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TOXINOUS SECRETIONS FROM INDIAN TOAD (*BUFO MELANOSTICTUS*) WITH POTENTIAL ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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ABSTRACT

Amphibian skin glands provide variety of compounds as a source of potential new chemical entities. Indian Toad Paratoid Glandular Secretions (TPGS) show anti-diabetic and cardio tonic activities and limited research is done on other activities. The present study focused to explore the antioxidant, anti-inflammatory effects. Male Wistar rats (n=6) used for acute toxicity study according to OECD 423 guidelines. I*n-vitro* and in-*vivo* antioxidant study was performed by DPPH and CCl_4 induced oxidative stress model respectively. Further, anti-inflammatory with paw edema and absorption kinetics of naproxen was done and LD50 found to be 500 mg/kg. TPGS shown potential antioxidant effect with IC 50 value of 33.53±1.26µg/ml. Significant decrease in serum enzymes and MDA levels and increase in GSH levels observed in TPGS and ascorbic acid pretreated rats when compared with CCL_4 induced rats and was evidenced with histopathological study. Percentage reduction of rat paw edema was 47.6±1.3 with TPGS alone and 49.6±0.9 was with TPGS + NPX. In the pharmacokinetics study between naproxen and TPGS shows significant decrease in $C_{max,}$ AUC, and an increase in Cl and V_d indicating the induction of NPX metabolism. Based on the results TPGS has anti oxidant, anti-inflammatory

and enzyme induction effects. So TPGS can be a good source for the potential new chemical compounds for different biological activities. Further studies are required to prove the activities.

Keywords: Acute toxicity study, Ascorbic acid, *Bufo melanostictus,* Carrageenan, Inflammation, Naproxen

INTRODUCTION

From the amphibians Indian toads plays an important role for the new chemical compounds from the animal source. Toads belongs to Anura order lives in diverse range of moist environment, during the demanding situations like self defending with their predators they expel noxious, venomous and poisonous chemical substances **[1]**. Toads possesses two types of glands one is cutaneous glands which secretes mucus for maintaining humidity and breathing, and the other is glandular (serous or poison) glands, which releases venomous chemicals for their own protection. The envenomation is characterized by salivation, excitation, paralysis, trembling, and convulsions, leads to the death of predators and even microbes **[2-4]**. Toads, depending on the species and surroundings wherein they stay, the toxins contains biogenic amines, steroids, alkaloids, proteins and peptides **[5]**. Different types of chemical substances are found in skin glands of toad make them unique to find out new chemical entities from their secretions **[6]**.

Several bioactive compounds from toad parotoid gland secretions with variety of biological activities determined in many folklore medicines and several cultural countries throughout the world. Chansu which is a traditional Chinese medication used for the treatment of cardio tonic, analgesic, anesthetic agent, for ulcer healing and also as an anti-cancer agent **[7]**. Earlier days several researchers focused mainly on these Chansu and now significant studies are examined on therapeutic potential of other toad species like Australian cane toad (*Bufo marinus*) **[8]**, Brazilian toad (*Bufo rubescens*) **[9]** , *Rhinella schneideri* **[10]**. Some previous studies are done on *bufo melanostictus* toad skin extract like lethal, neurotoxic, cardiotoxic, hemolytic, sleep potentiation activity **[11]**, immunomodulatory and antineoplastic activity **[12]**. Past investigations from our lab divulged that toad (*B.melanostictus*) parotoid glandular secretions (TPGS) has got some pharmacological activities like antidiabetic **[13, 14]** and cardio tonic **[15]**.

New compounds from animal sources are few and some drugs already developed are like batroxobin from *Bothrops atrox* and

Bothrops moojeni, a venomous pit viper used to forestall bleeding **[16]**. Captopril a anti hypertensive agent from Brazilian pit viper venom **[17]**, Eptifibatide from south-eastern pygmy rattle snake venom used as anti coagulant **[18]**. Exenatide from saliva of the Gila monster lizard *Heloderma suspectum* used in Type-2 diabetes **[19]**. Ziconitide a ω-conotoxin from cone snail used to treat intense ache also used as N-type calcium channel blocker **[20]**. Antioxidant activity of proteins from animal-derived products used in treating the diseases which might be associated with oxygen free radical mediated tissue damages. Free radicals are generated in oxidative stress leads to diseases like cancer, hypertension, neuronal problems, Alzheimer's disease, slight cognitive impairment, Parkinson's disease, alcohol prompted liver disease, ulcer, ageing and atherosclerosis **[21, 22]**. The development of novel non-steroidal anti inflammatory drugs (NSAIDs) with better activity with lower side effects is the required area of research. The inflammation is a tissue response to a damage/trauma characterized by the signs of heat, pain, redness, swelling, and lack of functioning **[23-31]**. Over production of free radicals leads to activation of a complex enzymes, and releases inflammatory and pro-inflammatory mediators and they are involved in the inflammatory process. Carrageenan causes edema with a biphasic phenomenon. Before 1 hr (initial phase) is a launch of bradykinin, histamine, serotonin and substance P. After 1 hr (late section) is a contribution of neutrophil infiltration, prostaglandin generation and launch of the neutrophil-derived free radicals, nitric oxide (NO) and pro-inflammatory cytokines including tumor necrosis factor(TNF-α), and interleukin-1 β (IL-1 β).

