Indonesia (No. 112/CMHC/KEPK-X/2021).

A total of 1 kg of wet AP leaves were cleaned and dried using an oven at a temperature of 60^{o}C. After the AP leaves were dry, then the AP leaf was refined to obtain AP Simplicia powder. The AP Simplicia powder was then extracted by the maceration method using 96% 1:10 ethanol solvent for 3x24 hours. Next, the pulp and macerate separation process is carried out. The macerate is evaporated so that it becomes a thick extract using a rotary evaporator. The DM induction process was carried out by intraperitoneal induction of Alloxan at a dose of 110 mg/kg BW. If the results of the examination of blood sugar levels by spectrophotometry have shown more than 200 mg/dL, the rat has been considered to have DM.

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Table 2 shows the potency of AP extract in reducing alpha-glucosidase enzyme activity. Groups P1, P2, and P3 showed that they were able to reduce the activity of the alpha-glucosidase enzyme better than the untreated group K1. Treatment groups P1 and P2 decreased the activity of the alpha-glucosidase enzyme not as optimally as K2, which received acarbose treatment. The P3 group was able to reduce the activity of the alpha-glucosidase enzyme more optimally than K2, which received acarbose treatment. The higher the dose of AP extract showed the ability of AP extract to reduce the activity of the alpha-glucosidase enzyme more optimally.

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4. Conclusion

Extract of *Andrographis paniculata* is effective in reducing blood sugar levels by inhibiting the activity of the alpha-glucosidase enzyme in the intestine in rats.

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Paper Accepted for publication (February 22th, 2022)

Eureka Herba Indonesia

HM Publisher

Letter of Acceptance

Manuscript “Effect of *Andrographis paniculata* on Blood Sugar Levels Through Regulation of Alpha-Glucosidase Enzyme Expression: An In Vivo Study“ by Rachmat Hidayat*, Patricia Wulandari, has been accepted to publish in Eureka Herba Indonesia Vol 3 issue 1 in February 2022.

Cordially,

Prof. Paula Magnano, PhD

Editor HM Publisher

(*) Corresponding author

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Eureka Herba Indonesia Vol 3 Issue 1 2022

Eureka Herba Indonesia
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ARTICLE INFO

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Diabetes Mellitus
Andrographis paniculata
Alpha-glucosidase
Glucose

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All authors have reviewed and approved the final version of the manuscript.

https://doi.org/10.37275/ehi.v3i1.56

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P2	3 11,2*
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The AP extract contains various secondary metabolites, especially flavonoids. Several studies show that AP extract is rich in flavonoids.[9,10] Other studies have shown that flavonoid compounds (quercetin, kaempferitrin, rutin, and kaempferol) have the ability to inhibit the activity of digestive enzymes in the duodenum, both maltase, sucrase, and lactase.[11,12] The ability to inhibit digestive enzymes in the duodenum causes the ability of flavonoids to prevent the breakdown of disaccharides into monosaccharides. Other studies have also shown that flavonoid compounds (quercetin and rutin) have the potential to inhibit alpha-glucosidase enzyme activity in vitro.[13] Enzyme alpha-glucosidase is an enzyme located in the brush border of the intestine that plays a role in the absorption of glucose in the intestine.[14] The ability of AP extract to reduce the activity of the alpha-glucosidase enzyme causes a decrease in the activity of glucose absorption in the intestine so that it will reduce blood sugar levels.[15]

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CERTIFICATE

OF PUBLICATION

Effect of Andrographis paniculata on Blood Sugar Levels Through Regulation of Alpha-Glucosidase Enzyme Expression: An In Vivo Study

Authored by;

Rachmat Hidayat, Patricia Wulandari

Published in

Eureka Herba Indonesia Volume 3 Issue 1 2022

Indexed in:

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