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In-vitro Free Radicals Scavenging and Antioxidant Activity of Rice Bran Extract

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ABSTRACT

Aim: To evaluate the antioxidant activities of the γ-oryzanol isolated from rice bran oil of Indian origin in various *in vitro* models. **Materials and Methods:** The hydrogen donating ability of the hydroalcoholic extract of γ-oryzanol was measured in the presence of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical. **Results:** A 100 μg mL^{-1} of γ-oryzanol and ascorbic acid exhibited 64.56 and 64.88% inhibition, respectively and the IC_{50} values were found to be 48 and 56.50 μg mL^{-1} for γ-oryzanol and ascorbic acid respectively. The effect of γ-oryzanol on reducing power was studied according to the reaction of Fe^{+3} to Fe^{+2}. The reducing power of the extract increased with the increasing amount of the concentration. The total polyphenolic content of the γ-oryzanol was tested using Folin-Ciocalteau reagent. It was found that γ-oryzanol contained 19.31±1.79 mg of GA/g, which is significant ($p<0.05$) when compared to gallic acid. γ-oryzanol hydrogen peroxide (H_2O_2) radical at IC_{50} values of 45 μg mL^{-1} against the corresponding standards ascorbic acid (IC_{50} = 35 μg mL^{-1}). Moreover, the results were observed in a concentration dependent manner. **Conclusion:** All the above *in-vitro* studies clearly designate that the hydroalcoholic isolated γ-oryzanol has a significant antioxidant activity.

Key words: Antioxidant, DPPH, γ-oryzanol, rice bran extract

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INTRODUCTION

Oxygen free radicals and nitrogen free radicals are generated within the cells due to the various metabolic processes and mitochondrial energy production[1-5]. These radicals are responsible for beneficial as well as deleterious response. Their beneficial role includes response to the noxious stimuli that is antagonizing infectious agents along with this, they are the part of cell-cell signaling processes[6-9]. In low or moderate concentration, they play vital role in mitogenic response plus they required for the activation of cyclic guanosine monophosphate (cGMP) as a second messenger[10].

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The destructive effect of these generated free radicals instigating potential biological damage is known as oxidative stress and nitosative stress[11-18]. This eventuates in biological systems when there is an excessive synthesis of Reactive Oxygen Species (ROS) or Reactive Nitrogen Species (RNS) on one side at the same time an insufficiency of enzymatic and non-enzymatic antioxidants on the other[19-28]. This can be expressed in different way that, oxidative stress emerges from the metabolic processes that utilize oxygen and shows a disturbance in the equilibrium status of pro-oxidant or antioxidant reactions in living tissues. The excess ROS can vitiate cellular lipids, fatty acids, proteins, or DNA impeding their normal functions. This results into oxidative stress induction[29-39]. This induced oxidative stress can cause number of human diseases as well as in the ageing process. The insubstantial balance

between beneficial and harmful effects of free radicals is a very key aspect of living organisms and is executed by mechanisms known as "redox regulation" (pro-oxidant-antioxidant homeostasis). The processes of "redox regulation" shields living tissues from numerous oxidative stresses formed within the cells and retain "redox homeostasis" via controlling the redox status *in-vivo*[40-48].

Strenuous exercise causes oxygen utilization and that results into disturbance of intracellular redox regulation. The mitochondrial electron transport chains, polymorphoneutrophil, along with xanthine oxidase have been recognized as crucial sources of intracellular free radical generation centers during exercise. Reactive oxygen species constitute a significant defilement to the cellular antioxidant defense mechanism, like decreased storage of antioxidant such as vitamins and glutathione and increased tissue propensity to oxidative damage[49-52]. Nevertheless, enzymatic and nonenzymatic antioxidants have signified great familiarization to acute and chronic exercise. The important balance between pro-oxidants and antioxidants propose that supplementation of antioxidants may be useful for physically active individuals under certain physiological conditions by imparting a larger protective margin[53-57]. Research from human and animal studies proved that strenuous physical exercise may produce a state wherein the antioxidant defense mechanisms of several tissues are affected by excess reactive oxygen. A wide range of physiological and dietary antioxidants exploit in concert to elude such a stress. Regular physical activity in combination with dietary supplement that provide adequate supply of a combination of appropriate antioxidants may be anticipated to yield desirable outcome[58].

Ramarathnam in 1989 first identified and reported that the components of rice bran as flavonoid, isovitexin, α-tocopherol and γ-oryzanol possess high antioxidant activity[59]. γ–oryzanol is composed of mixture of phytosteryl ferulates and comprises of cycloartenyl ferulates, 24-methylenecycloartanyl ferulate and campesteryl ferulate. This mixture has been studied for antioxidant activity and this effect has been achieved as it enables donation of electron and destroys the action of reactive oxygen species and reactive nitrogen species. γ-oryzanol blocks pyrogallol autoxidation moreover, γ-oryzanol is more effective than the synthetic antioxidants against hydroperoxide formation. Also, reports suggested that γ-oryzanol inhibits the cholesterol oxidation and prevents tissues through stabilization of cellular metabolism preventing disease formation[60]. But the reports of the γ-oryzanol isolated from Indian rice bran extract are less about its antioxidant property. So, the main objective of the present study was to isolate γ-oryzanol from Indrayani rice bran of Indian origin and to study the antioxidative potencies, scavenging activities against DPPH radical, reducing power and estimation of polyphenol contents.