Common Indian Toad (*bufo melanostictus*) belongs to family *bufonidae* genus *bufo* located in India and it's parotoid exudates is a good source of chemical compounds which may be successful to become new chemical entities with pharmacologically potential active substances those are not reported yet. This study is to discover the toxic dose and as well as effective dose through acute toxicity experimental protocol followed by the OECD 423 guidelines and also to evaluate the antioxidant, anti-inflammatory activity and enzyme modulatory effects of Toad (*bufo melanostictus*) parotoid gland secretion (TPGS).

MATERIALS AND METHODS

Secretion collection

Toads collected at Kakatiya University campus premises, Warangal, Telangana, and India and authenticated by the Department of

Zoology, Kakatiya University, Warangal, Telangana, and India. The secretion was obtained by mechanical compression of both paratoid glands from living individuals is called milking process of collection method. These yellowish and doughy secretions were collected on the surface of the watch glass and after, the toads were released.

Animals

Healthy young adult female Wister rats weighing about 180-200 gm are used for LD50 study and male Wistar rats were used for other studies. Rats fed with standard food pellets which are procured from Sainath Agencies, Hyderabad, Telangana state and acclimatized at UCPSc animal house. Animals are kept in separate polypropylene cages with rice husk bed and provided 12 hrs light and 12 hrs dark with standard pellet diet and water *ad libitum*. All the experiments conducted were approved by the IAEC (IAEC/35/UCPSc/KU/2018).

Chemicals

Naproxen got as a gift sample from Aurobindo Pharmaceuticals, Jadcherla. HPLC grade acetonitrile, diethyl ether and methanol, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), dimethyl sulphoxide (DMSO) and CCL_4 (Carbon tetrachloride) were purchased from Merck (Merck, Mumbai). Deionized water was purified by the Milli-Q50 SP Reagent Water System (Millipore Corporation, USA).Glacial acetic acid AR grade; ibuprofen, Pyridine, butanol, Reduced Glutathione, Trichloro Acetic Acid, Sulphanilamide, Tris Hydrochloride and carrageenan were purchased from (Sigma Aldrich, Bangalore). Ethylene Diamine TetraAcetic Acid (EDTA), ThioBarbituric Acid, DTNB (5,51- Dithio,Bis (2- Nitro Benzoic Acid), Sodium Dodecyl Sulphate , Sodium Dihydrogen Phosphate, 5-Sulfo Salicylic Acid, Glacial Acetic Acid were of Himedia. All other chemicals and reagents used were of AR grade and were obtained from commercial sources

Acute toxicity studies

According to OECD 423 guidelines animals were divided in to 5 groups (n=3) like Normal control, TPGS 5, 50,300 and 2000 mg/kg. The test substance (TPGS) is administered in a single oral dose; animals should be fasted prior to dosing. Mean body weight, food intake, water intake and locomotor activity changes are considered and recorded at starting day before first dose and daily thereafter for 14 days.

Observations

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the

first 4 hours, and daily thereafter, for a total of 14 days after single dose treatment whether the animals found abnormal and dead or not. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behavioral pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

***In vitro* Antioxidant activity**

DPPH radical scavenging assay activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is used to estimate the antioxidant activity of the test substance (TPGS). Samples were prepared ranging from 1 to 100 µg/ml from the readily prepared stock solution (1 mg/ml). Add 1 ml to all the samples from the freshly prepared 0.1mM DPPH solution and kept in dark for 30 min. Change in absorbance of the DPPH due to test substance is recorded by using a spectrophotometer (Elico UV–Visible Spectrophotometer SL 210) at 517 nm. Ascorbic acid was used as a standard. Control sample was prepared containing DPPH alone. Percentage inhibition is calculated from the following equation:

% Inhibition = A_{bs} control-Abssample / A_{bs} control x 100

A_{bs} control means absorbance of DPPH alone

A_{bs} sample means absorbance of DPPH and TPGS[32].

