International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 05 Issue 11 November 2025

Page No : 592-596

DOI: https://doi.org/10.47191/ijpbms/v5-i11-05, Impact Factor: 8.163

The Effect of Broccoli Extract *(Brassica Oleracea Var.)* on the Kidney Glomerulus of Male Wistar Rats (Rattus Norvegicus) Induced by Alloxan

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ABSTRACT

Aloxan causes an imbalance between antioxidants and free radicals, leading to health implications including kidney dysfunction, which can be observed, among other factors, by the extent of the kidney glomerulus area. Exogenous antioxidant intake is needed to prevent a decrease in the glomerulus area due to free radicals induced by aloxan, one of which is broccoli (Brassica oleracea var. italica) which has high levels of antioxidants. Objective: This study aims to determine the effect of broccoli extract (Brassica oleracea var. italica) on the changes in the glomerulus area of male Wistar rats induced by aloxan. Methodology: This research used an experimental method, namely RCT (Randomized Controlled Trial), with a post-test control group design on 32 male Wistar rats for 30 days, divided into 4 groups: negative control group, positive control group, and 2 treatment groups (100 mg/kgBW and 200 mg/kgBW). The calculation of the glomerulus area in the kidneys was performed with a magnification of 400x. The data obtained will be processed using ANOVA test to observe differences between groups. Research Results: This study showed differences in the glomerulus area of the kidneys in all groups (ANOVA, p=0.00). The increase in broccoli extract administration is directly proportional to the glomerulus area in the group given aloxan and yielded significant results (LSD, p<0.05). Conclusion: Administration of broccoli extract can increase the glomerulus area of Wistar rat kidneys induced by aloxan.

KEYWORDS: DM type 2, Hyperglycemia, Histopathological of kidney, Alloxan, Broccoli Extract.

ARTICLE DETAILS

Published On:
07 November 2025

Available on:
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INTRODUCTION

Diabetes mellitus is a disease characterized by hyperglycemia. Hyperglycemia occurs due to impaired insulin production and secretion, or insulin resistance (Takashi, 2004). Alloxan is a chemical used to induce diabetes in laboratory animals. Administration of alloxan is a rapid method for producing experimental diabetic conditions (hyperglycemia) in laboratory animals (Nugroho, 2004; Filipponi, 2009).

The increase in DM incidence is likely to be followed by an increase in the incidence of chronic complications of DM, namely the occurrence of blood vessel blockages, both microvascular and macrovascular, which occur due to changes in the vascular system. These changes are partly caused by free radical stress. Free radicals can react with cells and can cause cell damage (Kisaoglu et al., 2013). Free radicals also play a role in the progression of hyperglycemia due to decreased insulin secretion and insulin action (Sarian et al., 2017). One cause of these free radicals is diabetic nephropathy. Diabetic nephropathy is a structural abnormality in the glomerulus and renal tubular elements characterized by hypertrophy, increased thickness of the glomerular basement membrane and the formation of nodular glomerulosclerosis, accumulation of extracellular matrix components, increased Glomerular Filtration Rate (GFR) with intraglomerular hypertension, proteinuria, systemic hypertension, and loss of kidney function (Kajal and Singh, 2019).

Persistent hyperglycemia can increase glucose uptake and glycolysis in tubular and glomerular cells. This results in the production of Advanced Glycation Endproducts (AGEs) and the activation of protein kinase C and the polyol pathway. These three pathways activate IL-6, MCP-1, TGF-beta (transforming growth factor-beta), and VEGF (vascular endothelial growth factor) (Lin, J. S., & Susztak, K., 2016). This can make it difficult for the glomerulus to maintain homeostasis, resulting in increased glomerular arteriolar area. Broccoli contains flavonoids, phenols, vitamin C, sulforaphane, and glucosinolates, which act as antioxidants (Liebman et al., 2021). Flavonoids are a group of polyphenolic compounds that have antioxidant, antimicrobial, and anticancer properties (Panche, 2016). Sulfurophane also has antioxidant properties so it can reduce ROS levels (Liebman et al., 2021).

Based on this description, the researcher wants to know the effect of administering broccoli extract (Brassica oleracea var. italic) on the glomerular appearance of the kidneys of Wistar rats induced by alloxan.

