WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 13, 1468-1482. Research Article ISSN 2277– 7105

ANTIOXIDANT AND IMMUNOMODULATORY ACTIVITY OF VRISHYAKSHEERA YOGA GRANULES

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Article Received on
02 August 2022,

Revised on 23 August 2022,
Accepted on 13 Sept. 2022

DOI: 10.20959/wjpr202213-25593

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ABSTRACT

Vrishyaksheera yoga is a polyherbal formulation mentioned in the context of *Vajikarana.* It is prepared with the drugs like *Aswagandha, Shatavari, Yashtmadhu, Kharjura, Masha, Mridwika, Kharjuramastaka* and *Kapikacchu* in the form of *Ksheerapaka.* Considering the inconveniences in the preparation of *Ksheerapaka,* an attempt has been made to convert it into granule form which is to handle with. But for its wider acceptance and clinical applicability, further evaluation in the form of Antioxidant and immunomodulatory effect of VKG in Experimental models is essential. The antioxidant activity of VKG was assessed by the activity of SOD, Catalase, GSH and concluded with significant results. Similarly, the immunomodulatory effect of VKG was analyzed by haematological parameters, relative organ weight and bone marrow cellularity with the results of moderate immunomodulatory activity.

KEYWORDS: Antioxidant, Immunomodulatory, *Vrishyaksheera yoga*, Granules, new dosage form.

INTRODUCTION

In this modern industrialised era, people are habituated to stress, anxiety, improper lifestyle and food habits which can produce an oxidative stress in the body producing more number of free radicles. It can cause various ailments as a sequel like inflammatory diseases, diabetes, cancers, atherosclerosis, asthma, Alzheimer's diseases and ultimately ageing. In order to tackle these free radicles and subsequent disorders a powerful antioxidant is necessary.

Although the human body has its own defences against oxidative stress, these become weak with age or in the case of an illness. These cytotoxic free radicals not only raise the oxidative stress but also play an important role in the immune-system dysfunction. Immune cells use ROS in order to support their functions and therefore need adequate levels of antioxidant defences in order to avoid the harmful effect of an excessive production of ROS. So, each cell in immune system will undergo stress and it may be overcome by the help of antioxidants only. So antioxidants are the need for the hour.

The development of nutraceuticals is a great revolution in this regard and has been enlarged in this scenario. The *Vrishya ksheera yoga* mentioned in *Charaka Samhitha*[1] is one of the herbal formulation that offers protection against disease and helps to maintain health. The modification of that yoga to a more acceptable form can be a new innovation. But for its wider acceptance and clinical applicability, further evaluation in the form of Antioxidant and immunomodulatory effect of *Vrishyaksheera yoga* granules (VKG) in Experimental models will be helpful. Hence an attempt is made to review the conducted study.

MATERIALS AND METHODS

Drug preparation

The *Vrishyaksheera yoga* was prepared according to the classical reference which was later converted in the form of granules as per the reference of *Khanda kalpana.*[2]

Animals

All animal experiments were conducted after getting prior permission from the Institutional Animal Ethics Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), ministry of Environment and Forest, Government of India. IAEC approval number is ACR/IAEC/21(1)-P5. Male Swiss albino mice (25 - 30 g) were purchased from the Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala. They were housed in the animal house of Amala Cancer Research Centre, in well ventilated sterile polypropylene cages. Each cage contained 5 mice. They were maintained at a controlled temperature of 22±2 ^{0} C and relative humidity 60±10% and provided 12 h light/dark cycles. Experiments were started after acclimatizing the mice for 2 weeks. They were fed with normal pelleted chow (Sai Durga Feeds and Foods, Banglore, India) and water *ad libitum*.

Dose fixation

The doses for the study was fixed by converting human dose to animal dose on the basis of body surface area ratio by referring to the table of Paget and Barnes (1969)

Human dose × conversion factor (0.0026) for mice = X gm/20 gm mice

Human dose of *VKG* = 12 g/day ie, 12000 mg

Mice dose = 12000 × 0.0026 = 31.2 mg/g (for 20 gm mice)

Therapeutic dose administered: 31.2 mg /kg.b.wt

Lower dose administered: 15.6 mg /kg.b.wt

Route of drug administration

- *Vrishya Ksheera Yoga Granules* were mixed in water and administered through an oral feeding cannula attached to a 1 ml syringe.
- Sodium fluoride (NaF) in the prescribed dose was administered through drinking water.
- Cyclophosphamide (CTX) was mixed in water and administered orally through an oral feeding cannula attached to a 1 ml syringe.

