Journal of Scientific Research and Development (2024) Vol. 23 (1) 109 - 119

A bi-annual journal published by the Faculty of Science, University of Lagos, Nigeria

http://jsrd.unilag.edu.ng/index.php/jsrd

Intraocular pressure reducing effect of *Hibiscus sabdariffa* leaf aqueous extract in normotensive subjects

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(Received 12 August 2024/Revised 28 November 2024/Accepted 04 December 2024)

Abstract

This study is aimed to investigate the effect of *Hibiscus sabdariffa* leaf aqueous extract on intraocular pressure (IOP) in normotensive subjects. A total of fifty-two (52) individuals participated in this study with a mean age of 22.79 ± 2.645 years, with equal gender distribution of male and female. The study population was subdivided into two groups: an experimental group A and Control group B. Each group consisted of 26 normotensives (as study design) comprising of 13 males and 13 females who were age-matched. All the participants abstained from medication for one week and no breakfast on the day of the experiment. The baseline IOP value of all subjects in both groups were measured with Perkins non-contact tonometer thereafter, those in the experimental group were administered with aqueous extract of *Hibiscus sabdariffa.* The IOP was then measured at intervals of 30, 60, 90 and 120 mins after ingestion of the extract**.** The findings of this study showed that in group A, there was significant reduction in IOP in both the right and left eyes over time intervals ($p < 0.001$) post – ingestion of *Hibiscus sabdariffa* leaf aqueous extract. The peak of reduction in IOP occurred at 120 minutes on the right eye (10.15 ± 1.190 mmHg) and left eye (10.31 ± 1.192) compared to the baseline findings of 13.23 ± 1.996 mmHg and 13.04 ± 2.490 mmHg, respectively. Notably, group B did not exhibit any significant changes in IOP. Across the time intervals examined, it was observed that there was no significant relationship between the age and gender of the participants and the fluctuations in their IOP subsequent to ingesting *Hibiscus sabdariffa* leaf aqueous extract ($p > 0.05$). Conversely, there were significant relationships identified between the participants' baseline IOP levels and the alterations in their IOP after ingesting *Hibiscus sabdariffa* leaf aqueous extract ($p < 0.05$). This study suggests that eye care professionals could consider the integration of *Hibiscus sabdariffa* leaf aqueous extract as a complementary therapy in the management of IOP in some ocular conditions like glaucoma.

Keywords: Hibiscus drink, intraocular pressure, *Hibiscus sabdariffa*, normotensive subjects.

Introduction

Hibiscus sabdariffa, also known as Roselle or Zobo, holds deep cultural and medicinal significance. It's a flowering shrub which is native to tropical regions in Africa and Asia, it belongs to the Malvaceae family (Riaz and Chopra, 2018; Nwaiwu *et al*., 2020). Among the 300+ Malvaceae species, *Hibiscus sabdariffa* stands out as the most widely recognized (Hopkins *et al*., 2013; Aziz *et al*., 2013). Its leaves play a key role in traditional

medicine and cooking across several nations, such as Nigeria, Mexico, and Thailand (Akujobi *et al*., 2018). The infusion of its dried calyxes, known as Zobo drink, has been an integral part of dietary practices in various regions. This vibrant crimson beverage is not only admired for its refreshing taste but has also captured scientific interest due to its rich phytochemical composition. It is known to be abundant in antioxidants, flavonoids, and phenolic compounds, believed to contribute to potential health benefits (Ojulari *et al*., 2019).

The intricate balance of intraocular pressure (IOP) plays a pivotal role in maintaining ocular health, influencing both the structure and function of the eye. Deviations from this balance, particularly elevated IOP, have consistently emerged as a significant risk factor for various ocular pathologies, notably glaucoma, a leading cause of irreversible blindness globally (Weinreb *et al*., 2014). Regulating IOP involves delicate mechanisms that govern the equilibrium between the production and drainage of aqueous humor. As the prevalence of ocular disorders rises, eye care practitioners face challenges, compelling the exploration of alternative approaches to conventional treatments.