METHODS

This research is experimental research (Notoatmodjo, 2012). The research used post-test only control group design is laboratory experimental research to determine the cause-and-effect relationship. Laboratory experimental research is research conducted in a laboratory from a treatment given intentionally by the researcher (Nursyahidah, 2013). The research subjects were male Wistar rats aged 2-3 months with a body weight of 200-250 grams, totaling 32 rats divided into 4 groups, namely group 1 as a normal group that was only given standard food during the study, group 2 as a negative control group that was given alloxan induction, group 3 as the first treatment group that was given alloxan induction and broccoli extract 100mg/kgBW, group 4 as the second treatment group that was given alloxan induction and broccoli extract 200mg/kgBW.

The experimental animals were induced with alloxan at a dose of 120 mg/kgBW. Group 5, as a positive control, was given glimepiride, which has been proven effective in treating diabetes, as a comparison for the effect of broccoli extract on glomerular arteriole area. Glimepiride was given at a dose of 2 mg/kgBW, administered once daily with a gastric tube. The broccoli extract given to the treatment group was obtained through an extraction process using the maceration method dissolved in 1% Na CMC and distilled water. The broccoli extract was given once daily in suspension form using a gastric tube. The standard food and drink provided were 15 grams of pellet feed and drinking water provided ad libitum (Stevani, 2016).

Preparation of slides for data collection was carried out in the Anatomical Pathology Laboratory of the Faculty of Medicine, University of Surabaya in accordance with the SOP in the laboratory. After the slides were ready, they were observed in 5 fields of view with a binocular microscope at 400x magnification and arteriole area measurements were performed with 5 repetitions. The results of the arteriole area obtained were processed and analyzed using Statistical Product and Service Solution (SPSS) version 27. The collected data were pre-tested with the Shapiro-Wilk normality test and the Levene homogeneity test with a significance requirement of $p>0.05$. Furthermore, if the data were proven to be normal and homogeneous, they were tested with One Way ANOVA and Post Hoc LSD tests to compare the glomerular arteriole area in the five groups.

RESULTS

The mean value and standard deviation of each group based on table 1 are group I (1305.71 ± 30.076), group II (1907.00 ± 43.209), group III (1377.00 ± 15.652), group IV (1340.00 ± 31.024) and group V (1326.00 ± 22.749). Based on Figure 1, it can be seen that the normal control group (group I) is the group with the lowest arteriole area while the negative control group (group II) is the group with the highest arteriole area. Groups III, IV and V have decreased glomerular arteriole area. The prerequisite test shows that the glomerular arteriole area data is normally distributed ($p>0.05$) and homogeneous ($p>0.05$). Based on the results of the prerequisite test, a One Way ANOVA test was carried out with the result $p<0.001$ ($p<0.05$). This indicates a significant difference in glomerular arteriole area across all groups. The data were subjected to a post-hoc LSD test to compare the groups, with the following results:

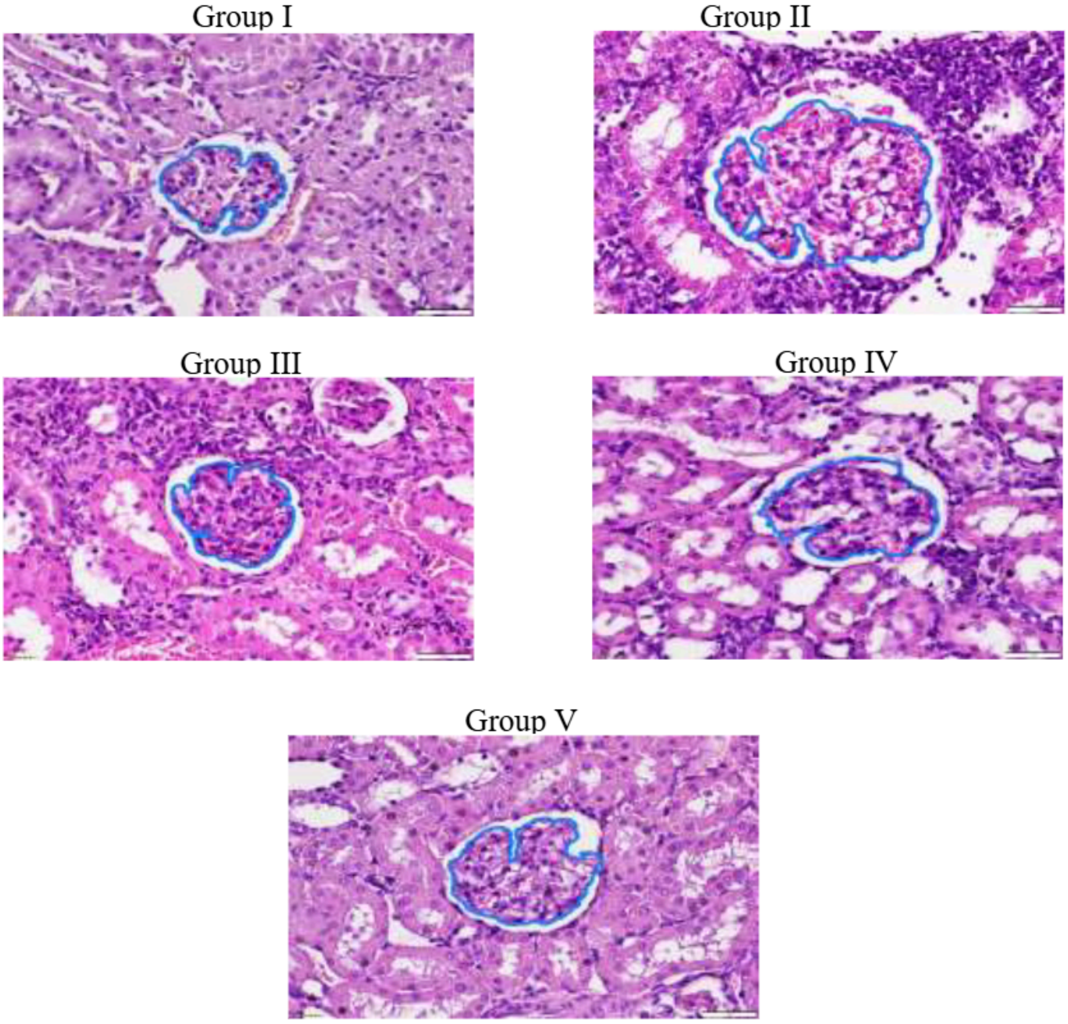

Figure 1. Results of comparison of the area of glomerular arterioles in rats with 400x magnification and HE staining.

Based on the results of the comparison between groups with the LSD Post Hoc Test in Table 2, it can be seen that there are significant differences (p<0.05) between group I and group II, group I and group III, group II and group III, and group III and group V. Comparison of other groups shows no significant differences (p>0.05).

Table 1. Description of the area and statistically results of the glomerular arterioles.

Group	Mean ± SD	Shapiro-Wilk	Levene' test	ANOVA	Least Significance Different (LSD)				
					I	II	III	IV	V
I	1305,7 ± 30,0	0,265							
II	1907,0 ± 43,2	0,918			<0.001*				
III	1377,0 ± 15,6	0,603	0,220	<0,001*	<0.001*	<0.001*			
IV	1340,0 ± 31,0	0,470			0.064	<0.001*	0.064		
V	1326,0 ± 22,7	0,846			0.261	<0.001*	0.013*	0.468	

*Significancy level <0.05 (CI=95%)

DISCUSSION

The results of the comparative study of group I (normal control group) which was only given standard food and drink for 22 days with an average arteriole area of 1305.71 ± 30.076 with group II (positive control group) which was given alloxan induction and standard food for 14 days with an average arteriole area of 1907.00 ± 43.209 showed a significant increase in glomerular arteriole area (p <0.05) in group II, this is in accordance with the research of Ighodaro et al (2018) which said that administration of alloxan can create hyperglycemic conditions by degrading pancreatic β cells. The dose of alloxan used was 120mg/kgBW based on the research of Swastini et al (2018).

The results of the study between group II (negative control group) which was given alloxan induction and standard feeding for 14 days with an average glomerular arteriole area of 1907.00 ± 43.209 and group V (positive control group) which was given alloxan induction and glimepiride 2mg/kgBW for 14 days with an average of 1326.00 ± 22.749 showed a significant decrease in glomerular arteriole area ($p<0.05$) in group V. Glimepiride is an antidiabetic drug of the sulfonylurea group. Glimepiride works on the calcium ATPase channel in pancreatic beta cells to stimulate insulin release (Pakaya, M, 2022). Sulfonylureas bind to the 65-kD protein of beta cells, and are able to improve insulin secretion (Al-Kaff, 2021).