PROTECTIVE EFFECT OF *VKG* ON SODIUM FLUORIDE INDUCED OXIDATIVE STRESS IN MICE

Experimental groups

The Swiss albino mice were divided into the following 5 groups of 5 animals;

- Group1 : Normal/untreated
- Group 2 : Control (NaF - 600 ppm/L/day)
- Group 3 : *VKG* (31.2mg/kg b.wt)) + NaF
- Group 4: *VKG* (15.2 mg/kg.b.wt.) + NaF
- Group 5: Standard (Vitamin C 15 mg/kg.b.wt.) + NaF

Experimental protocol

The Swiss albino mice were weighed and grouped into 5 according to their body weights, with 5 mice in each group. Animals in group 1 served as normal and received regular rodent feed and potable drinking water *ad libitum*. The animals in group 2, 3, 4 and 5 were given NaF (600 ppm/L/day) along with drinking water from the 8th day onwards and continued for another seven consecutive days to induce oxidative stress. *VKG* and vitamin C were given orally once a day to animals for seven days before NaF treatment and continued for another seven consecutive days (Nabavi et al., 2012). On the 15th day animals were weighed and

sacrificed in a carbon dioxide chamber. Blood samples were collected by cardiac puncture and used for the estimation of enzymatic antioxidants such as SOD (McChord and Fridovich, 1969) and catalase (Aebi, 1974) as well as non-enzymatic antioxidant, GSH (Moron *et al.*, 1979).

IMMUNOMODULATORY ACTIVITY OF *VKG*

Protocol without Cyclophosphamide

Experimental groups

The male Swiss albino mice were divided into the following 2 groups containing 3 animals.

Group 1: Normal/untreated

Group 2: *VKG* 31.2mg/kg b.wt

Experimental protocol

The Swiss albino mice were weighed and grouped into 2 according to their body weights, with 3 mice in each group. Animals in group 1 served as normal and received regular rodent feed and potable drinking water *ad libitum*. Animals in group 2 received *VKG* orally for 7 days. Any behavioural and clinical changes throughout the experiment were recorded. On the 7th day animals were weighed and sacrificed in a carbon dioxide chamber. Blood samples were collected by cardiac puncture for the estimation of haematological parameters and bone marrow also collected from both femurs. The spleen and thymus were dissected out carefully, washed with saline solution and preserved at -80^{0}C which were later taken and weighed.

PROTOCOL WITH CYCLOPHOSPHAMIDE

Experimental groups

The animals were divided into the following 3 groups containing 5 animals;

Group 1: Normal/untreated

Group 2: Control (CTX 50 mg/kg b.wt.)

Group 3 : *VKG* 31.2mg/kg b.wt. + CTX

Experimental protocol

The Swiss albino mice were weighed and grouped into 3 according to their body weights, with 5 mice in each group. Animals in group 1 served as normal and received regular rodent feed and potable drinking water *ad libitum. VKG* (31.2 mg/kg b.wt) were administered orally to group 3 for 7 days. Animals in group 2 and 3 were treated with Cyclophosphamide (CTX 50 mg/kg b.wt.) orally for 7 days to induce immunosuppression (Pratheeshkumar and Girija

Kuttan, 2010). Behavioural and clinical changes throughout the experiment were recorded. On the 7th day animals were weighed and sacrificed in a carbon dioxide chamber. Blood samples were collected by cardiac puncture for the estimation of haematological parameters and bone marrow was also collected. The spleen and thymus were dissected out carefully, washed with saline solution and preserved at -80^{0}C which were later taken and weighed.

STATISTICAL ANALYSIS

Values are expressed as mean ±SD and compared to control. The statistical analysis was done by one way analysis of variance (ANOVA) followed by Dunnetts test. P values-$p<0.05$* and $p<0.01$** were considered as significant and $p>0.05$ as non-significant compared to control group.