***In vivo* Antioxidant activity**

CCL_4 induced oxidative stress

Wistar rats weighing about 200-250 gm are randomly divided in to 5 groups. First group receives saline served as control. Group second receives TPGS (50 mg/kg) alone for 4 days. Animals of the third group are pre treated with TPGS and intoxicated with CCL_4 on the 4^{th} day after 2hr administration of the TPGS. Fourth group receives single dose (2 mL/kg) of 20% CCL_4 in olive oil. Group five receives Ascorbic acid (300 mg/kg) and also intoxicated with CCL_4 on the 4^{th} day after 2hr administration of the AA. At the end of the experiment on 5^{th} day all the rats were sacrificed by decapitation blood and livers were collected for biochemical estimation and histopathological studies. Livers were removed, washed with ice cold saline (0.9% NaCl), blotted dried and cut in to 2 pieces, weighed 1 g each for the estimation of GSH levels and MDA quantity. Tissues were homogenized in ice cold buffers and stored at -20 º C until analysis **[33, 34]**.

Estimation of serum enzymes

Blood was taken in an eppendorf tubes and serum was separated by centrifugation at 10000 rpm for 10 min at 4 ºC. Measurement of Alanine amino transferase (ALT), aspartate amino transferase (AST) activities and alkaline phosphatase (ALP) was done using semi automated biochemical analyzer (ERBA chem5).

Lipid peroxidation assay (MDA)

Malondialdehyde (MDA) in tissue homogenate were estimated **[35]** by using method briefly described as 0.2 ml of tissue homogenate was taken and to this 0.2ml of 8.1%SDS, 1.5 ml of 20% acetic acid and 1.5ml of aqueous TBA (0.8%) were added and volume was made up to 5ml with distilled water and heated in oil bath at 95 ºC for 1hr. Then the mixture of n-butanol and pyridine (15:1 v/v) was added and shaken vigorously. The mixture was centrifuged at 4000 rpm for 10 m, separate the organic layer and measure the absorbance at 532nm. The tissue MDA levels is measured from the standard curve and expressed as µ mol/g tissue.

Reduced glutathione assay (GSH)

Reduced glutathione was estimated according to the method described by Ellman **[36]** by using glutathione as standard with slight modifications. Tissue homogenate (1ml) was precipitated with 4% sulfosalicylic acid (1ml) and cold digested at 4 ºC for 1hr and cold centrifuged at 1200 x g for 15 min at 4 ºC and the supernatant was collected. To this 0.2ml of supernatant 2.3 ml of phosphate buffer (0.1M, pH 7.6) and 0.5ml of DTNB were added and incubated for 5mins at room temperature. The absorbance of yellow color produced was measured at 412 nm using systronics spectrophotometer.

Carrageenan-induced rat paw edema model

Male Wistar rats were divided in to 5 groups (n=6). Group 1; receives normal saline, group 2; carrageenan, group 3; TPGS (50 mg/kg) treated, group 4; NPX (25mg/kg p.o) treated and group 5; TPGS&NPX combination treated are used in this study. 1% w/v suspension of carrageenan is prepared freshly in normal saline and 0.1 ml is injected into sub plantar region of the right hind paw **[37]**. Rat paw volume was measured by using mercury filled plethysmometer at different time intervals and percentage paw edema reduction was calculated by the following equation:

Percentage reduction in edema= Vcontrol-Vtreated /Vcontrolx100

On the 14th day of the study, after an hour of drugs treatment blood samples were obtained from tail vein at time intervals of 0.5, 2, 4,

8,12 and 24 hours to estimate serum concentration of naproxen.

HPLC Analysis of Naproxen

Stock solutions (1mg/ml) of naproxen and internal standard ibuprofen were prepared. To 100 µl of plasma 300 ml of a 0.05 M sodium acetate buffer and 70% perchloric acid (50 µl) was added in a clean dry centrifugation tubes and vortexed for 1 min. Then again the mixture was vortexed for another 1 min by adding 50 µl of acetonitrile. Tubes are subjected to centrifugation at 5000 rpm for 10 min. Supernatant was separated and loaded into HPLC injection port before spiking 50 µl into the chromatogram system.

Analysis was performed on Merck C-18 Column of 25cm length and 4.6mm internal diameter packed with porous silica spheres of 5µ diameter using mobile phase acetonitrile: acetic acid (42:58 v/v) adjusted to pH 3 and a flow rate of 1ml/min at 280nm **[38]**.