MATERIALS AND METHODS

Chemicals: Standard γ–oryzanol was procured from Oryza Oil and Fat Chemical Co. Ltd. Japan. Toluene, ethyl acetate, methanol and other analytical grade reagents and chemicals were used in the study are purchased from Merck Specialties Private Limited, Mumbai, India. Double distilled water filtered via 0.45 μ filter paper was utilized in the research work. Microliter syringe (Hamilton, Bonaduz, Switzerland) was acquired from Anchrome Enterprises (I) Pvt. Ltd Mumbai, India. Precoated Thin Layer Chromatographic (TLC) silica gel plates were purchased from Merck, Bangalore, Karnataka, India, of Kieselgel 60, F-254, 0.2 mm. Precoated glass silica gel plates were utilized for preparative TLC. High-Performance Thin-Layer Chromatography (HPTLC) spectra analysis was carried out on a Linomat V (Camag, Muttenz, Switzerland). Butylated hydroxytoluene (BHT), L-ascorbic acid, gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, 2-deoxyribose, trichloroacetic acid (TCA) and ferric chloride were obtained from Sigma-Aldrich Co. LLC. Mumbai, India.

Extraction and isolation of γ-oryzanol Rice Bran Oil (RBO): The extraction of rice bran oil was carried out according to previously reported method[61]. Shortly, rice bran of Indrayani variety of rice was acquired from Radhe Shyam Poha mill, Pune. This bran was kept in a refrigerator before its use. Direct solvent extraction method was applied for getting crude rice bran oil from 1 kg rice bran. Petroleum ether (60-80) was used as the solvent for extraction and the solvent was properly kept for 24 h for getting more yields. This procedure was performed with same rice bran with fresh petroleum ether three times for getting more yields. These obtained three fractions were mixed with each other to get one petroleum fraction. The resultant fraction then concentrated on rotary evaporator to get oil. The percentage yield was 10.4% of petroleum ether extract.

This collected rice bran oil was kept in a refrigerator before its use. From this, 3 mL oil was mixed with 5 mL absolute ethanol. Ethanol-oil mixture was further vigorously mixed by using cyclo-mixer and kept aside for 5 min. The supernatant was collected. The same procedure was repeated four times, so that 25 mL of supernatant was obtained. All the ethanol fractions were mixed and concentrated on rotary evaporator. The resultant concentrate was used for HPTLC spotting, isolation and *in-vitro* antioxidant study of γ–oryzanol. Oil (lower layer) remained in the test tube was dissolved in 5 mL ethyl acetate to verify the complete extraction of γ–oryzanol.

Chromatography: A Camag High-pressure Thin-Layer Chromatography (HPTLC) system equipped with an automatic TLC sampler (Linomat 5) with TLC scanner 3 and integrated software Win-Cats version 4 was used for the analysis. The HPTLC was implemented on a precoated silica gel HPTLC 60F254 (20×10 cm) plate for the quantification of γ-oryzanol in concentrated fractions. The samples and the standards were spotted on the plate as 6 mm wide bands. The linear ascending development was performed in a camag twin trough chamber (20×10 cm), which was pre-saturated with 20 mL mobile phase with toluene: ethyl acetate: Methanol (15.0:1.7:3.3, v/v/v) for 15 min, at room temperature. The length of the chromatogram run was kept up to 80 mm. Subsequent to the development; the TLC plate was dried by using current of air and with a hair dryer. Quantitative evaluation of the plate was carried out in the absorption reflection mode at 317 nm using Win-Cats software. Isolation of active constituent from concentrated fraction was carried out by preparative HPTLC.

***In-vitro* antioxidant activity of 24-mCAF isolated from rice bran oil**

Free radical scavenging activity in 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay: The antioxidant activity using the DPPH assay was evaluated in terms of hydrogen donating or radical-scavenging capability using the stable radical DPPH. The DPPH solution of 0.1 mM was prepared in ethanol and from this 1 mL was added to γ-oryzanol solution in ethanol at varying concentrations ranging from 10-100 μg mL^{-1}. These test solutions are kept at 37°C for incubation for 30 min. After 30 min of incubation, the absorbance of sample solutions was measured at 517 nm using ethanol as blank. Ascorbic acid was used as positive control for the study. Lower absorbance of reaction mixture stipulates greater free radical scavenging activity. To get exact scavenging capacity of γ-oryzanol following mathematical formula has been used:

$$\text{DPPH scavenged (\%)} = (A_{control} - A_{test}/A_{control}) \times 100$$

where, $A_{control}$ indicates the absorbance of control reaction while A_{test} is for the absorbance in the presence of γ-oryzanol. The antioxidant capacity of the γ-oryzanol was manifested as IC_{50}. Here, IC_{50} value was described as the concentration of γ-oryzanol in μg mL^{-1} that inhibits the formation of DPPH radicals by 50%.