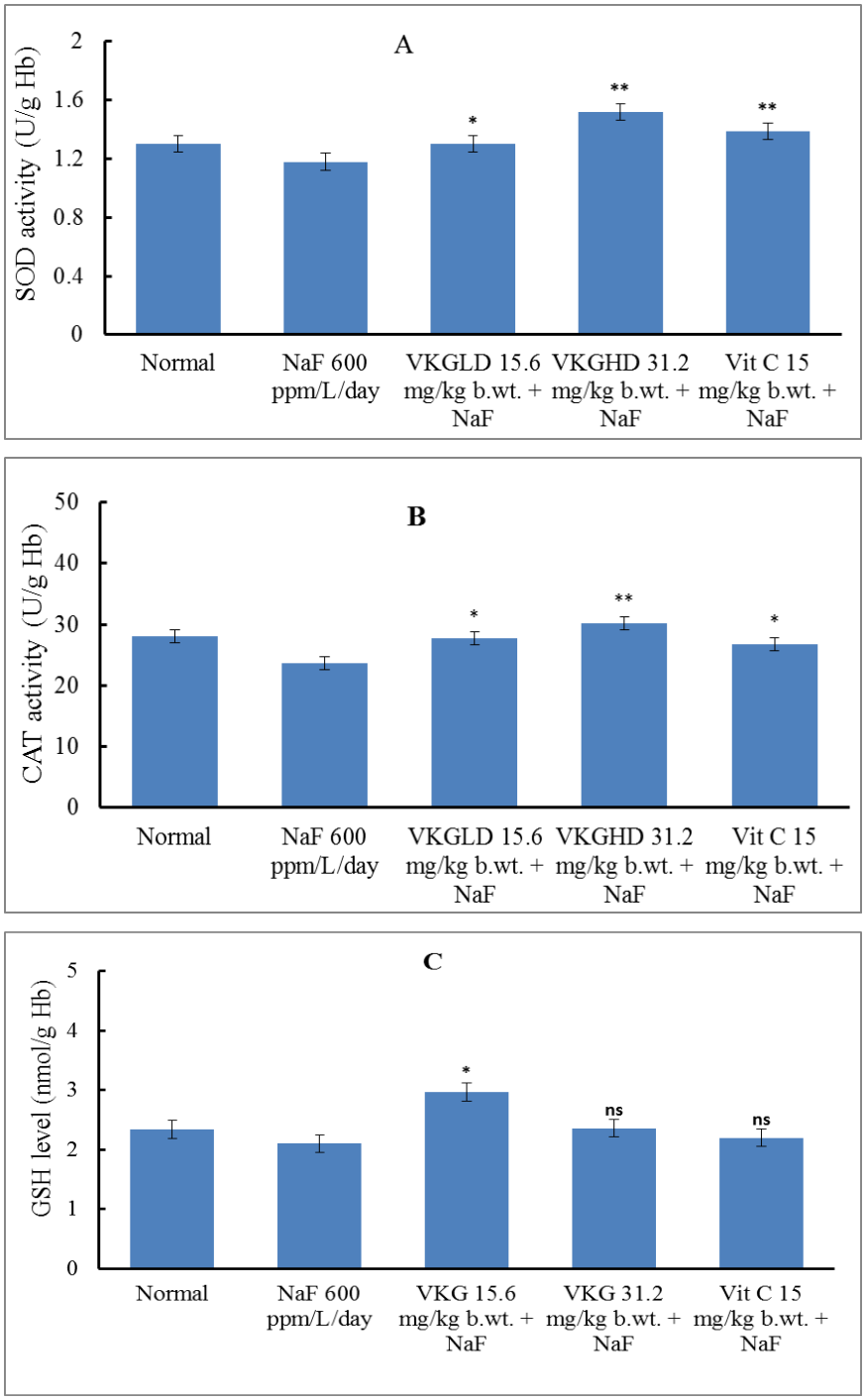
OBSERVATIONS AND RESULTS

PROTECTIVE EFFECT OF VRISHYAKSHEERA YOGA GRANULES (*VKG*) AGAINST SODIUM FLUORIDE (NAF) INDUCED OXIDATIVE STRESS

1. Effect of *VKG* on blood antioxidant enzymes

Graph 1 illustrated the alterations in blood antioxidant status in mice as a result of NaF intoxication. Administration of NaF causes a drop in the activities of antioxidant enzymes including SOD (1.18± 0.135 U/g Hb) and catalase (23.57± 0.65 U/g Hb) compared to normal (1.3 ± 0.019 and 28.08 ± 3.18 U/g Hb) animals. The treatment with *VKG* at the dose of 15.6 and 31.2 mg/kg b.wt. have increased the activity of SOD to 1.3 ± 0.019 and 1.52 ± 0.008 U/mg protein, respectively. In case of catalase, *VKG* treatment enhanced the activity of catalase to 27.67 ± 2.35 and 30.15 ± 0.502 U/mg protein in mice. Additionally, treatment of vitamin C has also shown significant improvement in the activity of SOD (1.39 ± 0.01) and catalase (26.74 ± 0.96) compared to control animals. (graph 1A&B).

The level of reduced glutathione (graph 1C) was lowered to 2.1 ± 0.04 nmol/g Hb in control animals as a result of the NaF challenge. The pre-treatment of vitamin C and *VKG* at the doses of 15.6 and 31.2 mg/kg b.wt have enhanced the GSH level to 2.2 ± 0.02, 2.97 ± 0.82 and 2.36 ± 0.27 nmol/g Hb respectively.



Graph 1: Effect of *VKG* administration on blood antioxidant enzymes - (A) SOD, (B) CAT and (C) GSH. Values are expressed as mean ± SD for 5 animals per group; $^{**}p<0.01$, $^{*}p<0.05$ and $^{ns}p>0.05$ compared to control.

