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INDEX

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Nanoformulations of Curcumin for the Treatment of Diabetes Mellitus and Associated Complications

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ABSTRACT

Diabetes Mellitus (DM), the most prevalent metabolic disorder mainly characterized by chronic hyperglycemia, is increasing at an alarming level. DM can affect various organ systems in the body and over time, can lead to associated complications like diabetic cardiomyopathy, diabetic neuropathy, diabetic retinopathy, diabetic nephropathy, diabetic wound, etc. Many medicinal plant based therapeutic approaches were reported for DM prevention and management. Out of these medicinal plants, turmeric (Curcuma longa) has been used for the treatment of diabetes and associated complications. Curcumin, a bioactive phytocompound derived from rhizomes of turmeric is gaining great matter of interest amongst the scientific community due to it's antioxidant, antidiabetic, anticancer, anti-inflammatory, antimicrobial, cardioprotective, nephroprotective, hepatoprotective, neuroprotective, diabetic wound healing properties. However, it's clinical application is limited due to poor solubility, poor absorption, low bioavailability, rapid metabolism, rapid systemic elimination. So, various nanotechnology-based applications have been used to improve the clinical efficacy of curcumin. This review summarizes various nanoformulations of curcumin in the management of diabetes mellitus and associated complications.

KEYWORDS

Diabetes mellitus (DM), Associated complications, Phytocompound, Curcumin, Nano formulations.

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1. DIABETES MELLITUS

Diabetes mellitus is a multifactorial chronic metabolic disorder that involves the inability to produce insulin or to use it properly, resulting in altered metabolism of carbohydrates, fats, proteins and long-lasting hyperglycemia [1,2]. This disease is one of the most prevalent chronic diseases in the world and one of the serious public health challenges of the 21st century and it's incidence is increasing in both underdeveloped and developing countries [3,4,5]. Over the past few decades, the occurrence of diabetes has rised exponentially. In 2014, approximately 422 million

people constituting 8.5% of the total global population were diagnosed diabetic [6,7]. It is estimated that the number of diabetic individuals aged 20–79 years were 8.8% in 2015, corresponding to 415 million people [8]. The World Health Organization (WHO) has estimated that in 2030, 439 million people will be diabetic [9]. It has been predicted that approximately 642 million people will suffer from diabetes mellitus by 2040 [8].

In 2017, the American Diabetes Association (ADA) classified diabetes mellitus as follows: 1) type 1 diabetes, which is characterized by autoimmune β-cell destruction with consequent absolute insulin deficiency; 2) type 2 diabetes (90-95% of all diabetes cases), which is distinguished by a progressive loss of β-cells involved in insulin secretion and insulin resistance so, the body cannot properly use produced insulin; 3) gestational diabetes mellitus, a disorder that is diagnosed in the second or third trimester of approximately 7% of all pregnancies; and 4) specific types of diabetes due to other causes including monogenic diabetes syndromes, diseases of the exocrine pancreas (e.g. cystic fibrosis related diabetes) and drug or chemical induced diabetes (e.g. use of nicotinic acid and glucocorticoid), which represent about < 5% of patients with diabetes [10].

Diabetes is a silent killer which can give rise number of slowly or rapidly developing pathogenesis such as nephropathy, retinopathy, neuropathy, cardiomyopathy, peripheral arterial disease, coronary artery disease and stroke [11,12,13,14,15]. For type 1 diabetic patients, major treatment includes regular dose of long-acting insulin to maintain a basal level of insulin, in combination with bolus injections of fast-acting insulin at meal times [16]. Several hypoglycemic agents alone or in combination with insulin were clinically used for the treatment of type 2 diabetes mellitus [7,14]. However, the clinically available antidiabetic agents disappoint both clinicians and patients owing to their unexpected effects, which obviously shifted the focus in the direction of novel antidiabetic agent's discovery. On the other hand, numerous phytochemicals that found in nature have shown huge prospects against diabetes and diabetic complications in preclinical assays via aiming at multiple targets [17,7].

