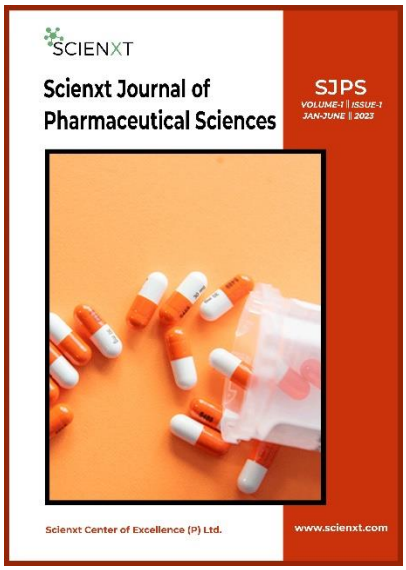


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Preventive effect of lepidium sativum seed extract and Zinc containing nutritional supplement on age related cataract progression in wistar rats

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Abstract:

Our study aimed to investigate properly about the potential preventive effect of a nutritional supplement containing Lepidium Sativum seed extract with psidium guajava fruit extract and zinc on age related progression in Wistar rats.Our supplement was formulated to include vitamins (C, B3, E, B1, B2, B5, B6, folic acid, A, D3, biotin, B12) with minerals (zinc, magnesium, chromium, molybdenum) and natural extracts (Lepidium Sativum seed, Psidium Guajava fruit extract).Wistar Rats were divided into control and experimental groups and with their health markers and aging related parameters were assessed by us over a specified period. Our results indicated a very potential role of the supplement in mitigating age related progression in the experimental group.

Keywords:

Lepidium Sativum seed, multivitamin, Psidium Guajava fruit extract, Wistar Rats, anti-inflammatory, Sorbitol dehydrogenase

1. Introduction:

We know aging is a complex of biological process associated with various physiological changes as including oxidative stress, inflammation with declining nutrient absorption. The nutritional interventions by using bioactive compounds and essential nutrients have shown promise in attenuating age related decline. Lepidium Sativum seed extract, Psidium Guajava fruit extract and zinc have individually demonstrated potential health benefits including antioxidant and antiinflammatory properties. Our study investigates the combined effect of these components in preventing about age related progression in a Wistar rat model.

2. Materials and Proper Methods:

Our formulated multivitamin tablets as this our invented formulation done by a company with Lepidium Sativum seed extract and psidium guajava fruit extracts we collected from Gujrat by Lab. Our formulated one tablet contains Lepidium sativum seed extract with psidium guajava fruit extract to other constituents as described in Table-1 was perfectly crushed and dissolved in 100ml of the normal saline 0.09% sodium chloride solution. After subcutaneously the solution injected to different treatment groups (37,72 and 152mg/kg). As glutathione reductase, Sodium Selenite, NADPH, Reduced glutathione, TCA, TBA and DTNB collected by our Lab. As we have one touch glucometer (Accu-Chek Active) with glucose oxidase peroxidase reactive strips our lab purchased. Sorbitol dehydrogenase, Aldose reductase and catalase assay kits also available in our lab.Glucose-6-phosphate dehydrogenase and ATP kits also available and others like potassium chloride, sodium azide, sodium hydroxide, sodium chloride, EDTA all others we collected by an agent. We investigate that the reagents were properly analytical grade. As phosphate buffers were prepared everyday as all reagents except and properly stored in a refrigerator of +4°C.At room temperature for 29min56sec before use the reagents were equilibrated as start of the analysis or when it will be refilled by reagent containers. For one month phosphate buffers were stable at +4°C for 1month1day.

Table. 1: Composition of our formulated tablet.

Ingredients	**Per tablet contains**
Vitamin C	70 mg
Vitamin B^3	55 mg

Vitamin E	13 mg
Vitamin B^1	12 mg
Vitamin B^2	8 mg
Vitamin B^5	8 mg
Vitamin B^6	2 mg
Folic acid	0.5 mg
Vitamin A	4500 IU
Vitamin D^3	200 IU
Biotin	100 mcg
Vitamin B^{12}	8.5 mcg
Zinc	15 mg
Magnesium	12 mg
Chromium	15 mg
Molybdenum	15 mg
Lepidium Sativum seed Extract	15 mg
Guava fruit extract	15 mg

2.1. Animals:

Wistar rats pups of 8 days old with their mother were properly housed in separate polypropylene cages maintained under very stable conditions of 23-239C,11hr58min dark/11hr58min light cycle relatively air humidity of 41-59%.To normal calorie rats had continuously access of rat pellet diet and to the aquaguard purified water. Animals were

acclimatized after grouping to the laboratory conditions for one week before experiment start. From their mother animals described as fasted were properly separated and for 16hr deprived of food but free access of water allowed. We must follow the ethical guidelines after we use.