Reducing power assay: Various concentrations of γ-oryzanol (10-100 μg mL^{-1}) of 1 mL were mixed with 2.5 mL of 0.2 M sodium phosphate buffer of pH 6.6 plus 2.5 mL of 1% potassium ferricyanide. The mixture was kept for incubation at 50°C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid (w/v) was added followed by centrifugation at 3000 rpm for 10 min. The upper layer 2.5 mL was taken and mixed with 2.5 mL deionised water plus 0.1% of 0.5 mL of ferric chloride was added. The absorbance of final mixture was analyzed on UV spectrophotometer at 700 nm by using sodium phosphate buffer having pH 6.6 with 0.2 M strength as blank solution. Higher absorbance at 700 nm denotes higher reducing power. The assay was performed in triplicate. Reducing power is indicated in Ascorbic Acid Equivalent (ASE) with unit ASE/mL that shows amount of ascorbic acid expressed in milli-Moles those reducing power is the same than that of 1 mL of sample.

Determination of total phenolic content: A 0.5 mL of sample i.e., 24-mCAF was mixed with 0.5 mL of Folin and Ciocalteu's phenol reagent. Above mixture was mixed thoroughly and was kept aside for three minutes. After 3 min, 3 mL of 2% sodium carbonate solution was added to the mixture. Final volume at 10 mL of mixture was made by adding sufficient amount of distilled water. This final mixture was allowed to stand for 2 h with intermediate shaking. The absorbance of the blue colored solution was read at 760 nm. Total phenolic content of 25-mCAF was performed in triplicate. The concentration of total phenolic compounds in the gallic acid was measured in terms of μg of gallic acid equivalent using an equation acquired from the standard gallic acid graph.

$$\text{Absorbance} = 0.0008 \times \text{gallic acid (μg)}$$

Hydrogen peroxide scavenging assay: The assay was done by adding 0.1 mL of 1 mM of EDTA, 0.01 mL of 10 mM FECl3, 0.1 mL of 10 mM H_2O_2 and 0.36 mL of 10 mM deoxyribose along with 1.0 mL of different dilutions of 24-mCAF (10-100 μg mL^{-1}) dissolved in ethanol plus 0.33 mL of phosphate buffer of pH 7.4 of strength 50 mM and 0.1 mL of ascorbic acid in sequence. The mixture was kept for incubation at 37°C aside for 1 h. for the development of pink color to the incubated mixture was taken 1 mL and mixed with 1.0 mL of 10% TCA and 1.0 mL of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA). The absorbance of the final mixture was read at 532 nm. The hydroxyl radical scavenging activity of the compound is reported in terms of % inhibition of deoxyribose degradation and is calculated as per following formula:

$$H_2O_2 \text{ scavenged } (\%) = (A_{cont} - A_{test})/A_{cont} \times 100$$

where, "A cont" is absorbance of the control reaction whereas "A test" is the absorbance in the presence of the sample of the compound.

Statistical analysis: Experimental results were expressed in Mean±SD of three measurements. The values $p<0.05$ recorded were considered as significant.

RESULTS AND DISCUSSION

Preparative HPTLC of petroleum extract: The method of extraction of phytochemicals from rice bran is the most significant route in the progress of pharmaceutical development of any plant species which is identified or described to have medicinal value. Preparative HPTLC is one of the simplest, robust and precise and yet highly steadfast procedure in the desire of collecting pure isolated compound from the rice bran extract. The rice bran extract revealed the brown fluorescent band and light brown band at Rf 0.72 for γ-oryzanol under the TLC scanner at 317 nm. The clear, bright fluorescent band was confirmed by comparing with the bands of standard γ-oryzanol and having same Rf value was scarped and dissolved in absolute ethanol and filtered. Ethanol was evaporated under vacuum and γ-oryzanol was isolated. The same procedure was repeated to get sufficient quantity of γ-oryzanol.

***In-vitro* antioxidant activity of isolated compound:** A wide range of *in-vitro* schemes have been used to evaluate radical scavenging ability and antioxidant activity of a compound. Antioxidant capacity is broadly applied as a criterion for medicinal bioactive components[62,63]. Different artificial entities have been utilized like 1, 1-diphenyl-2-picrylhydrazyl (DPPH), OH radicals scavenging activity, Fe^{3+}–Fe^{2+} reducing activity, ferrous ions (Fe^{2+}) chelating activity, H_2O_2 scavenging activity. In this study, the antioxidant activity of the γ-oryzanol was studied. The antioxidant activity of the γ-oryzanol has been studied in a succession of *in-vitro* tests namely: DPPH, free radical scavenging, reducing power, scavenging of superoxide anion radical-generated non-enzymatic system, hydrogen peroxide scavenging and metal chelating activities.