Statistical analysis

All the values are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using WinNonlin software and graph pad prism software version 8.1.2

RESULTS

Acute toxicity studies

Toad parotoid gland secretions (TPGS) at different dose ranges (5, 50,300 and 2000mg/kg) administered to rats and there was the evidence of mortality at 2000 mg/kg, and no symptoms of toxicity at 5, 50 and 300mg/kg was observed for the duration of the 14 days period of study is given in **Table 1**. The animals did not display any modifications in preferred appearance all through the observational period. Morphological traits (skin, eyes and nose) appeared ordinary. No salivation, diarrhea, lethargy have been located. But convulsions were found after treatment of 2000mg/kg earlier than the demise occurred, as a result LD 50 dose of TPGS is 300 mg/kg

The mean weight at dose 300 mg/kg (213.33±6.66 to 181.66±7.26) when in comparison with 5mg/kg (186.66±6.66 to 198.33±6.01) and 50 mg /kg (185.00±5.00 to 187.66±3.71) shown in **Figure 1A**. In food intake a significant difference was observed at 5 mg/kg (37.00±11.93 to 56.66±4.41) when compared with a LD 50 dose (23.33±3.33 to 31.66±1.66) and at 50 mg/kg (40.33±0.88 to 43.66±1.33) shown in **Figure 1B**. Water intake was decreased at300 mg/kg dose (33.33±13.33 to 25.00±1.00) but it was increased at 5 mg/kg (29.66±4.91 to 50.00±2.88) and at 50 mg/kg (38.33±6.01to 46.66±1.66) which is shown in **Figure1C**.

Locomotor activity is enormously raised at 300 mg/kg (98.33±1.66to 313.6±23.59) and a little difference was observed at 5 mg/kg (395.00±75.71 to 426.00±75.85) and at 50 mg/kg (405.00±83.73 to 477.00±110.41) when compared to control groups are shown in **Figure1D**.

DPPH radical scavenging assay activity

Change in color from deep violet to light yellow because of scavenging of DPPH radical by way of the glandular secretion which ends due to decrease in absorbance of the samples. All the test samples from 1 µg/ml to 100 µg/ml shows free radical scavenging activity in terms of percentage inhibition with increasing concentration and IC50 value of TPGS is 33.53±1.26µg/ml which is given in **Figure 2D**. All the values obtained were in comparison with standard ascorbic acid which concludes that TPGS has potential antioxidant activity.

Estimation of serum enzymes

A significant difference (*P<0.5*) is observed between treatment groups (TPGS+CCL_4, AA+ CCL_4) and CCL_4 group. Serum enzymes ALP, ALT and AST levels were significantly increased in CCL_4 (250.28 ±10.92, 113.32 ±1.75 and 301.75±10.89) treated rats. However the enzyme levels were significantly (*P<0.5*) brought down to lower in TPGS+CCL_4 (136.80±8.52, 48.63±10.61 and 168.60±3.76) and AA+ CCL_4 (75.98 ±8.44, 40.28 ±4.33 and 153.07±4.13) pretreated rats. Results are shown in **Table 2**.

MDA levels in the liver

Increase in the MDA quantity is observed in CCL_4 (0.052±0.002) treated rats. A significant (*P<0.5*) decline in the MDA levels is noticed in TPGS+CCL_4 (0.027±0.004) and AA+ CCL_4 (0.022±0.006) groups when compared with CCL_4 treated rats; the results are shown in **Table 2**.

GSH levels in the liver

Depletion in the GSH levels is recorded in CCL_4 alone (0.043±0.010) intoxicated rats. These values were augmented to a significant (*P<0.5*) levels in TPGS+CCL_4 (0.146±0.016) and AA+ CCL_4 (0.150±0.030) groups which were shown in **Table 2**. Histopathological studies showed the decreased hepatocellular damage or necrosis with TPGS when compared with CCL_4 induced hepatotoxicity **(Figure 2)**.

λ-Carrageenan-induced rat paw edema model

Figure 3 C indicates there's a marked difference in reducing the mean paw volume when treated with combination of NPX and TPGS than TPGS alone. Percentage inhibition at 4^{th}hr in combination treated group (49.6±0.9%) is more than TPGS alone treated (47.6±1.3%) and nearer to naproxen treated group (59.1±1.3).

Pharmacokinetics of Naproxen

The pharmacokinetic study of naproxen alone and when in combination with TPGS were conducted after oral administration; 2 groups of rats received 25 mg/kg of naproxen and 2 groups of rats acquired TPGS. **Figure 3A** and **Figure 3B** shows the naproxen plasma concentration (mean ± SEM) against the time profiles following oral doses of naproxen alone (25mg/kg) and when co administered with TPGS (50 mg/kg) in rats at single (SDT) and multi dose treatment (MDT) levels. The pharmacokinetic parameters of naproxen with and without TPGS are given in **Table 3**. According to these data, the same doses of naproxen alone or when combined with TPGS have been significantly different and the serum concentration of naproxen decreases when given with TPGS and thus results endorse that our sample ought to be an enzyme inducer which will increase the metabolism of Naproxen that is catalyzed by using CYP1A2 enzyme.