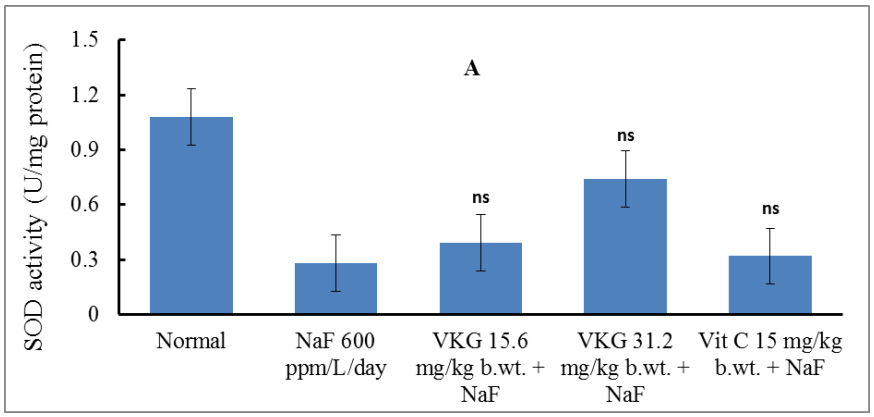
2. Effect of *VKG* on hepatic antioxidant enzyme status

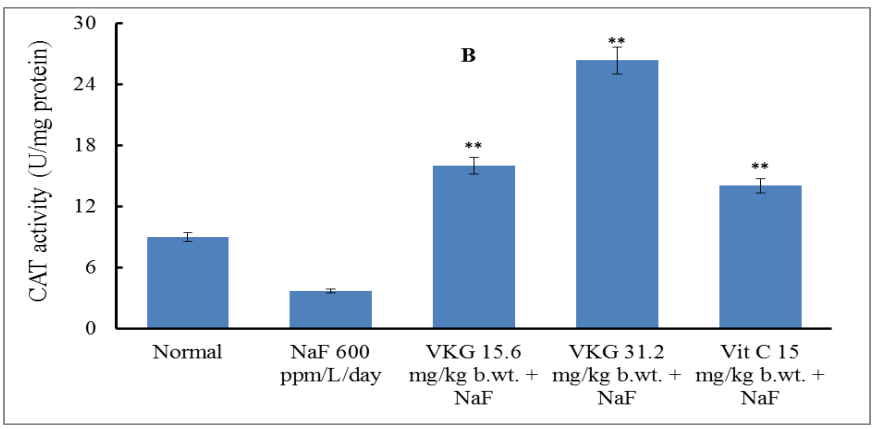
Sodium fluoride intoxication created an imbalance of redox status which was evidenced from the hike in lipid peroxidation with a concomitant drop in antioxidant enzymes in hepatic tissue of mice (graph 2). The activities of SOD (0.28 ± 0.06 U/mg protein) and CAT (3.69 ±

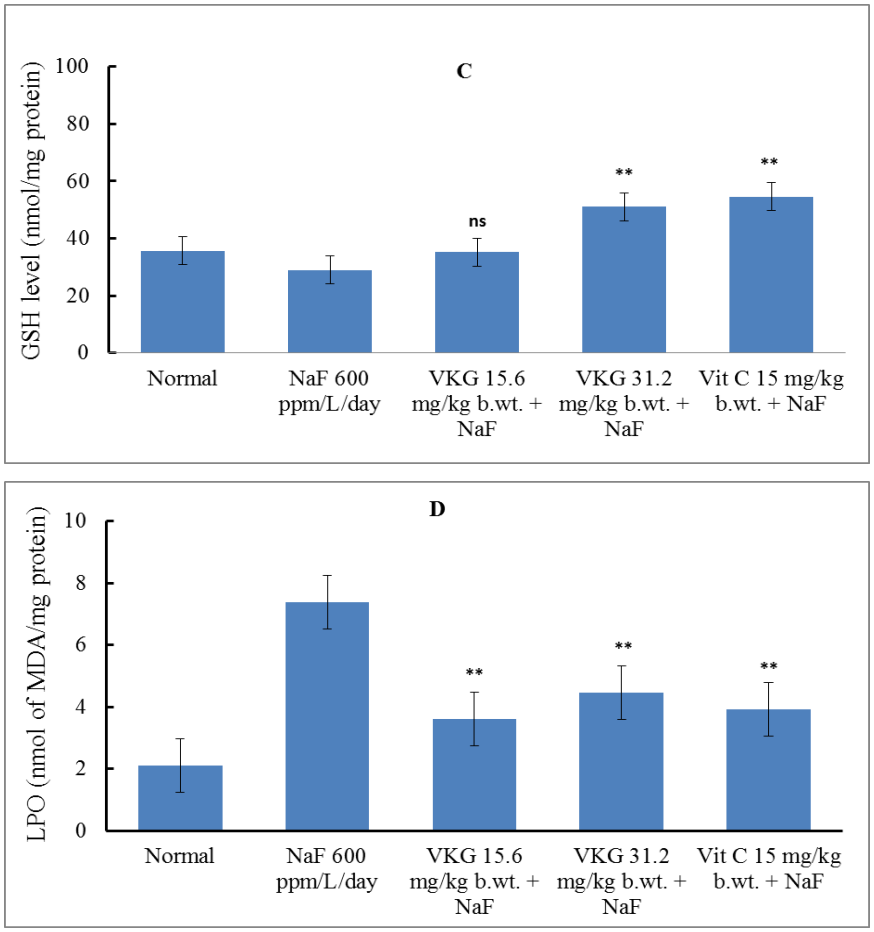
0.97 U/mg protein) in hepatic tissue were found to be declined in control animals. Upon the treatment with *VKG* at the doses of 15.6 and 31.2 mg/kg b.wt. the activity of catalase was increased to 16.03 ± 0.32 and 26.38 ± 0.54 U/mg protein, respectively (graph 2B). Similarly, the declined activity of SOD was elevated to 0.392 ± 0.07 and 0.74 ± 0.32 U/mg protein by *VKG* administration (15.6 and 31.2 mg/kg b.wt. respectively) (graph 2A). The administration of vitamin C significantly elevated the SOD and CAT activity to 0.3 ± 0.14 and 14.05 ± 3.78 U/mg protein, respectively compared to control animals.