2. PHYTOCOMPOUNDS

Plants have always been an excellent source of traditional medicines or natural drugs. Phytocompounds are natural components isolated from plants, which have been paid increased attention with progressively in-depth research of modern medicine in the field of alternative medicine [18]. Nowadays, out of total Food and Drug Administration (FDA) approved drugs approximately 50 % of the drugs are plant derived compounds or their derivatives [19]. For example, a biguanidine-type antidiabetic drug- metformin is developed from galegine which is primarily isolated from *Galega officinalis* L. (Fabaceae) [20] and is currently used as the first-line oral

medication for type 2 diabetes mellitus (T2DM) management [21,22]. Plant-derived compounds are more affordable and accessible as compared with conventional therapies with minor side-effects, hence pharmaceutical research is progressively engaged in the discovery of plant based novel antidiabetic drugs [23,24]. Approximately 1200 plants have been claimed to contain compounds with antidiabetic potential and more than 400 plants and their bioactive compounds have been scientifically assessed in the T2DM treatment [24,25]. Phytocompound-based remedies are able to be developed as new approaches to treat T2DM or as adjuvants to support the existing treatment.

2.1 Curcumin

Curcumin (CM) is a natural polyphenolic compound isolated from the rhizome of *Curcuma longa* L. commonly known as turmeric (family Zingiberaceae). Curcumin has been widely used as herbal medicine in many Asian countries for thousands of years [26]. Curcuminoid known as diferuloylmethane is major constituent of CM (77 wt %); the other two curcuminoids are demethoxycurcumin (17 wt %) and bis-demethoxycurcumin (3 wt %) [27]. Curcumin is used in traditional medicine for the treatment of various disorders like anorexia, hepatic diseases, biliary complaints, cough and sinusitis [28]. Studies revealed that CM possesses antibacterial, antioxidant, anti-proliferative, anti-inflammatory, anti-carcinogenic and anti-amyloidogenic effects in vitro and in vivo models [29,30,31]. Other biological properties related to curcumin include wound healing, antirheumatic, hepatoprotective and anti-HIV [32,33,34,35].

Curcumin is a hydrophobic polyphenol and various studies have disclosed extremely low stability, low water-solubility, rapid metabolism and poor absorption of this component that markedly reduces its bioavailability and consequently decreasing the health benefits associated with this important compound [36,37,38]. To overcome these problems, drug delivery systems (DDS) have been taken into consideration to provide longer circulation times, increased permeability and resistance toward metabolic presystematic degradation [39,40]. Nanotechnology based formulations of curcumin has emerged as effective strategies for the treatment of diabetes mellitus and associated complications.

3. NANOTECHNOLOGY

The prefix ‘nano’ has been derived from Latin ‘nanus’, which means ‘dwarf’. Nanoscience deals with the objects with dimensions of 10^{-9} to 10^{-7} m. In recent years, nanotechnology has obtained huge interest in diagnosis and therapy of medical science. It has been shown that nanoscaled materials acquire some special physical, chemical and biological properties, which make them contributing for enormous biomedical applications [41]. Curative agents at the nanoscale dimension have been set up to break the barrier between therapeutic effects and pharmaceutical

incompetence. The development of different types of nanocarriers such as nanoparticles, micelles, liposomes, dendrimers has become a new approach in drug delivery over conventional drug delivery systems in the matter of bioavailability, effectiveness, stability, release of drugs and target specificity [42]. Nanocarrier-based drug colloidal nanoparticles with a dimension of less than 500 nm offer a high surface area to volume ratio [42]. A recent report has brought to the light that the global market of drug nanoformulations is rising continuously with an annual growth rate of 22 % [43].

4. NANOFORMULATIONS IN DIABETES TREATMENT

Nanotechnology-based approaches offer improved therapeutic management of diabetes mellitus with a minimized risk of acute and chronic associated complications [44]. For the treatment of diabetes mellitus, multiple nanoformulations with varying architectures have been fabricated [45]. Nanocarrier-based formulations ensure the efficient delivery of drugs to the target site with the desired release pattern [44,45]. In addition, nanoformulations allow the delivery of drugs through various routes [45,46]. Fabrication of nanocarriers can reduce the dose of drug and frequency of administration and the risk of toxic manifestations [44,47]. Thus, suitably designed nanoformulations of hypoglycemic agents may offer enhanced therapeutic management of diabetes in the near future. The subsequent section of this review emphasized the advancement and effectiveness of nanobased formulations of antidiabetic agent- curcumin.