The average glomerular arteriole area in group II (the negative control group) compared to the average glomerular arteriole area in groups III and IV showed a significant decrease ($p<0.05$). Thus, groups III and IV with broccoli extract doses of 100 mg/kgBW and 200 mg/kgBW were effective in reducing glomerular arteriole area. The decrease in glomerular arteriole area due to broccoli extract administration is due to the extract's content of sulforaphane, glycosinolates, polyphenols, and hydroxynanic acids.

Flavonoids work by increasing insulin levels by donating a hydrogen atom to neutralize the toxic effects of free radicals. Glucosinolates protect DNA from damage and thus stabilize free radicals (Juliana, 2018). The flavonoids found in broccoli have a similar mechanism to sulforaphane: increasing heme oxygenase-1 expression by inhibiting NRF2 degradation, which in turn increases pancreatic antioxidant enzymes, leading to the production of superoxide dismutase and glutathione peroxidase. This results in reduced ROS production (Liebman et al., 2021). Sulforaphane inhibits KEAP1-mediated NRF2 degradation, which leads to transcriptional production of antioxidant enzymes such as glutathione S-transferase (GST), catalase (CAT), and glutathione peroxidase (GPx), thereby increasing antioxidant levels in the body and reducing blood pressure (Liebman et al., 2021).

The measurement of glomerular arteriole area between groups V and III showed a significant difference ($p<0.05$), while the measurement of glomerular arteriole area between groups V and IV showed an insignificant difference ($p>0.05$). Based on these results, the more effective dose of broccoli extract is 200 mg/kgBW.

CONCLUSION

Based on the study results, it can be concluded that broccoli (Brassica oleracea var. italic) extract is effective in preventing renal arteriolar dilation. Of the two doses administered, the 200 mg/kg body weight broccoli extract dose was more effective but not comparable to glimepiride.

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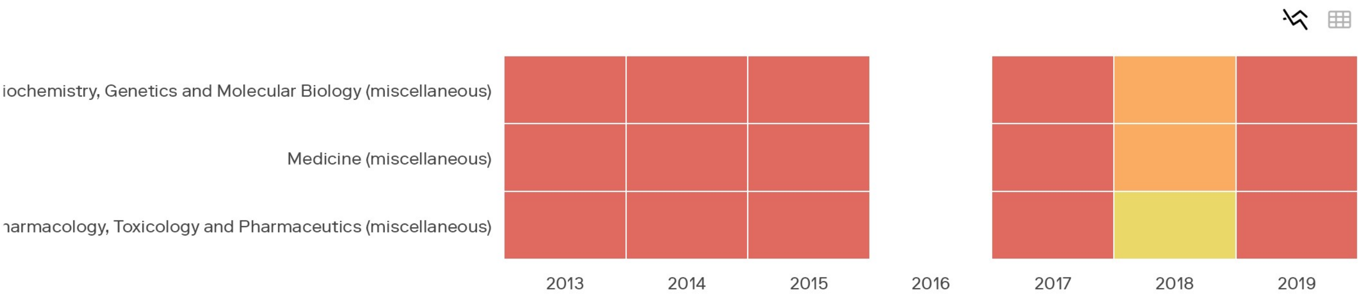