Materials and Methods

This was a clinic-based experimental study design carried out at the University of Benin Optometry Teaching Clinic, Benin City, Edo State. A convenience sampling technique was used to select fifty- two (52) healthy young undergraduates of the University of Benin. The study was carried out within a period of two months (January and February).

Research Materials:

Hibiscus sabdariffa leaf extracts, Perkins non-contact tonometer, U-MEC mercurial Sphygmomanometer, A weight scale, Keeler Ophthalmoscope, Sprague Stethoscope, Measuring cylinder, Sterile dry plastic containers, Pen and paper and A stop watch

Inclusion Criteria

Age between 18 and 40 years, Normal blood pressure (systolic < 140 mmHg, diastolic < 90 mmHg), Normal intraocular pressure (11-21mmHg), No history of ocular disease or family history of glaucoma, Not taking any forms of medication at the time of the study, participants that provide informed consent to participate in the study, participants in generally good health, without significant chronic medical conditions and participants with no known allergies to *Hibiscus sabdariffa*.

Exclusion Criteria

Age above 40 years, High blood pressure (systolic > 140 mmHg, diastolic > 90 mmHg), High intraocular pressure (>21mmHg), Pregnant individuals or those planning to become pregnant, History of ocular disease or family history of glaucoma, Participants taking any forms of medication at the time of the study, participants that did not provide informed consent to participate in the study, participants with significant chronic medical conditions,

participants with known allergies to *Hibiscus sabdariffa*.

Data Collection Procedures

A total of 50 subjects participated in this study. Subjects were screened to ensure they meet the inclusion criteria before they were able to participate in this study. The screening covered case history, external and internal examination of the anterior and posterior segments of the eyes of each participant, blood pressure measurement, blood sugar measurement, external and internal ocular examinations, using standard testing procedures.

Preparation of the *Hibiscus sabdariffa* Drink

Based on the study by Abubakar *et al*. (2019) and Harmili *et al*. (2021), 30 grams of dried *Hibiscus sabdariffa* leaves were obtained from Uselu market in Benin City. It was weighed respectively with a weigh scale. The leaves were properly rinsed in cold water and thereafter 1 L of boiling water at 100^0C for 10 minutes, which was covered and made to brew for few hours. The extract was sieved and transfer into sterile collecting bottles and administered. A 250 mL of the *Hibiscus sabdariffa* drink was administered to each participant in the intervention group A. Group B were administered orally with 250 mL of distilled water without *Hibiscus sabdariffa* leaf. Thereafter, IOP was measured across the different assessment time 30, 60, 90, and 120 minutes after oral administration of *Hibiscus sabdariffa* using pulsair non-contact tonometer.

Measurement of IOP

The participant was seated comfortably and the procedure was explained to alleviate any anxiety. The participant's chair was adjusted so that their eyes were at the same level as the tonometer. The participant was instructed to look straight ahead and keep their head still. The participant was informed that a light puff of air was directed toward their eye. The tonometer settings were adjusted based on the participant's age and corneal thickness if necessary. The tonometer was properly aligned by looking through the eyepiece and centering the instrument on the participant's eye. The trigger button was pressed to release a short burst of air towards the cornea. The tonometer calculated the intraocular pressure based on the eye's response to the air puff. The IOP measurement for each eye was recorded. Multiple measurements were taken, and the average or the most consistent readings were noted. Where necessary, the procedure was repeated for accuracy, especially if the participants were anxious or if the readings varied significantly. The date, time, and the measurements obtained from the participants were recorded.

Data Analysis

All data in this study was analyzed using the Statistical Package for Social Sciences version 25, (SPSS). A one-way analysis of variance (ANOVA) was used to determine if *Hibiscus sabdariffa* leaf aqueous extract has significant effect on IOP. The Bonferroni repeated measures ANOVA was used to determine at what minute was the difference

most significant. A p-value < 0.05 was considered statistically significant.