Inhibition of DPPH radical: With this method, it was promising to conclude the antiradical power of an antioxidant by measuring of a decrease in the absorbance of $DPPH^-$ at 517 nm. Color change from purple to yellow, the absorbance decreased when the $DPPH^-$ was scavenged by an antioxidant during donation of hydrogen to form a stable $DPPH^-$ molecule. In the radical form, this molecule had an absorbance at 517 nm, which vanished after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. Table 1 demonstrate a significant ($p<0.05$) reduce in the concentration of DPPH radical due to the scavenging ability of isolated γ-oryzanol and the standard ascorbic acid as a reference compound, conferred the maximum activity at all concentrations. A 100 μg mL^{-1} of γ-oryzanol and ascorbic acid exhibited 64.56 and 64.88% inhibition, respectively and the IC_{50} values were found to be 48 and 56.50 μg mL^{-1} for γ-oryzanol and ascorbic acid, respectively.

Table 1: DPPH radical scavenging activity of γ-oryzanol and ascorbic acid

Concentration (μg mL^{-1})	Inhibition (%) (Extracts)	Inhibition (%) (Standard)
10	22.42±0.257	33.14±0.150
20	27.14±0.144	48.93±0.076
40	44.96±0.160	62.93±0.117
60	50.10±0.163	70.58±0.139
80	61.32±0.175	76.87±0.127
100	64.46±0.122	81.53±0.125

Data is presented as the percentage of DPPH radical scavenging, Mean±SD

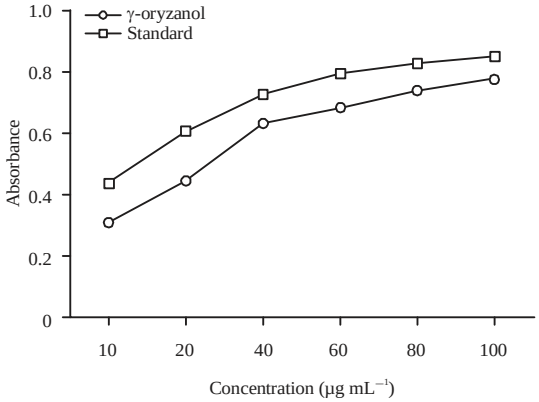

Fig. 1: Reducing power of γ-oryzanol isolated from rice bran oil. Each value is expressed as Mean±SD (n = 3)

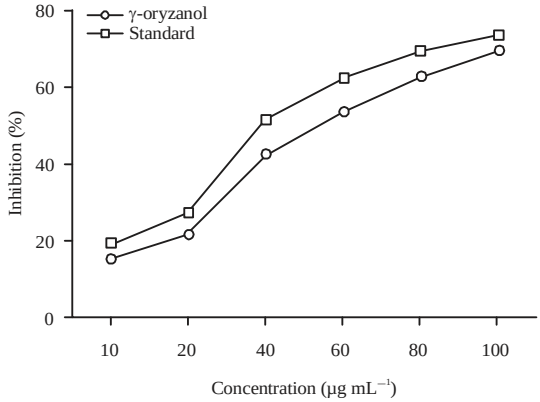

Fig. 2: Inhibition of hydroxyl radical by γ-oryzanol isolated from rice bran oil. Each value is expressed as Mean±SD (n = 3)

Reducing ability: The reducing capability of a compound generally depends on the presence of reductants which have been demonstrating antioxidant potential by breaking the free radical chain and donating a hydrogen atom. The presence of deductants in γ-oryzanol causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Hence, the Fe^{2+} can be monitored by measuring the production of Perl's Purssian blue at 700 nm. Figure 1 shows the reductive capabilities of the "γ-oryzanol" extract compared to ascorbic acid. The results on reducing power reveals the electron donor properties of γ-oryzanol thus neutralizing free radicals by forming stable products. The outcome of the reducing reaction is to conclude the radical chain reactions that may otherwise be very damaging.

Amount of total phenolic compounds: Phenolic compounds are identified as powerful chain breaking antioxidants. Phenols are very significant plant constituents because of their scavenging capacity owing to their hydroxyl groups. The phenolic compounds may provide directly to antioxidant action. It is recommended that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1 g daily from a diet rich in fruits and vegetables. The total phenolic content expressed in terms of Gallic Acid Equivalent (GAE) and was found to be 19.31±1.79 mg of GA g^{-1}. The total phenolic contents were calculated using the following linear equation based on the calibration curve of gallic acid:

$$y = 0.0025X+0.013, R2= 0.964$$

where, A is absorbance and X is amount of gallic acid in μg.