A drop in GSH (29.05 ± 4.89 nmol/mg protein) level was observed in control animals indicates redox imbalance by NaF. The level of GSH was increased to 35.18 ± 4.54 and 51.06 ± 5.5 nmol/mg protein by the treatment of *VKG* (15.6 and 31.2 mg/kg b.wt.). The vitamin C administration also significantly elevated the GSH level to 54.63 ± 3.91 nmol/mg protein in mice (graph 2C).

The high MDA (nmol/mg protein) level was observed in NaF challenged mice (7.39 ± 0.84) which were reduced to 3.61 ± 0.72 and 4.47 ± 0.13 by the administration of *VKG* at the doses of 15.6 and 31.2 mg/kg b.wt. respectively (graph 2D). The administration of vitamin C also inferred the reduction in LPO (3.93 ± 0.44).







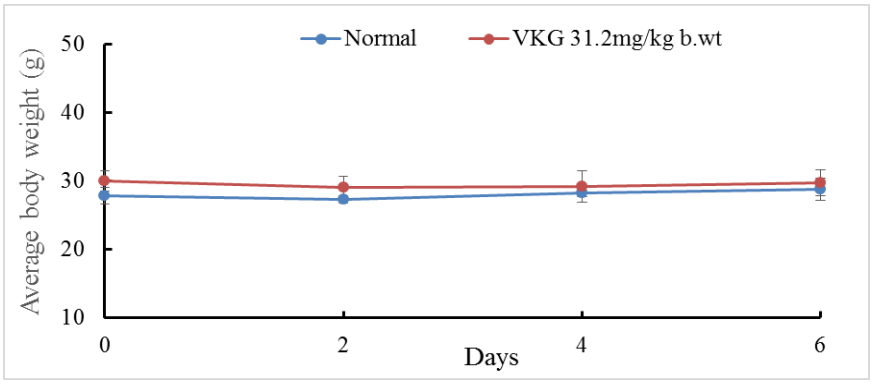
Graph 2: Effect of *VKG* administration on hepatic antioxidant enzymes - (A) SOD, (B) CAT, (C) GSH and (D) LPO. Values are expressed as mean ± SD for 5 animals per group; $^{**}p<0.01$, $^{*}p<0.05$ and $^{ns}p>0.05$ compared to control.

IMMMUNOMODULATORY ACTIVITY OF VRISHYAKSHEERA GRANULES

EFFECT OF *VKG* ON IMMUNOMODULATORY EFFECT

1. Effect of *VKG* on body weight

Treatment with *VKG* has weight gain compared to the normal group but not significant (graph 3).



Graph 3. Effect of *VKG* administration on body weight.

2. Effect of *VKG* on weight of spleen and thymus

The organ weight of spleen and thymus in *VKG* group was (0.372 ± 0.02, 0.233± 0.04 respectively) was not elevated compared to the organ weight of spleen and thymus in normal group (0.328 ± 0.05, 0.258 ± 0.02 respectively) (table 1).

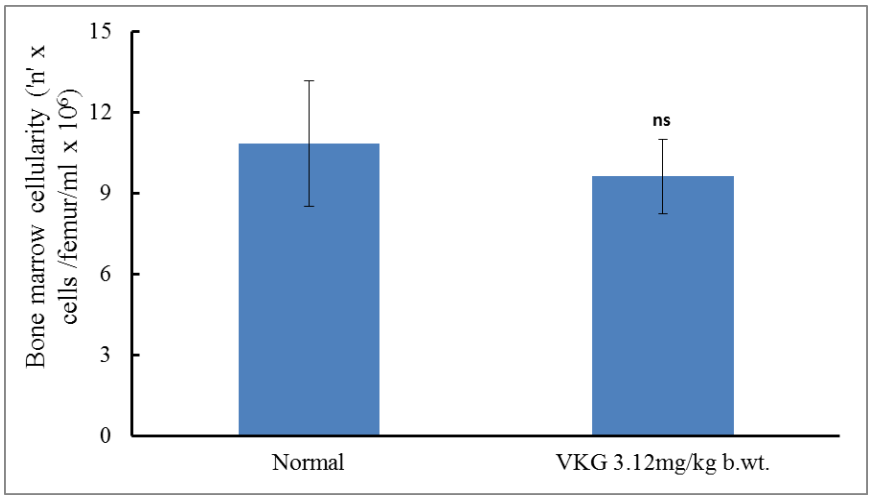
Table 1: Effect of *VKG* administration on relative organ weight.