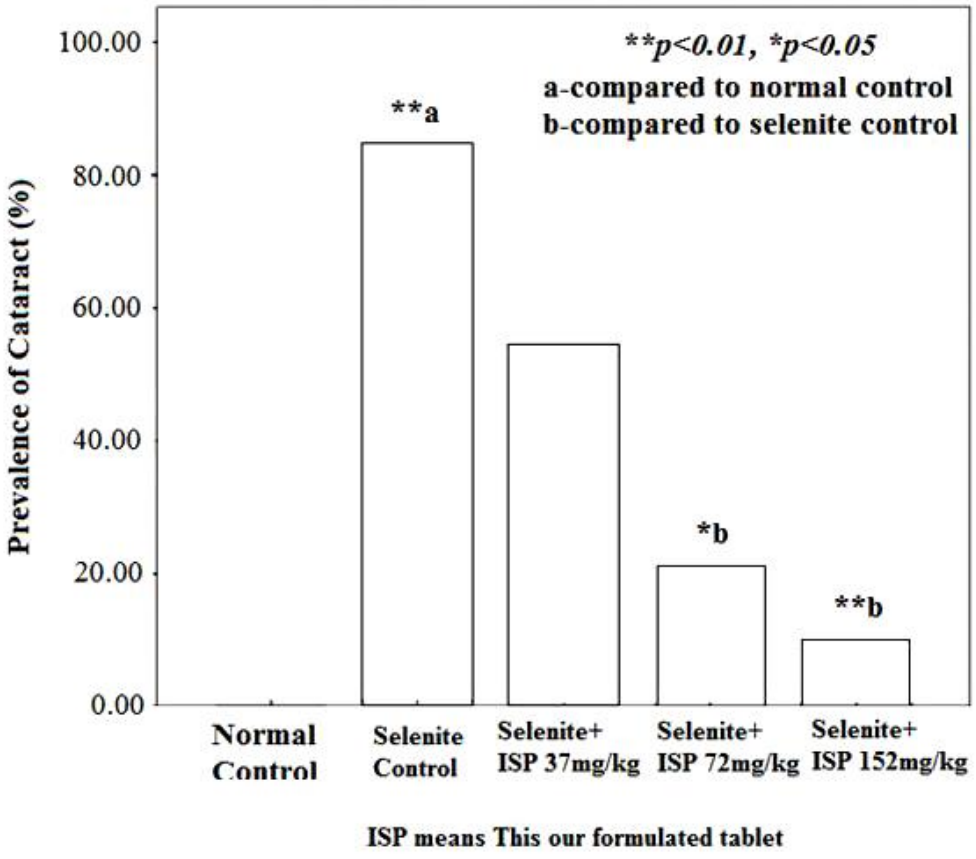


Figure. 1: Effect of different doses of our formulation of Lepidium seed extract, Guava fruit extract with our formulated tables on prevalence of the sodium selenite induced semile cataract among experimental groups

2.2. Design of the Experiment:

30 of wistar rats pups we divided in the five groups. Our formulated tablets with lepidium sativum seed extract and psidium guajava fruit extract were properly administered of one day prior of sodium selenite 10µmoles/kg subcutaneously as addition of regular treatment. As we continued treatment of day 8 to day 20 of their post natal life as follow.No1 Group-we give control rat pups 0.1ml of normal saline subcutaneously, No. 2 Group-As cataract control of sodium selenite induced rat pups were properly given 0.1ml subcutaneously, No.3 Group-As cataract control of sodium selenite induced we must given our formulated tablet 37mg/kg/day

with psidium guajava fruit extract and Lepidium sativum seed extract subcutaneously.No4 group-by cataract of rat pups sodium selenite induced of by given our formulated tablet with lepidium sativum seed extract and psidium guajava fruit extract 72mg/kg/days ubcutaneously.No5 group-cataract rats pups of sodium selenite induced were given our formulated tablet with lepidium sativum seed extract and psidium guajava fruit extract of 152mg/kg/day subcutaneously.

2.3. Collection of blood, lens and processing:

When rat pups first opened their eyes approximately 17days after birth the cataract formation and prevention properly monitored by examining the eye as in the lens cloudy area with a mini pen light illumination thereafter end of the experiment and for isolated lenses subsequent photography was done 21st day at the end of the experiment each of the rat pup body weighted properly before start. all the rat pups were sacrificed for the overdose of Thiopentone Sodium. As dissected the eye lenses of posterior approach and stored at -70°C after analysis. Each rat pups lens were weighted. As drawn the fasting blood samples on from retro orbital plexus of all experimental animals by using the capillary tube for proper estimation of blood glucose by help of glucose oxidase-peroxidase reactive strips. As lens perfectly homogenates 10%w/v were well prepared from three of the five pooled lenses of 50mM of potassium phosphate buffer pH-7.4 by using Remi homogenizer. The cell debris and unbroken cells perfectly removed by centrifugation at 10000rpm for 20minutes by using Remi C-24 refrigerated centrifuge. At -79°C the resulting supernatant stored. In the soluble fraction all of the biochemical parameters analyzed of lens homogenate except for the malondialdehyde determined for proper homogenate as our research following done in totally triplicate manner and we read properly about the optical density of reagent.