Inhibition of hydroxyl radical: The highly reactive hydroxyl radicals can origin oxidative damage to DNA, lipids and proteins. The effect of γ-oryzanol on the inhibition of free radical-mediated deoxyribose damage was evaluated by means of the Iron (II)-dependent DNA damage assay. The Fenton reaction creates hydroxyl radicals (OH) which demean DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may assault DNA either at the sugar or the base, giving production of large number of products. Figure 2 shows the effect of the γ-oryzanol on the Iron (II)$^{-}$ dependent deoxyribose damage. The γ-oryzanol was the capable of sinking DNA damage at all concentrations. (IC_{50} =53 μg mL^{-1}). Ascorbic acid, used as a standard was highly effective in inhibiting the oxidative DNA damage, showing an IC_{50} = 38.50 μg mL^{-1}.

Inhibition of H_2O_2 radical: Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell since it may give mount to hydroxyl radical in the cells. Therefore, removing H_2O_2 as well as O_2^- is very significant for protection of food systems. The scavenging ability of γ-oryzanol on hydrogen peroxide is shown in Fig. 3 and compared with that of ascorbic acid as standard. γ-oryzanol at 45 μg mL^{-1} and α-tocopherol

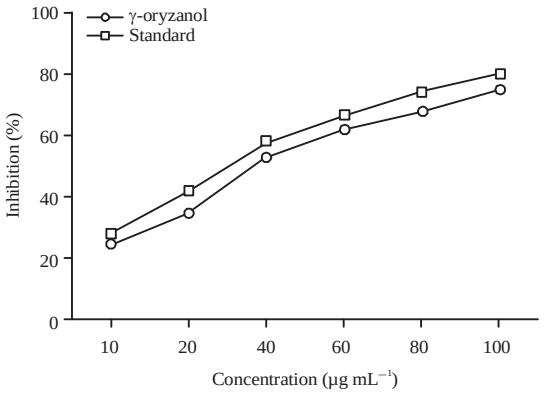

Fig. 3: Inhibition of H_2O_2 radical by γ-oryzanol isolated from rice bran oil. Each value is expressed as Mean±SD (n = 3)

at 35 µg mL^{-1} exhibited 50% scavenging activity, respectively, on H_2O_2 radical. There was statically significant correlation between γ-oryzanol value and standard ($p<0.05$).

CONCLUSION

According to data obtained from the present study, γ-oryzanol isolated from Indian rice bran oil extract was found to be an effective antioxidant in different *in-vitro* assay including reducing power, DPPH radical. Hydrogen peroxide scavenging and metal chelating activities when it is compared to standard antioxidant compounds such as BHT; γ-oryzanol plays equal role as natural antioxidant. Based on the discussion above, it can be applicable for minimizing and preventing lipid oxidation in food products, retarding the formation of toxic oxidation products, maintaining nutritional quality and lengthening the shelf life of foods and pharmaceuticals.

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REFERENCES

1. Adil, M., A. Visnagri, V.S. Kumar, A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2014. Protective effect of naringin on sodium arsenite induced testicular toxicity via modulation of biochemical perturbations in experimental rats. Pharmacologia, 5: 222-234.
2. Aswar, U.M., A.D. Kandhare, V. Mohan and P.A. Thakurdesai, 2015. Anti-allergic effect of intranasal administration of type-a procyanidin polyphenols based standardized extract of cinnamon bark in ovalbumin sensitized BALB/c mice. Phytother. Res., 29: 423-433.
3. Badole, S.L., S.M. Chaudhari, G.B. Jangam, A.D. Kandhare and S.L. Bodhankar, 2015. Cardioprotective activity of *Pongamia pinnata* in streptozotocin-nicotinamide induced diabetic rats. BioMed Res. Int. 10.1155/2015/403291
4. Bhalsinge, R.R., P.S. Worlikar, A.V. Tilak, S.L. Bodhankar and P. Ghosh *et al*., 2012. Effect of Nicotine on Brain Gaba Levels in Depressed Rats. Am. J. PharmTech Res., 2: 556-563.
5. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Pharmacokinetics, tissue distribution and excretion study of a furostanol glycoside-based standardized fenugreek seed extract in rats. Renal Failure, 24: 1-11.
6. Valko, M., C.J. Rhodes, J. Moncol, M. Izakovic and M. Mazur, 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact., 160: 1-40.
7. Ghosh, P., A.D. Kandhare, D. Gauba, K.S. Raygude and S.L. Bodhankar, 2012. Determination of efficacy, adverse drug reactions and cost effectiveness of three triple drug regimens for the treatment of *Helicobacter pylori* infected acid peptic disease patients. Asian Pac. J. Trop. Dis., 2: S783-S789.
8. Ghosh, P., A.D. Kandhare, V.S. Kumar, A.R. Rajmane, A. Mohammad and S.L. Bodhankar, 2012. Determination of clinical outcome and pharmacoeconomics of anti-rheumatoid arthritis therapy using CDAI, EQ-5D-3L and EQ-VAS as indices of disease amelioration. Asian Pac. J. Trop. Dis., 2012: S671-S678.
9. Ghosh, P., A.D. Kandhare, K.S. Raygude, D. Gauba, T.P. Gosavi and S.L. Bodhankar, 2012. Cigarette smoking and *H. pylori* infection: A meta-analysis of literature. Der Pharmacia Lettre, 4: 128-134.
10. Ridnour, L.A., J.S. Isenberg, M.G. Espey, D.D. Thomas, D.D. Roberts and D.A. Wink, 2005. Nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1. Proc. Natl. Acad. Sci. USA., 102: 13147-13152.
11. Kovacic, P. and J.D. Jacintho, 2001. Mechanisms of carcinogenesis focus on oxidative stress and electron transfer. Cur. Med. Chem., 8: 773-796.