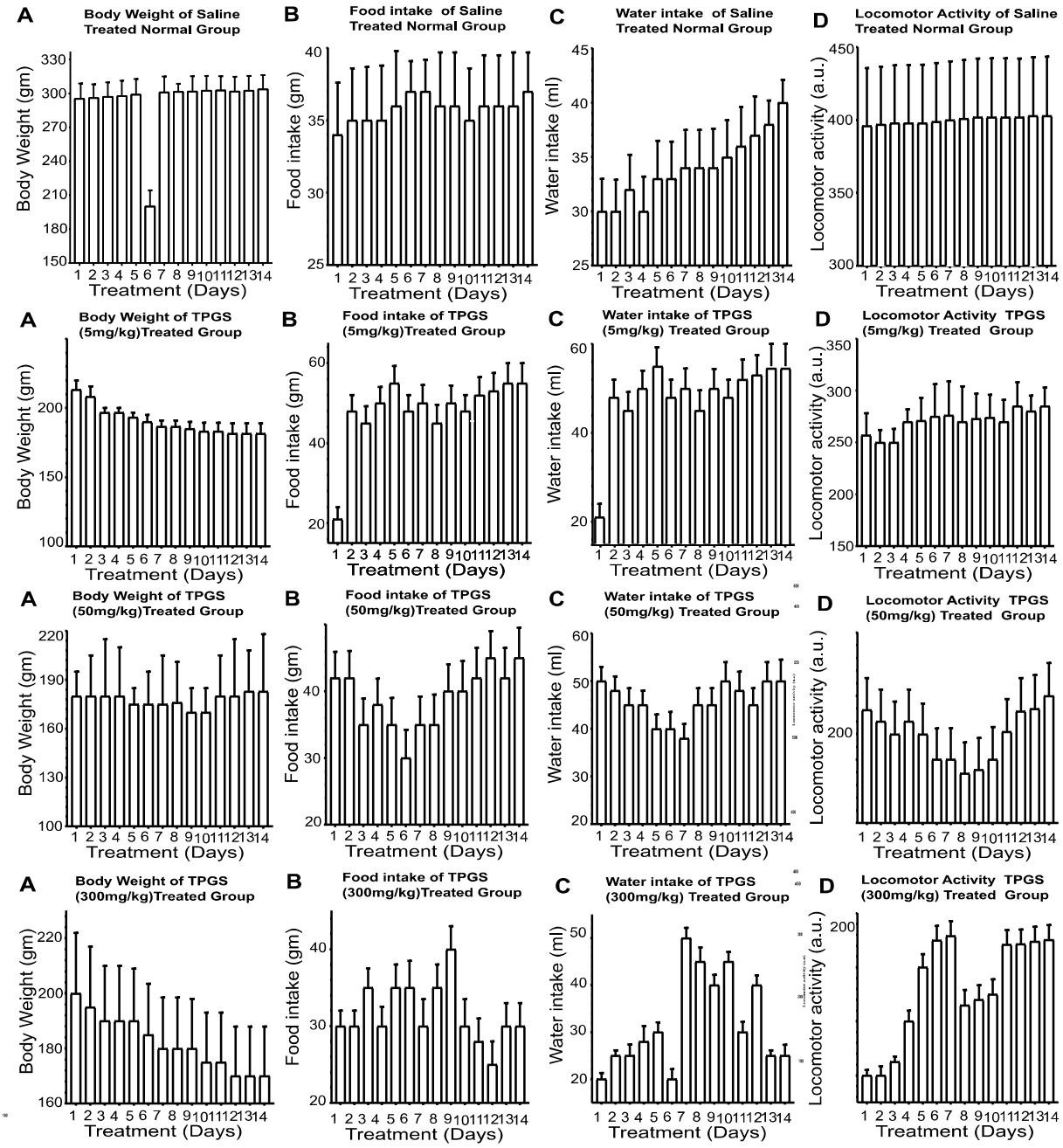

Figure 1: Toxicity observational study in female rats (n=3) for 14 days. A). Body weight changes B) Food intake changes, C) Water intake changes D) Locomotor activity changes. TPGS-Toad Parotid Gland Secretions, a.c-arbitrary units