2.4. Malonadlehyde level determination:

As homogenate sample of 20µl lens, 200µl of 0.67% of thiobarbituric acid and 100µl of 20% trichloroacetic acid were added was centrifuged at 12000rpm of 4min59sec and as supernatant of 100µl was transferred to the 96 proper wells of micro test plate. Supernatant of optical density was read of 500nm by using Elisa.

2.5. Reduced glutathione level determination:

100µl of lens tissue homogenate mixture with 100µl of trichloro acetic acid was properly centrifuged at 5000rpm for 4min59sec. After tissue supernatant of 25µl, 150µl of sodium

phosphate buffer as pH-8, PBS-0.2M with 25µl DTNB of 0.6mM added together of the 96wells of micro test plate and incubated for 9min59sec at room temperature after we read the absorbance it will be 380nm by using ELISA. Glutathione peroxidase determination-0.1mM EDTA pH will be 6.5 by 550µl phosphate buffer will be 100mM,50µl lens tissue homogenate, 50µl of sodium axide 2mM, glutathione reductase of 100µl of 2.5U/ml and glutathione (100mM, GSH) incubated at 36°C for 9min 57sec and added together. After in the above mixture 100µl hydrogen peroxide (1.5mM) and optical density read 315nm at one minute of the interval for 4min59sec by taking UV-2450 Spectrophotometer.

Table. 2: Effect of our formulation of Lepidium sativum seed extract, guava fruit extract with our formulated tablets on reduced glutathione (µmoles/mg) and glutathioneperoxidase (µmoles/ml) with Glucose-6-phosphate dehydrogenase (µmoles/min/ml) in the lens tissue homogenate.

Groups (n=6)	**GSH (Q1, Q3)**	**GPx (Q1, Q3)**	**G6PD (Q1, Q3)**
I- Normal control (2% gum acacia)	67.87 (44.13, 103.07)	334.69 (203.78, 457.85)	40.20 (25.93, 76.58)
II- Selenite control (2% gum acacia)	25.41 (22.57, 61.62)	89.79 (75.92, 270.57)	16.45 (14.55, 43.71)
III- Selenite +ISP (37 mg/kg/day)	20.90 (15.35, 26.10)	90.66 (58.31, 117.32)	15.33 (10.73, 18.74)
IV- Selenite + ISP (72mg/kg/day)	16.76 (14.29, 56.83)	88.33 (57.32, 237.91)	19.18 (10.63, 39.09)
V- Selenite + ISP (152 mg/kg/day)	76.75 (58.44, 105.52)	324.00(158.58, 360.24)	51.88(42.05, 77.82)
	p = 0.004	p = 0.017	p = 0.027

n- Number of the rats in each of the group. As GSH- Reduced glutathione, GPx- Glutathione peroxidase with G6PD- Glucose-6-phosphate dehydrogenase. Data are expressedproperly as the median (quartiles- Q1, Q3) and there are different treatments were analyzed properly by non-parametric K Independent sample test followed by Kruskal-Wallis H test.

Table. 3: Effect of our formulation of Lepidium sativum seed extract, guava fruit extract with our formulated tablets on protein thiol (µmoles/mg), adenosine triphosphate(mmoles/ml) with malondialdehyde (nmoles/g) in lens tissue homogenate.

Groups (n=6)	PT (Q3, Q3)	ATP (Q3, Q3)	MDA (Q3, Q3)
I- Normal control (2% gum acacia)	823.88 (485.09, 1090.42)	0.92 (0.14, 1.96)	25.40 (20.32, 29.64)
II- Selenite control (2% gum acacia)	368.12 (310.98, 840.68)	0.26 (0.14, 1.48)	25.40 (22.86, 54.20)
III- Selenite +ISP (37 mg/kg/day)	282.08 (209.27, 317.10)	0.185 (0.06, 0.51)	33.87 (19.48, 49.12)
IV- Selenite + ISP (72mg/kg/day)	219.73 (206.54, 402.17)	0.19 (0.12, 0.27)	22.01(20.32, 24.55)
V- Selenite + ISP (152 mg/kg/day)	1083.89 (472.74, 1547.86)	0.84 (0.62, 1.72)	18.62 (16.08, 20.32)
	p = 0.020	p = 0.042	p = 0.022

n- Number of rats in each OF THE group. PT- Protein thiol with ATP- Adenosine triphosphate and MDA- Malondialdehyde. Data are expressed as for the median (quartiles- Q1, Q3) and about the different treatments were properly analyzed by non-parametric K Independent sample test followed by the Kruskal-Wallis H test.