12. Ghosh, P., A.D. Kandhare, K.S. Raygude, V.S. Kumar, A.R. Rajmane, M. Adil and S.L. Bodhankar, 2012. Determination of the long term diabetes related complications and cardiovascular events using UKPDS risk engine and UKPDS outcomes model in a representative western Indian population. Asian Pac. J. Trop. Dis., 2012: S642-S650.
13. Gosavi, T.P., P. Ghosh, A. Kandhare and S.L. Bodhankar, 2011. Unwrapping homeopathic principles in the wake of research: Serendipity, placebo or true therapeutic milestones? Pharmacologyonline, 1: 894-906.
14. Gosavi, T.P., A.D. Kandhare, K.S. Raygude, P. Ghosh and S.L. Bodhankar, 2011. A comparative study on the efficacy, safety and cost effectiveness of *Viscum album* and *Rauwolfia serpentina* mother tincture in hypertensive patients. Deccan J. Nat. Prod., 2: 29-35.
15. Gosavi, T.P., P. Ghosh, A.D. Kandhare, V.S. Kumar, M. Adil, A.R. Rajmane and S.L. Bodhankar, 2012. Therapeutic effect of *H. pylori* nosode, a homeopathic preparation in healing of chronic *H. pylori* infected ulcers in laboratory animals. Asian Pac. J. Trop. Dis., 2: S603-S611.
16. Gosavi, T.P., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2012. Anticonvulsant activity of *Argentum metallicum*, a homeopathic preparation. Der Pharmacia Lettre, 4: 626-637.
17. Gosavi, T.P., A.D. Kandhare, K.S. Raygude, P. Ghosh and S.L. Bodhankar, 2011. Evaluation of Clinical Outcome in Arthritis with AIMS2. Deccan J. Pharmacol., 2: 10-30.
18. Goswami, S., A. Kandhare, A.A. Zanwar, M.V. Hegde and S.L. Bodhankar *et al*., 2014. Oral l-glutamine administration attenuated cutaneous wound healing in wistar rats. Int. Wound J. 10.1111/iwj.12246
19. Honmore, V., A. Kandhare, A.A. Zanwar, S. Rojatkar, S. Bodhankar and A. Natu, 2015. *Artemisia pallens* alleviates acetaminophen induced toxicity via modulation of endogenous biomarkers. Pharmaceut. Biol., 53: 571-581.
20. Kamble, H., A.D. Kandhare, S. Bodhankar, V. Mohan and P. Thakurdesai, 2013. Effect of low molecular weight galactomannans from fenugreek seeds on animal models of diabetes mellitus. Biomed. Aging Pathol., 3: 145-151.
21. Kandhare, A.D., M.V.K. Patil and S.L. Bodhankar, 2015. L-Arginine attenuates the ethylene glycol induced urolithiasis in ininephrectomized hypertensive rats: Role of KIM-1, NGAL and NOs. Renal Fail., 37: 709-721.
22. Kandhare, A.D., K.S. Raygude, P. Ghosh and S.L. Bodhankar, 2011. The ameliorative effect of fisetin, a bioflavonoid, on ethanol-induced and pylorus ligation-induced gastric ulcer in rats. Int. J. Green Pharm., 5: 236-243.
23. Kandhare, A.D., J. Alam, M.V.K. Patil, A. Sinha and S.L. Bodhankar, 2015. Wound healing potential of naringin ointment formulation via regulating the expression of inflammatory, apoptotic and growth mediators in experimental rats. Pharmaceut. Biol. 10.3109/13880209.2015.1038755
24. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Prophylactic efficacy and possible mechanisms of oligosaccharides based standardized fenugreek seed extract on high-fat diet-induced insulin resistance in C57BL/6 mice. J. Applied Pharma. Sci., 5: 35-45.
25. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Acute and repeated doses (28 days) oral toxicity study of glycosides based standardized fenugreek seed extract in laboratory mice. Regul. Toxicol. Pharmacol., 72: 323-334.
26. Kandhare, A.D., S.L. Bodhankar, V. Mohan and P.A. Thakurdesai, 2015. Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fibrosis in rats: Decisive role of Bax, Nrf2, NF-κB, Muc5ac, TNF-α and IL-1β. Chemico-Biol. Interact., 237: 151-165.
27. Kandhare, A.D., S.L. Bodhankar, V. Singh, V. Mohan and P.A. Thakurdesai, 2013. Anti-asthmatic effects of type-A procyanidine polyphenols from cinnamon bark in ovalbumin-induced airway hyperresponsiveness in laboratory animals. Biomed. Aging Pathol., 3: 23-30.
28. Kandhare, A.D., P. Ghosh and S.L. Bodhankar, 2013. Morin attenuates airway hyperresponsiveness of allergic asthma via down regulation of immune-inflammatory biomarkers. Proceedings of the 15th International Congress of Immunology, August 22-27, 2013, Milan, Italy.
29. Kandhare, A.D., P. Ghosh and S.L. Bodhankar, 2014. Naringin, a flavanone glycoside, promotes angiogenesis and inhibits endothelial apoptosis through modulation of inflammatory and growth factor expression in diabetic foot ulcer in rats. Chemico-Biol. Interact., 219: 101-112.