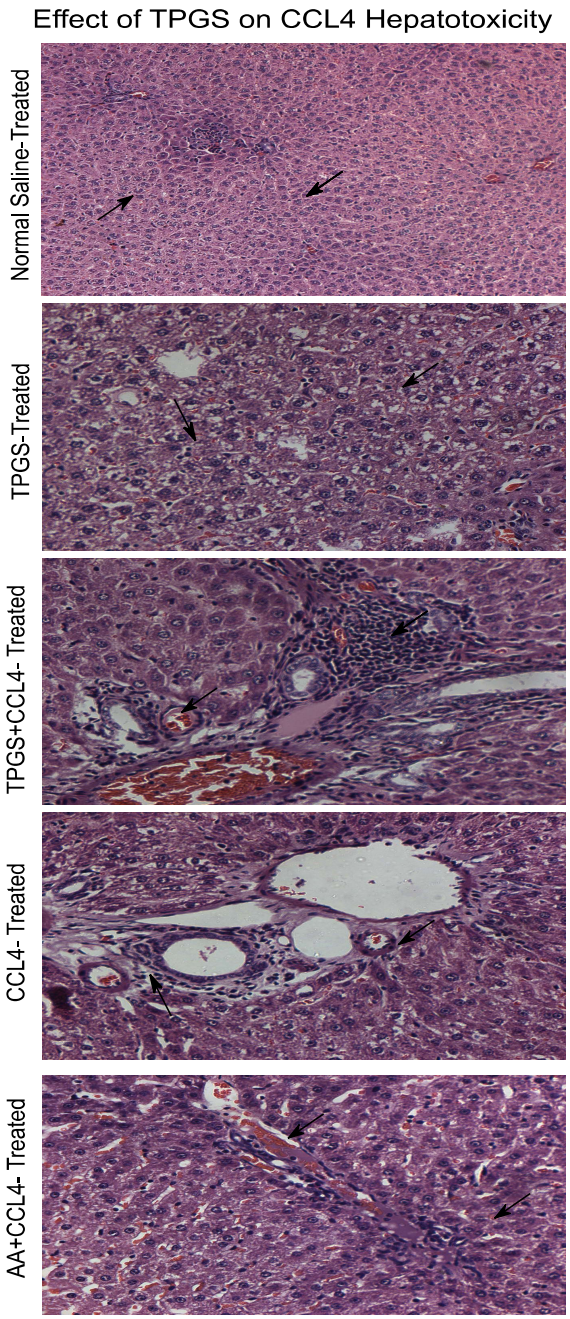

Figure 2: Histopathology of liver section (400 X) of normal, TPGS treated, TPGS+CCL4 treated, CCL4 treated and, CCL4+AA groups taken on 5 day of the study. The arrows indicate the appearance of hepatocellular changes of different groups. TPGS-Toad Parotid Gland Secretion, CCL4-Carbontetra chloride and AA-Ascorbic acid