Ethical Considerations

Ethical approval was obtained from the Department of Optometry Research Ethics Committee. It was ensured that all participants were fully aware of the potential risks and benefits associated with participating in the study. This experimental study was performed with strict adherence to the Declaration of Helsinki. Informed consent was obtained from every participant using standardised protocol.

Limitations of Study

The study investigating the effects of *Hibiscus sabdariffa* leaf aqueous extract on IOP in normotensive subjects is notable for its clinic-based experimental design. While such a design offers controlled conditions for data collection, it inherently restricts the study's applicability beyond the clinical setting. Findings derived from a controlled environment may not fully reflect real-world scenarios or encompass the diverse demographics encountered outside of the clinic. This limitation could impact the generalizability of the study's conclusions, potentially skewing its relevance to broader populations. Furthermore, the study's utilization of a convenience sampling technique introduces another limitation. Although convenient, this sampling method can introduce selection bias and compromise the representativeness of the sample. As such, the study's sample may not accurately represent the broader population of normotensive individuals, raising concerns about the external validity of its findings. The study's narrow focus on healthy young students at the University of Benin further limits its generalizability. Moreover, the relatively short duration of the study, conducted over two months, may have provided limited insight into the sustained effects of *Hibiscus sabdariffa* leaf extract on IOP regulation. A longer study duration would be necessary to comprehensively evaluate the extract's long-term efficacy and safety profile. Future research endeavours should aim for larger, more diverse samples, rigorous study designs, and extended follow-up periods to enhance the robustness and generalisability of findings in this area.

Results

The proportion of the gender were equally distributed among the participants. However, majority of the participants (34.6%) were aged 24-26 years with a mean age of 22.79 ± 2.645 years (Table 1).

Table 1: Socio-demographics of participants

Variable	Frequency (n)	Percent (%)
Gender		
Male	26	50.0
Female	26	50.0
Age		
18-20	14	26.9
21-23	17	32.7
24-26	18	34.6
27-29	3	5.8
(22.79 ± 2.645)		

As shown in Table 2 of the IOP in the right and left eyes of the experimental group, IOP was measured at baseline, after 30 minutes, after 60 minutes, after 90 minutes and after 120 minutes. The mean IOP in the right eye was 13.23 ± 2.00, 12.54 ± 2.20, 11.77 ± 2.08, 10.92 ± 1.32 and 10.15 ± 1.19, respectively. The mean IOP in the left eye was 13.04 ± 2.49 at baseline, 12.50 ± 2.47 after 30 minutes, 11.65 ± 2.35 after 60 minutes, 11.12 ± 1.71 after 90 minutes and 10.31 ± 1.19 after 120 minutes.

Table 2: Descriptive Statistics of Intraocular Pressure at Baseline, After 30 minutes, 60 minutes, 90 minutes and 120 minutes of *Hibiscus sabdariffa* Leaf Extract Intake

Variable	Mean ± S.D. of IOP measurement	Minimum	Maximum
IOP (RE)			
Baseline	13.23 ± 2.00	10	17
After 30minutes	12.54 ± 2.20	10	17
After 60 minutes	11.77 ± 2.08	10	18
After 90 minutes	10.92 ± 1.32	10	14
After 120 minutes	10.15 ± 1.19	8	13
IOP (LE)			
Baseline	13.04 ± 2.49	10	19
After 30minutes	12.50 ± 2.47	10	18
After 60 minutes	11.65 ± 2.35	9	20
After 90 minutes	11.12 ± 1.71	9	16
After 120 minutes	10.31 ± 1.19	9	13

As shown in Table 3 of the IOP measured in participants who received *Hibiscus sabdariffa* leaf extract. Measurements were done at baseline, 30 minutes after, 60 minutes, 90 minutes and 120 minutes after the extract was ingested. Repeated measures ANOVA showed that the extract had a significant effect of IOP reduction on the right eye across the time points [F (2.582, 64.557) = 27.720, $p < 0.001$]. Similarly, there was a significant effect of IOP reduction on the left eye [F (2.206, 55.161) = 17.897, $p < 0.001$].