30. Kandhare, A.D., P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2013. Elucidation of molecular mechanism involved in neuroprotective effect of Coenzyme Q10 in alcohol-induced neuropathic pain. Fundm. Clin. Pharmacol., 27: 603-622.
31. Kandhare, A.D., P. Ghosh, A.E. Ghule, G.N. Zambare and S.L. Bodhankar, 2013. Protective effect of *Phyllanthus amarus* by modulation of endogenous biomarkers and DNA damage in acetic acid induced ulcerative colitis: Role of phyllanthin and hypophyllanthin. Apollo Med., 10: 87-97.
32. Kandhare, A.D., V.S. Kumar, M. Adil, A.R. Rajmane, P. Ghosh and S.L. Bodhankar, 2012. Investigation of gastro protective activity of *Xanthium strumarium* L. by modulation of cellular and biochemical marker. Orient. Pharmacy Exp. Med., 12: 287-299.
33. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. Fitoterapia, 83: 650-659.
34. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Therapeutic role of curcumin in prevention of biochemical and behavioral aberration induced by alcoholic neuropathy in laboratory animals. Neurosci. Lett., 511: 18-22.
35. Kandhare, A.D., K.S. Raygude, P. Ghosh, A.E. Ghule, T.P. Gosavi, S.L. Badole and S.L. Bodhankar, 2012. Effect of hydroalcoholic extract of *Hibiscus rosa sinensis* Linn. leaves in experimental colitis in rats. Asian Pac. J. Trop. Biomed., 2: 337-344.
36. Kandhare, A.D., K.S. Raygude, P. Ghosh, T.P. Gosavi and S.L. Bodhankar, 2011. A comparative cross sectional descriptive study of individual pharmacy vs mall pharmacies to evaluate customer satisfaction as well as purchasing behavior. Der Pharmacia Lettre, 3: 71-83.
37. Kandhare, A.D., K.S. Raygude, P. Ghosh, T.P. Gosavi and S.L. Bodhankar, 2011. Patentability of animal models: India and the globe. Int. J. Pharm. Biol. Arch., 2: 1024-1032.
38. Kandhare, A.D., K.S. Raygude, V.S. Kumar, A.R. Rajmane and A. Visnagri *et al*., 2012. Ameliorative effects quercetin against impaired motor nerve function, inflammatory mediators and apoptosis in neonatal streptozotocin-induced diabetic neuropathy in rats. Biomed. Aging Pathol., 2: 173-186.
39. Kandhare, A.D., V. Shivakumar, A. Rajmane, P. Ghosh and S.L. Bodhankar, 2014. Evaluation of the neuroprotective effect of chrysin via modulation of endogenous biomarkers in a rat model of spinal cord injury. J. Nat. Med., 68: 586-603.
40. Droge, W., 2002. Free radicals in the physiological control of cell function. Phsyol. Rev., 82: 47-95.
41. Ketkar, S., A. Rathore, A. Kandhare, S. Lohidasan, S. Bodhankar, A. Paradkar and K. Mahadik, 2015. Alleviating exercise-induced muscular stress using neat and processed bee pollen: Oxidative markers, mitochondrial enzymes and myostatin expression in rats. Integ. Med. Res. 10.1016/j.imr.2015.02.003
42. Kumar, V.S., A.R. Rajmane, M. Adil, A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2014. Naringin ameliorates acetic acid induced colitis through modulation of endogenous oxido-nitrosative balance and DNA damage in rats. J. Biomed. Res., 28: 132-145.
43. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2011. Pharmacological evaluation of ameliorative effect of aqueous extract of *Cucumis sativus* L. fruit formulation on wound healing in wistar rats. Chron. Young Sci., 2: 207-213.
44. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Anti-inflammatory effect of *Daucus carota* root on experimental colitis in rats. Int. J. Pharm. Pharmaceut. Sci., 4: 337-343.
45. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Anti-arthritic and Anti-inflammatory activity of *Xanthium srtumarium* L. ethanolic extract in freund's complete adjuvant induced arthritis. Biomed. Aging Pathol., 2: 6-15.
46. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Effect of aqueous extract of *Cucumis sativus* Linn. fruit in ulcerative colitis in laboratory animals. Asian Pac. J. Trop. Biomed., 2: S962-S969.
47. Patil, M.V.K., A.D. Kandhare and S.D. Bhise, 2012. Pharmacological evaluation of ethanolic extract of *Daucus carota* Linn root formulated cream on wound healing using excision and incision wound model. Asian Pac. J. Trop. Biomed., 2: S646-S655.