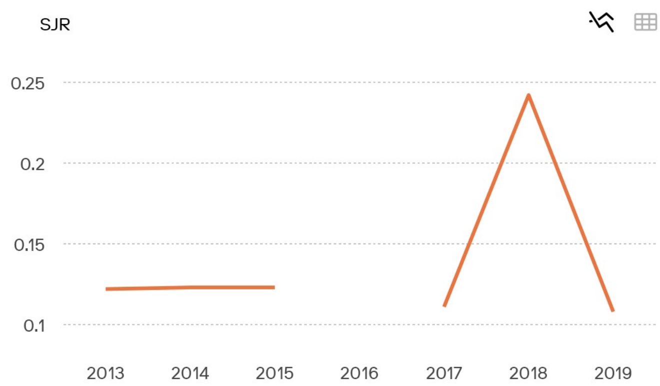

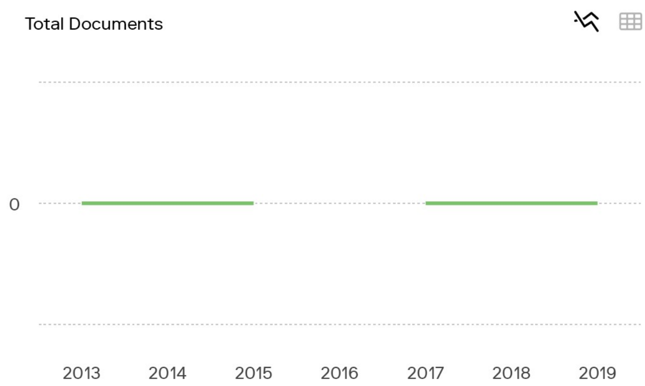

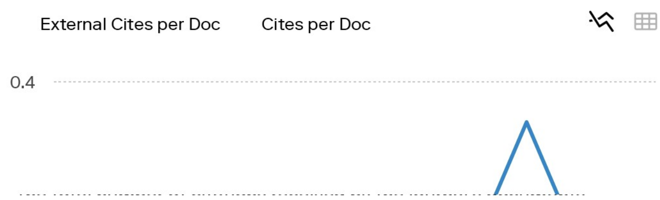

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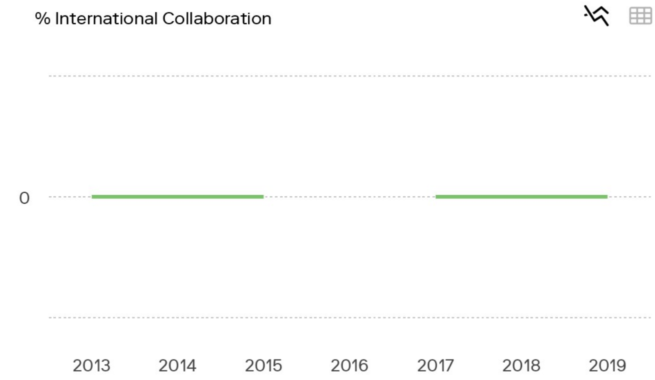

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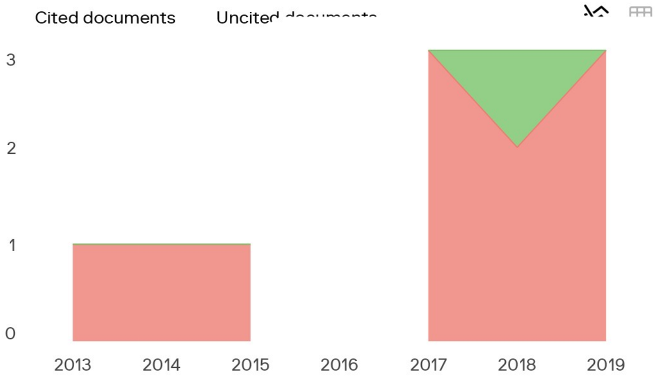

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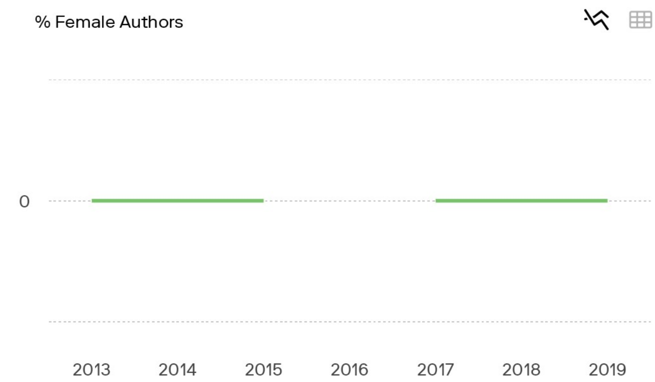

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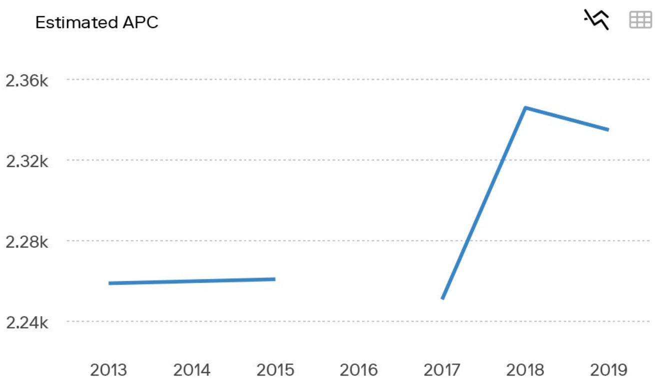