2.6. Protein thiol level determination:

Homogenate sample of 20µl of lens tissue properly added of 180µl of disodium edetate as disodium edetate of 2mM in 0.2 M disodium hydrogen phosphate of the buffer solution with DTNB solution of 4µl as DTNB 10mM of the 0.2M disodium hydrogen phosphate as wells of

96 of the micro test plate. After we read the optical density of 380nm by using ELISA.

2.7. Superoxide dismutase activity determination:

By using homogenate lens tissue 25µl the 925µl of sodium carbonate buffer (0.1M, pH-10-11) and adrenaline bitrate 50µl of 1mM was properly added and absorbance (A0s-A60s) we read 440nm by using UV-2450_spectrophotometer. Catalase activity determination- As we measured catalase activity in lens homogenate to the standard protocol given along with bioassay systems of catalase assay kit.

2.8. Glucose-6-Phosphate dehydrogenase activity determination:

In lens homogenate glucose-6-phosphate dehydrogenase according we maintained standard protocol of the glucose-6-phosphate assay kit by using ELISA reader of Biotek Instruments EL×800- MS.

2.9. Adenosine Triphosphate level determination:

As measured according to the standard protocol Adenosine Triphosphate given with Adenosine Triphosphate assay kit as using ELISA reader EL×800- MS. Aldose Reductase Level determination-In lens homogenate was measured aldose reductase concentration to standard protocol given with aldose reductase assay kit.

2.10. Sorbitol dehydrogenase level determination:

In lens homogenate sorbitol dehydrogenase level properly measured by kit.

Statistical Analysis-For statistical analysis we use ANOVA as follow post hoc tukey test. We did this statistical analysis. As quartiles Q1 and Q3 expressed as median by data with non-uniform distribution and for K-independent samples test which is non-parametric analyzed and we followed by Kruskal-Wallis H test.As $p \leq 0{\cdot}05$ was two-sided considered to statistically significant.

3. Results and Discussion:

Effect of Lens Morphology-In 17th day about the cataract formation with its prevention properly monitored of post natal of life after first opening of eyes._As received 0.1ml normal saline received for all six rat pups as in normal control group will exhibited a very completely transparency of lens._As we out 12lenses of eyes of six rat pups must induced sodium selenite

senile cataract control group as 10 of the eye lenses for exhibited a very dense opacification. As in the contrast the 2,6 and 1among lenses received our formulation with lepidium sativum seed extract with psidium guajava fruit extract tablets 37,72 and 152mg/kg exhibited a very mild lenticular opacification.

3.1. Body weight and isolated lens weight effect:

As our evaluation we follow that there will be no significant changes in body as well of isolated lens weight of the experimental group.

3.2. Biochemical parameters effect:

We followed the significant changes of glutathione (p=0.004), glutathione peroxidase of (p=0.017), glucose-6-phosphate dehydrogenase (p=0.027), protein thiol (p=0.020) and phosphate triphosphate (p=0.042) in as lens of the rat pups treated with our formulation with lepidium seed extract and guava fruit extract.

In table. 2 and table. 3 we used our formulation in a dependent manner when compared with the senile cataract control of rat pups of sodium selenite induced (p=0.022) as we saw that lens malondialdehyde level is significantly lower of rat pups treated with our formulation with lepidium sativum seed extract and guava fruit extract in table. 3. In fasting blood glucose level there will be no significant changes and lens catalase, aldose reductase, superoxide dismutase and sorbitol dehydrogenase among the experimental groups. Our preliminary findings suggest about the nutritional supplement containing Lepidium Sativum seed extract, psidium guajava fruit extract and zinc may have a potential preventive effect on age related progression in Wistar rats. Our experimental group displayed improved antioxidant status that reduced inflammation markers and attenuated cellular senescence compared about the control group. Additionally, we say that the combination of vitamins, minerals, and natural extracts contributed to overall positive impact on various health parameters.

Our results determinants further investigation about the molecular mechanisms underlying the observed effects and for long term implications of this nutritional intervention. Our research study suggests that the combination of Lepidium Sativum seed extract, psidium guajava fruit extract and zinc along with a comprehensive array of nutrients, could hold promise as a preventive strategy against age related decline. Further research is mostly needed to validate these findings and explore about the potential applications for animal and human aging.

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