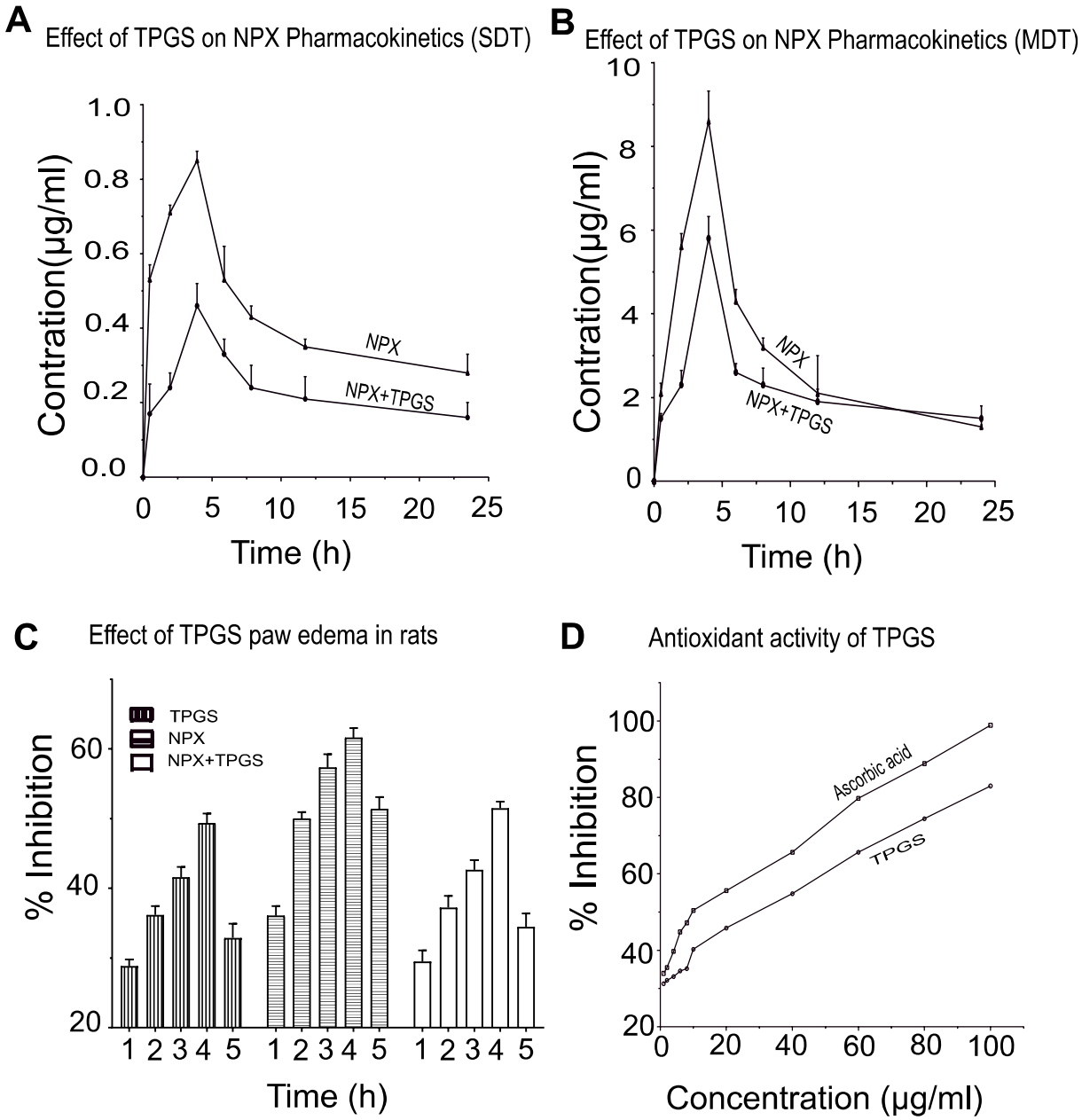

Figure 3: A) Influence of TPGS on NPX pharmacokinetics in rats (SDT). B) Influence of TPGS on NPX in rats (MDT) data expressed as Mean±SD (n=6) ***p<0.001. C) % Inhibition of paw edema with TPGS and statistical analysis was performed using two way ANOVA (Bonferroni post test) with p<0.05 as significant. D) Antioxidant activity of TPGS by using DPPH method. TPGS- Toad Parotid Gland Secretions, NPX-Naproxen. SDT-Single dose treatment, MDT-Multiple dose treatment

Table 1: Acute toxicity study of TPGS

Group	Treatment	Sign of toxicity(ST/NB)	Mortality(D/S)
Normal Control	Normal Saline	0/3	0/3
TPGS	5mg/kg	0/3	0/3
TPGS	50mg/kg	0/3	0/3
TPGS	300mg/kg	0/3	0/3
TPGS	2000mg/kg	2/1	2/1

TPGS = Toad Parotoid Glandular Secretion, ST=Sign of toxicity, NB=Normal behavior, D=Died, S= Survived. Values are expressed as number of animals (n=3)

Table 2: Data depicts the serum ALP, ALT, AST and Liver MDA and GSH levels of different experimental animal groups

Parameter	Control	TPGS	TPGS+CCL_4	CCL_4	AA+CCL_4
ALP (U/L)	185.10± 5.40	170.92±8.86	136.80±8.52***	250.28 ±10.92	75.98 ±8.44***
ALT (U/L)	66.53±10.26	54.77±8.59	48.63±10.61***	113.32 ±1.75	40.28 ±4.33***
AST (U/L)	194.18± 8.88	193.03±12.69	168.60±3.76***	301.75±10.89	153.07±4.13***
MDA (µM/g)	0.014±0.002	0.017±0.001	0.027±0.004***	0.052±0.002	0.022±0.006***
GSH (µM/g)	0.203±0.025	0.188±0.037	0.146±0.016***	0.043±0.010	0.150±0.030***

Results were expressed as Mean ±Sem, n=6 animals in each group, analyzed by one-way ANOVA followed by Tukey's multiple comparisons test. Comparison between (CCl_4) vs. (TPGS+CCL_4) group: ***$p<.001$. Comparison between (CCl_4) vs. (AA+CCl_4) group: ***$p<.001$

Table 3: Mean Pharmacokinetic parameters of Naproxen in presence of TPGS in inflammatory rats

PK Parameter	NPX (SDT)	NPX (MDT)	NPX +TPGS (SDT)	NPX+TPGS (MDT)
C_{max} (µg/ml)	0.95±0.3	8.6±0.72	0.46±0.02	5.80±0.5
t_{max} (h)	4.0±0	4.0±0	4.0±0	4.0±0
AUC $_{0-t}$ (µg/ml/h)	2.21±0.05**	4.81±1.8**	1.25±0.024	2.42±1.5
$t_{½}$ (h)	10.2±0.02**	10.7±0.05**	6.49±0.022	5.6±0.03
Clearance (l/hr)	4.23±0.03	4.83±5.45	3.11±0.05	2.50±2.85
V_d (ml)	12.26±0.08	12.92±8.68	14.6±0.32**	15.96±7.32**