Table 3: Repeated Measures ANOVA of IOP in Both Eyes

Variable	Type III Sum of Squares	df	Mean Square	f	*p*-value
IOP, RE	157.108	2.582	60.840	27.720	<0.001*
IOP, LE	122.492	2.206	55.516	17.897	<0.001*

*: *statistically significant*

As shown in Table 4 of the right eye, after 30 minutes of extract ingestion, there was a mean difference of 0.692 mmHg in IOP compared to baseline, which was not statistically significant ($p = 0.679$). However, after 60 minutes, there was a significant reduction in IOP by 1.462 mmHg ($p =$

0.018). Subsequent time intervals showed further significant reductions: at 90 minutes post-ingestion, the mean IOP decreased by 2.308 mmHg compared to baseline (p = 0.000), and at 120 minutes post-ingestion, the mean IOP decreased by 3.077 mmHg compared to baseline (p = 0.000).

Table 4: Pairwise Comparison Between IOP (RE) Across at Different Levels of Measurements

Variables	Mean Difference	Std Error	*P*-value
Baseline IOP RE * After 30 minutes	0.692	0.363	0.679
Baseline IOP RE * After 60 minutes	1.462*	0.420	0.018*
Baseline IOP RE * After 90 minutes	2.308*	0.354	0.000*
Baseline IOP RE * After 120 minutes	3.077*	0.350	0.000*

As shown in Table 5 of the left eye, following the ingestion of the extract, there was a mean difference of 0.538 mmHg in IOP compared to baseline after 30 minutes, which did not show statistical significance (p = 1.000). Similarly, after 60 minutes, there was a non-significant reduction in IOP by 1.385 mmHg (p = 0.125). However, subsequent time intervals demonstrated significant reductions: at 90 minutes post-ingestion, the mean IOP decreased by 1.923 mmHg compared to baseline (p = 0.003), and at 120 minutes post-ingestion, the mean IOP decreased by 2.731 mmHg compared to baseline (p = 0.00).

Table 5: Pairwise Comparison Between IOP (LE) Across at Different Levels of Measurements

Variables	Mean Difference	Std Error	*P*-value
Baseline IOP LE * After 30 minutes	.538	.373	1.000
Baseline IOP LE * After 60 minutes	1.385	.515	.125
Baseline IOP LE * After 90 minutes	1.923*	.457	.003*
Baseline IOP LE * After 120 minutes	2.731*	.413	.000*

As displayed in Table 6, the IOP in the right and left eyes of the control group was measured at baseline, after 30 minutes, after 60 minutes, after 90 minutes and after 120 minutes. The mean IOP in the right eye was 12.73 ± 2.089, 12.77 ± 2.006, 12.73 ± 1.909, 12.62 ± 1.981 and 12.69 ± 1.828, respectively. The mean IOP in the left eye was 12.92 ± 2.382 at baseline, 12.73 ± 2.127 after 30 minutes, 12.62 ± 2.174 after 60 minutes, 12.54 ± 2.177 after 90 minutes and 12.50 ± 2.177 after 120 minutes.

Table 6: Descriptive Statistics of Intraocular Pressure at Baseline, After 30 minutes, 60 minutes, 90 minutes and 120 minutes in Control Group

Variable	Mean ± S.D.	Minimum	Maximum
IOP (RE)			
Baseline	12.73 ± 2.09	10	17
After 30minutes	12.77 ± 2.01	10	17
After 60 minutes	12.73 ± 1.91	10	17
After 90 minutes	12.62 ± 1.98	10	17
After 120 minutes	12.69 ± 1.83	10	16
IOP (LE)			
Baseline	12.92 ± 2.38	9	17
After 30minutes	12.73 ± 2.13	10	18
After 60 minutes	12.62 ± 2.17	9	17
After 90 minutes	12.54 ± 2.18	10	17
After 120 minutes	12.50 ± 2.18	10	17

As shown in Table 7, the IOP was measured in the control participants. Measurements were done at baseline, 30 minutes after, 60 minutes, 90 minutes and 120 minutes. The Pearson distribution of the intra ocular pressure that there was no significant difference in IOP in the right eye across the time points [F (2.362, 59.051) = 0.424, p = 0.689]. Similarly, there was no significant difference on IOP in the left eye [F (2.436, 60.911) = 2.768, p = 0.06]