48. Patil, M.V.K., A.D. Kandhare, P. Ghosh and S.D. Bhise, 2012. Determination of role of GABA and nitric oxide in anticonvulsant activity of *Fragaria vesca* L. ethanolic extract in chemically induced epilepsy in laboratory animals. Oriental Pharmacy Exp. Med., 12: 255-264.
49. Raygude, K.S., A.D. Kandhare, P. Ghosh, T.P. Gosavi and S.L. Bodhankar, 2011. Consumption of alcohol and *H. pylori* infection: A cumulative meta-analysis of literature. Asian J. Biochem. Pharm. Res., 1: 338-345.
50. Raygude, K.S., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2012. Anticonvulsant effect of fisetin by modulation of endogenous biomarkers. Biomed. Preventive Nutr., 2: 215-222.
51. Raygude, K.S., A.D. Kandhare, P. Ghosh, A.E. Ghule and S.L. Bodhankar, 2012. Evaluation of ameliorative effect of quercetin in experimental model of alcoholic neuropathy in rats. Inflammopharmacology, 20: 331-341.
52. Saraswathi, K.Y., A. Muthal, A. Kandhare, S. Rojatkar and S. Bodhankar, 2014. Study of methanolic extract of *Artemisia pallens* wall on endurance of laboratory animals. Pharmacologia, 5: 298-309.
53. Ji, L.L., 1999. Antioxidants and oxidative stress in exercise. Proc. Soc. Exp. Biol. Med., 222: 283-292.
54. Sarkar, S., A. Sengupta, A. Mukhrjee, A. Guru, A. Patil, A.D. Kandhare and S.L. Bodhankar, 2015. Antiulcer potential of morin in acetic acid-induced gastric ulcer via modulation of endogenous biomarkers in laboratory animals. Pharmacologia, 6: 273-281.
55. Shivakumar, V., A.D. Kandhare, A.R. Rajmane, M. Adil and P. Ghosh *et al*., 2014. Estimation of the long-term cardiovascular events using ukpds risk engine in metabolic syndrome patients. Indian J. Pharmaceut. Sci., 76: 174-178.
56. Visnagri, A., A.D. Kandhare and S.L. Bodhankar, 2015. Renoprotective effect of berberine via intonation on apoptosis and mitochondrial-dependent pathway in renal ischemia reperfusion-induced mutilation. Renal Fail., 37: 482-493.
57. Visnagri, A., A.D. Kandhare, S. Chakravarty, P. Ghosh and S.L. Bodhankar, 2014. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. Pharmaceut. Biol., 52: 814-828.
58. Sen, C.K., 1995. Oxidants and antioxidants in exercise. J. Applied Physiol., 79: 675-686.
59. Ramarathnam, N., T. Osawa, M. Namiki and S. Kawakishi, 1989. Chemical studies on novel rice hull antioxidants. 2. Identification of isovitexin, a C-glycosyl flavonoid. J. Agric. Food Chem., 37: 316-319.
60. Kim, J.S., J. Godber, J. King and W. Prinyawiwatkul, 2001. Inhibition of cholesterol autoxidation by the nonsaponifiable fraction in rice bran in an aqueous model system. J. Am. Oil Chem. Soc., 78: 685-689.
61. Muthal, A.P., S.R. Rojatkar and S.L. Bodhankar, 2014. Development and validation of HPTLC method for quantitative analysis of 24-methylcycloartenol ferulic acid extracted from rice bran. Der Pharma Chemica, 6: 354-360.
62. Visnagri, A., A.D. Kandhare, P. Ghosh and S.L. Bodhankar, 2013. Endothelin receptor blocker bosentan inhibits hypertensive cardiac fibrosis in pressure overload-induced cardiac hypertrophy in rats. Cardiovasc. Endocrinol., 2: 85-97.
63. Visnagri, A., A.D. Kandhare, V.S. Kumar, A.R. Rajmane and A. Mohammad *et al*., 2012. Elucidation of ameliorative effect of co-enzyme Q10 in streptozotocin-induced diabetic neuropathic perturbation by modulation of electrophysiological, biochemical and behavioral markers. Biomed. Aging Pathol., 2: 157-172.