The data were mean ± SEM. Mean values with asterisk are statistically significant at ** $p < 0.01$

DISCUSSION

From the effects we expedited the mortality and observed the poisonous signs at 2000mg/kg which states that LD 50 dose is 500mg/kg. There is a significant growth in body weight at LD50 dose whilst as compared to 5 and 50 mg/kg that offers us data approximately the elevated fats absorption and also a substantial change is visible in food consumption, water consumption and locomotor activity at 300mg/kg than 5 and 50mg/kg given in **Figure 1** in a 14 days treatment.

Antioxidants are the substances that stabilize and prevent the free radicals that reasons damage to the internal organs by means of presenting electrons to the broken cells **[39]**. So consumption of antioxidant wealthy fruits and vegetables will decrease the chance of many sicknesses that are due to free radicals. Bioactive peptides from the amphibian skin secretions endowed with excellent antioxidant defense system. *Rhinella schneideri* parotoid gland secretions found to have compounds related to antioxidant enzyme (catalase and peroxiredoxin) system and described that these antioxidant enzymes may protect the animal against ROS damage generated endo- and exogenously **[40]**. Indole alkylamines identified in various toad species as reported to have antioxidant effect in various biological systems **[41]**. In the present study for the first time we described *in vitro* and *in vivo* anti oxidant activity of *Bufo melanostictus* (Common Indian Toad)

parotoid gland secretion crude extract with the DPPH free radical scavenging assay and CCl_4 induced oxidative stress model **[42]**. We found that TPGS is having antioxidant activity via showing decreasing absorbance values at 517 nm which results due to the decolorization of samples from deep violet in to light yellowish in color whilst as compared with the standard ascorbic acid. Furthermore TPGS had shown its antioxidant activity by decreasing the serum enzymes (ALP, ALT and AST) and MDA levels which were increased at oxidative stress condition. Similarly GSH levels were also increased significantly ($P<0.5$) when compared with the CCl_4 treated rats.

The anti-inflammatory effect of Chansu the use of BV2 microglial cells in which he confirmed the NO, COX-2, PGE2, and ILs synthesis inhibition. In another study the bufalin anti-inflammatory activity with λ - carrageenan paw edema model with toad parotoid glandular secretions**[43]**. Toad lectins were purified from specific tissues together with gonads, skin, and granular glands of amphibians belonging to diverse species. These proteins can inhibit the production of pro-inflammatory cytokines, stimulate the production of anti-inflammatory cytokines, and have an effect on the release and migration of leukocytes **[44]**. Naturally isatin is observed within the parotoid gland secretions of *Bufo* frog a by-product of indole which is present in all *bufo* species. Similarly Indian toad (*bufo melanostictus*) may also contain bufalin and proteins like lectins and derivatives of isatins and for this reason anti inflammatory interest of TPGS is tried for the first time and were given promising results, in the present study, we explored that TPGS decreased the inflammatory effect induced by carrageenan and proven to have anti inflammatory effect alone and in combination with naproxen. Pharmacokinetic evaluation of naproxen significantly suggests that serum concentration of naproxen in inflammation induced rats is decreased likely to be due to the CYP1A2 enzyme induction which metabolizes the NSAID when co administered with TPGS in rats.

The pharmacokinetic drug interaction by co administered with naproxen (NPX), the decrease in C_{max}, t_{max}, AUC_{0-t} of NPX significantly ($p<0.05$). Fast elimination of NPX was found with decreased $T_{1/2}$ when co administered with AN. Similarly we discovered that treatment of naproxen together with TPGS led to significant decrease ($p<0.05$) in mean C_{max}, AUC, $T_{1/2}$ indicates that reduced exposure of naproxen after TPGS treatment and a significant

increase in mean Vd, Cl of naproxen values suggests the increasing of elimination of naproxen. Hence, it turned into hypothesis that any substance influencing the CYP1A2 induction enzyme is probably have such effect on the metabolism of NPX, and TPGS has the similar effect with metabolism of NPX **[45]**.

CONCLUSION

In the present work TPGS is proved to be toxic at higher doses, LD 50 found to be 500 mg/kg. Toad paratoid glandular secretions shown antioxidant, anti inflammatory and may be CYP1A2 enzyme inducer activities. Therefore further studies are required to isolate the new chemical compounds in pure form from Toad parotoid gland secretion of *Bufo melanostictus* as they may become new drug candidates.

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