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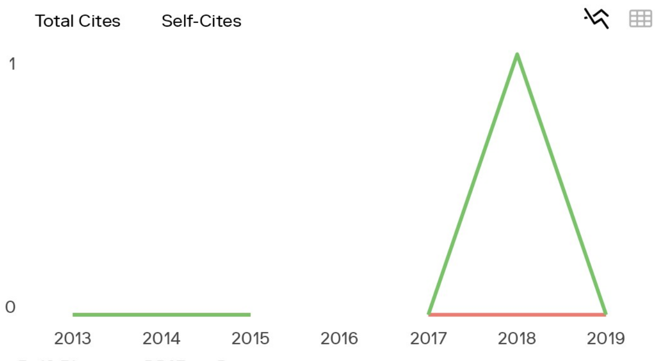

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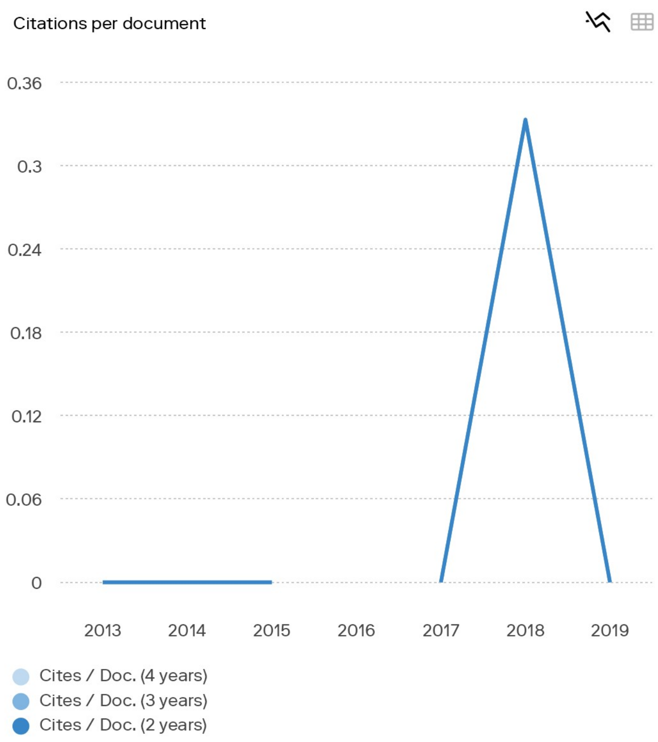

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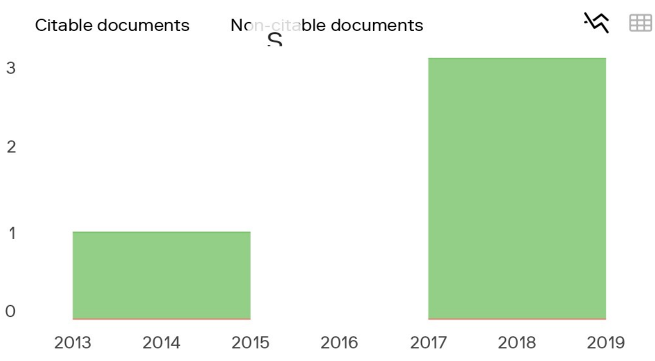

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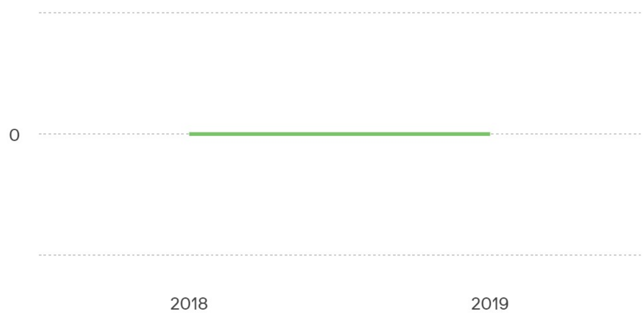

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