Group	Relative organ weight of spleen	Relative organ weight of thymus
Normal	0.328 ± 0.05	0.258 ± 0.02
VKG 31.2 mg/kg b.wt.	0.372 ± 0.02 ^{ns}	0.233 ± 0.04 ^{ns}

Values are expressed as mean ± SD for 5 animals per group and $^{ns}p>0.05$ compared to normal.

3. Effect on bone marrow cellularity

The graph 4 shows the bone marrow cellularity level in normal (10.82 ± 2.33) and *VKG* (9.62 ± 1.38) groups respectively. There was no significance difference in BMC.



Graph 4: Effect of *VKG* administration on BMC Values are expressed as mean ± SD for 5 animals per group and $^{ns}p>0.05$ compared to normal.

4. Effect of *VKG* in haematological parameters

The haemoglobin, level in *VKG* (13.7 ± 1.2g/dL) has slight increase compared to the normal (12.5 ± 1.9) but not significant. The RBC (millions/ cu mm) and platelets (Lakhs/ cu mm) values also slightly rose in *VKG* (8.6 ± 0.2 million/ cu mm, 8.1 ± 0.8 Lakhs/ cu mm) compared to the normal (7.6 ± 1.8 million/ cu mm, 7.5 ± 1.48 Lakhs/ cu mm). The WBC (cells/ cu mm) count reduced in *VKG* (5400 ± 707) compared to the normal (6400 ± 424). The percentage of Neutrophils, Lymphocytes, Eosinophils are in *VKG* 10.7 ± 2.1, 85.3 ± 2.9, 4.0 ± 1.0 respectively (table 2).

Table 2: Effect of *VKG* administration on haematological parameters.

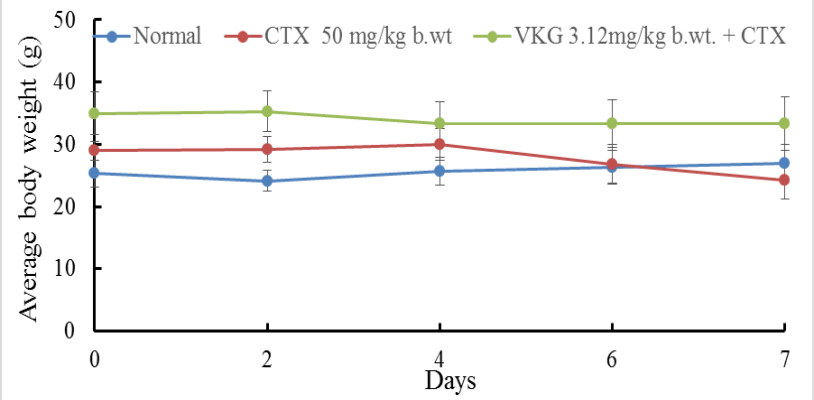
Group	Normal	*VKG* 31.2mg/kg b.wt
Hb (g/dL)	12.5 ± 1.9	13.7 ± 1.2 ^{ns}
RBC (millions/ cu mm)	7.6 ± 1.8	8.6 ± 0.2^{ns}
Platelets (Lakhs/ cu mm)	7.5 ± 1.48	8.1 ± 0.8^{ns}
WBC (cells/ cu mm)	6400 ± 424	5400 ± 707^{ns}
Neutrophils (%)	10.0 ± 1.0	10.7 ± 2.1^{ns}
Lymphocytes (%)	84.7 ± 0.58	85.3 ± 2.9^{ns}
Eosinophils (%)	5.3 ± 0.58	4.0 ± 1.0^{ns}

Values are expressed as mean ± SD for 5 animals per group and $^{ns}p>0.05$ compared to normal.

EFFECT OF *VKG* IN CTX INDUCED IMMUNOSUPPRESSED MICE

1. Effect of *VKG* on body weight

Reduction in body weight of cyclophosphamide treated Swiss albino mice after 7days of exposure is indicative of impending toxicity (graph 5). Treatment with *VKG* failed to attenuate toxicant induced weight change while normal group have weight gain.



Graph 5: Effect of *VKG* administration on body weight.

2. Effect of *VKG* on weight of spleen and thymus

Administration of cyclophosphamide decreased the weight of spleen (0.181 ± 0.02, 0.184 ± 0.02) and thymus (0.126 ± 0.02, 0.125 ± 0.01) in both *VKG* and CTX group respectively (table 3).