Table 7: The Pearson distribution of the intra ocular pressure

Variable	Type III Sum of Squares	df	Mean Square	F	*P*-value
IOP, RE	.354	2.362	.150	.424	.689
IOP, LE	3.031	2.436	1.244	2.768	.060

As shown in Table 8 of the right eye, following ingestion, there was a negligible mean difference of -0.038 mmHg in IOP compared to baseline after 30 minutes, which showed no statistically significant change (p = 1.000). Similarly, after 60 minutes, the mean IOP remained unchanged at 0.000 mmHg compared to baseline, with no significant difference observed (p = 1.000). Subsequent time intervals demonstrated further insignificant reductions: at 90 minutes post-ingestion, the mean IOP decreased by 0.115 mmHg compared to baseline (p = 1.000), and at 120 minutes post-ingestion, the mean IOP decreased by 0.038 mmHg compared to baseline (p = 1.000).

Table 8: Pairwise Comparison Between IOP (RE) Across at Different Levels of Measurements

Variables	Mean Difference	Std Error	*P*-value
Baseline IOP RE * After 30 minutes	-.038	.130	1.000
Baseline IOP RE * After 60 minutes	.000	.157	1.000
Baseline IOP RE * After 90 minutes	.115	.178	1.000
Baseline IOP RE * After 120 minutes	.038	.152	1.000

As shown in Table 9 of the left eye, following ingestion, there was a minimal mean reduction of 0.192 mmHg in IOP compared to baseline after 30 minutes, which was statistically insignificant (p = 1.000). Similarly, after 60 minutes, the mean reduction in IOP compared to baseline was 0.308 mmHg, with no significant difference observed (p = 1.000). Subsequent reductions were also insignificant: at 90 minutes post-ingestion, the mean IOP decreased by 0.385 mmHg compared to baseline (p = 0.476), and at 120 minutes post-ingestion, the mean IOP decreased by 0.423 mmHg compared to baseline (p = 0.246).

Table 9: Pairwise Comparison Between IOP (LE) Across at Different Levels of Measurements

Variables	Mean Difference	Std Error	*P*-value
Baseline IOP LE * After 30 minutes	.192	.184	1.000
Baseline IOP LE * After 60 minutes	.308	.182	1.000
Baseline IOP LE * After 90 minutes	.385	.185	.476
Baseline IOP LE * After 120 minutes	.423	.177	.246

As shown in Table 10 of all time intervals examined, there was no significant relationship between the age and gender of participants and the fluctuations in their IOP subsequent to ingesting *Hibiscus sabdariffa* leaf aqueous extract (p > 0.05). Conversely, there were significant relationships identified between the participants' baseline IOP levels and the alterations in their IOP subsequent to ingesting *Hibiscus sabdariffa* leaf aqueous extract (p < 0.05).

TABLE 10: Variations in Response of *Hibiscus sabdariffa* Leaf Aqueous Extract and Participants' Socio-Demographics

Eye	Variables	Chi-Square P-Value
OD	**Age**	0.066
	Gender	0.577
	Baseline IOP	0.006
OS	**Age**	0.252
	Gender	0.107
	Baseline IOP	0.000

Discussion

The subsequent sections explored the observed changes in IOP among both experimental and control groups over various time intervals (baseline, 30, 60, 90, and 120 minutes), along with an examination of how factors like age, gender, and baseline IOP influenced the response to the extract.