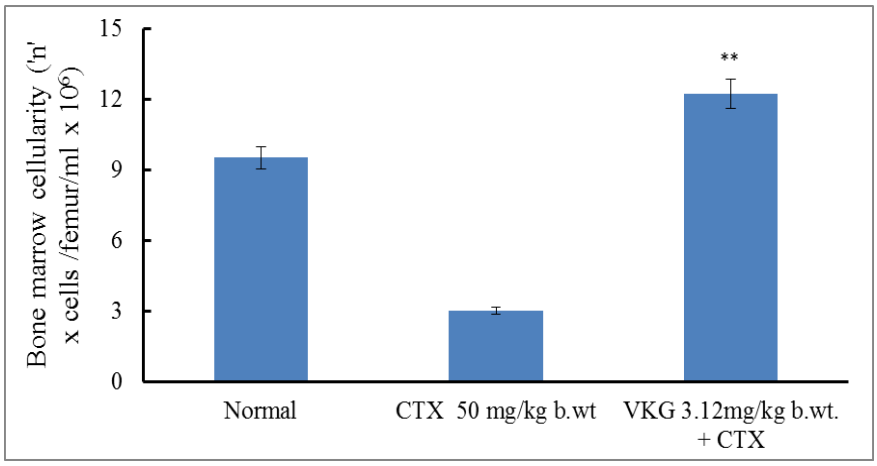
Table 3: Effect of *VKG* administration on relative organ weight.

Group	Relative organ weight of spleen	Relative organ weight of thymus
Normal	0.319 ± 0.04	0.232 ± 0.10
CTX 50 mg/kg b.wt	0.184 ± 0.02	0.125 ± 0.01
VKG 31.2mg/kg b.wt. + TX	0.181 ± 0.02^{ns}	0.126 ± 0.02^{ns}

Values are expressed as mean ± SD for 5 animals per group and $^{ns}p>0.05$ compared to control.

3. Effect of *VKG* on bone marrow cellularity

The graph 6 shows the bone marrow cellularity level increase in *VKG* (12.25 ± 2.21) compared to the CTX (3.005 ± 0.48). The result was showing significant in *VKG* compared to the CTX.



Graph 6: Effect of *VKG* administration on bone marrow cellularity. Values are expressed as mean ± SD for 5 animals per group and $^{**}p<0.01$ compared to control.

4. Effect of *VKG* in haematological parameters

Administration of cyclophosphamide leads to a decrease in the counts of total WBC (2667 ± 208), RBC (7.4 ± 0.2), Hb (11.9 ± 0.6) and platelets (10.1 ± 3.2) in *VKG* group. Percentage of neutrophils (12.4 ± 0.9), and Eosinophils (7 .0 ± 1.9) were increased in *VKG* administered group but it's not significant. In case of lymphocytes (80.6 ± 2.6), it is decreased compared to the control, but there is no significance result in *VKG* administered group (table 4).

Table 4: Effect of *VKG* administration on haematological parameters.

Group	Normal	CTX 50 mg/kg b.wt.	*VKG* 31.2 mg/kg b.wt. + CTX
Hb (g/dL)	12.6 ± 1.2	11.9 ± 0.3	11.9 ± 0.6^{ns}
RBC (millions/ cu mm)	7.6 ± 0.7	7.6 ±0.5	7.4 ± 0.2^{ns}
Platelets (Lakhs/ cu mm)	10.0 ± 2.1	9.7 ± 3.2	10.1 ± 3.2^{ns}
WBC (cells/ cu mm)	10200 ± 173	2125 ± 221	2667 ± 208^{ns}
Neutrophils (%)	10.6 ± 0.9	11.6 ±1.3	12.4 ± 0.9^{ns}
Lymphocytes (%)	86.4 ± 1.1	82.2 ± 3.0	80.6 ± 2.6^{ns}
Eosinophils (%)	3.0 ± 1.0	6.2 ± 1.9	$7 .0 \pm 1.9^{ns}$

Values are expressed as mean ± SD for 5 animals per group and $^{ns}p>0.05$ compared to control.

DISCUSSION

VKG possess the ingredients which are having the properties of antioxidant, immunomodulatory and free radical scavenging activities. When all these ingredients are made into a single formulation their pharmacodynamic actions may differ from their individual effects. The hypothesis of this study was all the ingredients may act synergistically to have potent phytochemical combination by which more antioxidant and immunomodulatory activities could be shown than the individual ingredients had. Human genome is very much similar to rodents. Therefore due to genome similarity it permits scientists to induce disease in them and use them as model organisms to study disease progression and treatment.

Stress is one of the major etiological factors for the manifestation of diseases through excessive generation of free radicals within the body. Excessive generation of free radicals is involved in the pathogenesis of several diseases and toxicity of a wide range of compounds (Nabavi *et al*., 2012). Many studies have reported that excessive fluoride exposure can cause the redox balance of the cells in tissues, decrease antioxidant defense capacity in brain, and increase the toxic effects on visceral organs mediated by generation of ROS and lipid peroxidation. A close association between chronic fluoride toxicity and increased oxidative stress has been reported in humans and in experimental animals. Neurotoxicity induced by fluoride has been linked with oxidative stress. It has been demonstrated that high concentration of fluoride can decrease learning ability and memory in some animal experiments and result in dysfunction of the central nervous system. The increase of reactive oxygen species (ROS) and lipid peroxidation (LPO) has been considered to play an important role in the pathogenesis of chronic fluoride toxicity. Fluoride has been shown to induce lipid peroxidation and decrease levels of several antioxidant molecules as well as enzyme activities in the blood and liver. Mainly, fluorides attack polyunsaturated fatty acids to yield hydroperoxides which in turn produce marked increases in MDA and reactive products of thiobarbituric acid, which are indicators of lipid peroxidation caused by increased free radicals. Additionally, increased LPO can be counteracted by administrating antioxidant molecules (Houghton *et al*., 1995). The present study demonstrates that the fluoride decreased antioxidant enzyme activity in both blood and liver of NaF alone treated control animals. Additionally, in these groups MDA levels was found to be increased which indicates NaF mediated hepatic toxicity. *VKG* pre-treatment significantly lowered the level of MDA after NaF challenge indicates its antioxidant activity.

The body has its own antioxidant defense mechanisms which stabilize oxidative molecules and keeps them in balance. The endogenous antioxidants both enzymatic and non-enzymatic are equally participated for preventing incidence and progression of diseases (Jacob, 1995). Fluoride is capable of interrelating with metals and thus can alter the activity of enzymes that contain a transition metal as part of their cofactors or in their active site (Chinoy, 2003). The endogenous antioxidant enzymes such as SOD and glutathione peroxidase bear manganese and selenium, respectively as cofactors. In the present study, the exposure of NaF leads to the reduction in activity of SOD and CAT in liver indicating an impaired function of the hepatic antioxidant defense system (Blaszczyk *et al*., 2011). In the present study, NaF intoxication leads to reduction of endogeneous enzymatic activities in both liver and blood. Administration of *VKG* restored antioxidant enzymes indicating its ability to restore antioxidant homeostasis in both liver and blood.

On analysing the result of the study one can infer that both doses are effective as an antioxidant agent. On comparing both of the doses, 31.2 mg/kgb.wt. is found to possess better efficacy than 15.2mg/kg b.wt. Hence for obtaining the health benefits of *Rasayana*, 31.2 mg/kg b.wt. dose will be ideal. Thus the study proves the usage of *Vrishya Ksheera Yoga Granules* as antioxidant.

Plant bioactive compounds can either stimulate or suppress the immune system and hence can be utilized to treat various disorders involving the immune system. Immunosuppression is a manifestation of abnormal immune function in the body. Under the action of single or multiple pathogenic factors, the damages of body immune system will make it prone to infection or the formation of immune diseases. Cyclophosphamide monohydrate (CTX), a cancer chemotherapeutic agent, facilitates cell apoptosis and decreases the homeostatic proliferation of regulatory T cells. CTX is used as an effective chemotherapeutic drug in tumor treatment. In addition, CTX can treat a variety of immune diseases such as rheumatoid arthritis, lupus erythematosus and colitis. However, as an immunosuppressive agent, CTX can also kill normal cells and decrease the body's immune function while treating diseases.

In first set of experimental protocol which was conducted with normal and *VKG* treated group, there was no significant change in *VKG* treated group compared to normal group. So in the next step cyclophosphamide (CP) induced experimental study was under taken as the *VKG* may not have direct role in the normal cells. So we need some abnormality in the cells. Reduction in body weight of CTX treated mice after 7 days of exposure is indicative of

impending toxicity. This was expected because of its tendency to produce cytotoxicity, especially in fast multiplying cells. The cytotoxicity in the gut may interfere with the absorption of nutrients. Treatment with *VKG* attenuated CTX induced weight loss, but the reversal did not reach statistically significant level.

The weight of immune organs is a reflection of body's innate immune function. Thymus, a central immune organ, is the place for immune cells differentiation and maturity. Spleen is a peripheral immune organ, where mature immune cells colonize and respond to the immune response. Thus, changes in the thymus and spleen indices reflect the strength of body's innate immune function. In this study, administration of CTX decreased the weight of spleen and thymus in both control and treatment group compared to the normal group. The observed decrease may be due to toxic effect of CTX as reported earlier studies. Treatment with *VKG* failed to attenuate CTX induced weight change in different immune organs.

Administration of CTX leads to a significant decrease in the counts of total WBC, neutrophils, lymphocytes, RBC and platelets. This data confirms the myelo-suppressive activity of the toxicant. Treatment with *VKG* non‑significantly attenuated almost all the CTX induced changes in hematological parameters. In bone marrow cellularity *VKG* shows significant result. On analysing the result we can observe that *VKG* has no significant result in immunomodulatory activity.

CONCLUSION

From the properties of ingredient drugs, it is evident that all ingredients have the potential to fight against a vast variety of diseases and thus ensure the longevity and health. *Vrishyaksheera yoga* granules possesses significant antioxidant activity and moderate immunomodulatory activity. Among the two doses 31.2 mg/kgb.wt. was found to have better activity profile. Hence it can be concluded that it is better to give31.2 mg/kgb.wt. dose to get desired pharmacological activity.

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