The study findings indicated a significant reduction in IOP in both the right and left eyes over time intervals, with statistically significant effects observed ($p < 0.001$). The peak reduction in IOP occurred at 120 minutes post-ingestion. Notably, the control group did not exhibit any significant changes in IOP, suggesting that the observed effects in the experimental group were likely attributable to the ingestion of the extract. These significant reductions in IOP following the consumption of *Hibiscus sabdariffa* leaf aqueous extract suggest its potential as a natural intervention for enhancing ocular health. In light of previous studies demonstrating the blood pressure-lowering effects of rosella (*Hibiscus sabdariffa*) flowers in hypertensive patients (Ritonga *et al*., 2017; Harmili *et al*., 2021), the significant reduction in IOP observed following the ingestion of *Hibiscus sabdariffa* leaf aqueous extract in our study underscores it potential on ocular health. Furthermore, studies indicating a correlation between decreased blood pressure in hypertensive patients and reduced IOP further support the notion that *Hibiscus sabdariffa* may offer promising avenues for reducing IOP (Devadas *et al*., 2017; Tiambeng *et al*., 2022).

Furthermore, at all time intervals examined, it was observed that there was no significant relationship between the age and gender of participants and the fluctuations in their IOP subsequent to ingesting *Hibiscus sabdariffa* leaf aqueous extract ($p > 0.05$). Conversely, there were significant relationships identified between the participants' baseline IOP levels and the alterations in their IOP after ingesting *Hibiscus sabdariffa* leaf aqueous extract ($p < 0.05$). The lack of significant correlation between age and gender and IOP fluctuations implies that the effects of the extract on IOP may not be influenced by demographic factors such as age or gender. However,

the significant relationship between baseline IOP levels and changes in IOP suggests that individuals with higher baseline IOP levels may experience greater reductions in IOP after consuming the extract. This highlights the importance of considering baseline IOP levels when assessing the efficacy of *Hibiscus sabdariffa* leaf aqueous extract as a potential treatment for managing IOP. A study conducted by Asaoka *et al*. examined the effect of various systemic factors on the longitudinal alteration of IOP. They identified age and gender, along with other factors such as aspartate aminotransferase, hemoglobin, platelet count, calcium levels, alanine aminotransferase, guanosine triphosphate, white blood cell count, and red blood cell count, as significant systemic variables associated with changes in IOP, a finding inconsistent with our own study results (Asaoka *et al*., 2022). However, it is worth noting that their investigation did not specifically target the consumption of *Hibiscus sabdariffa* leaf aqueous extract.

In contrast, Agarwal *and coworkers* conducted a study examining the relationship between baseline IOP levels and changes in IOP following the administration of a 0.5% drop of *Ocimum basilicum* herb extract (Agarwal *et al*., 2019). Their results showed a significant correlation between baseline IOP levels and fluctuations in IOP, mirroring our own findings, thus offering a relevant comparison point for our study outcomes.

Conclusion

In conclusion, this study showed that the aqueous extract of *Hibiscus sabdariffa* leaf exerts a significant reduction in intraocular pressure in both eyes of normotensive subjects. Additionally, no statistically significant associations were found between the age and gender of normotensive individuals and the variations in their intraocular pressure following the administration of *Hibiscus sabdariffa* leaf aqueous extract. Conversely, significant correlations were detected between the baseline intraocular pressure levels and the changes observed in intraocular pressure subsequent to the consumption of *Hibiscus sabdariffa* leaf aqueous extract.

Recommendation

This study recommends the conduct of additional research to explore the long-term effects of *Hibiscus sabdariffa* leaf aqueous extract on intraocular pressure. Longitudinal studies could provide valuable insights into the sustained efficacy and safety profile of this extract over extended periods.

This study recommends an investigation into the optimal dosage of *Hibiscus sabdariffa* leaf aqueous extract for intraocular pressure reduction. This could involve testing different concentrations or formulations of the extract to determine the most effective and well-tolerated dose.

This study recommends the initiation of clinical trials to evaluate the efficacy of *Hibiscus sabdariffa* leaf aqueous extract as a potential treatment for ocular conditions characterized by elevated intraocular pressure, such as glaucoma.

This study suggests that eye care professionals could provide education and information to patients about the potential benefits and risks of using *Hibiscus sabdariffa* leaf aqueous extract for managing intraocular pressure.

This study recommends the integration of *Hibiscus sabdariffa* leaf aqueous extract as a complementary therapy in the management of some ocular conditions.

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