5. CURCUMIN NANOFORMULATIONS (TABLE. 1)

The development of curcumin nanoformulations have emerged as one of the most prospective approaches to improve bioavailability, stability, solubility and therapeutic efficacy of curcumin as an antidiabetic agent.

5.1 Curcumin Nanoformulations for Diabetes Mellitus Treatment

Curcumin (50 mg/kg body weight) nanoencapsulated in chitosan-based complexes caused significant decrease in hyperglycemia within 7 days of treatment [48]. Nanoencapsulation of curcumin in chitosan-based polyelectrolyte complexes has increased the chemotherapeutic effectiveness of curcumin [48]. Curcumin-loaded polylactic acid (PLA)-polyethylene glycol (PEG) polymeric nanoparticles were found to be effective through the oral route in reciprocating hypoinsulinemia, hyperglycemia and diabetes-provoked hepatotoxicity more effectively than free curcumin [49]. This study revealed curcumin-PLA-PEG nanoparticles could impair hepatotoxicity by mitigating hepatic oxidative stress, inflammation, and fibrosis through suppression of respective signalling events [49].

Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) of polypeptide-k and curcumin exhibited better control of serum glucose level and biochemical tests

including liver parameters, lipid profiles, antioxidant levels and histological evaluation of pancreatic tissues as compared with their naive forms [50]. Oral delivery of curcumin-loaded pluronic nanomicelles has been shown to attenuate hyperglycemia, hyperlipidemia, hypoinsulinemia and oxidative stress via suppressing β-cell damage, promoting β-cell regeneration and triggering the activation of Pancreatic and Duodenal Homeobox 1 (PDX-1) and NK6 homeobox-1 (NKx6.1) genes beyond the values of controls [51].

Curcumin nanoparticles prepared by a modified emulsion-diffusion-evaporation method was found to decrease fasting blood glucose and glycosylated hemoglobin levels significantly via increasing the expression of insulin and insulin receptor (IR) mRNAs in diabetic rats [52]. Curcumin-nanoparticles (CUR-NPs) were found to significantly regulate glucose and insulin levels, restored BH4 (tetrahydrobiopterin) levels, improved oxidative stability and improved the bioavailability of Nitogen Oxide (NO) in the vascular wall [53]. Another study revealed that curcumin nanoparticles significantly alleviated the decreased phosphorylation of AKT pathway. Also Curcumin nanoparticles markedly reduced diabetes-induced oxidative stress and inflammation in the internal hepatic and pancreatic tissues [54]. Curcumin-ZnO (10 mg/kg, for 21 days) nanoparticles were claimed to be more effective than curcumin nanoparticles (50 mg/kg, for 21 days) in diabetes therapy in terms of reduction of blood glucose, improvement in serum insulin and activation of glucokinase and Glucose Transporter 2 (GLUT2) genes in pancreas and liver of type 2 diabetic rats [55]. Formulation of curcumin-loaded Poly Lactic-co-Glycolic Acid (PLGA) nanoparticles for oral delivery demonstrated enhanced bioavailability of curcumin and better therapeutic efficacy in the management of hyperlipidemia and inflammation in diabetic rats [56].

Curcumin-loaded chitosan nanoparticles were found to enhance muscle cell glucose uptake capacity of curcumin by inducing Glucose Transporter Type 4 (GLUT-4) translocation in vitro via enhancing its solubility [57]. Experiments showed significant reduction in body weight and fasting blood glucose levels and significant rise in insulin levels, liver glucokinase and glycogen synthase activities in nanocurcumin treated diabetic rats and mRNA expression of insulin, insulin receptor A, glucokinase and glucose transporter 2 significantly upregulated in diabetic rats received nanocurcumin [58]. Nano-curcumin showed enhancement in the antioxidant capacity in diabetic mice by increasing Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) activity when compared to both control and curcumin group [59]. Treatment of nanocurcumin decreased islet or beta cell death, shown by TUNEL assay and hematoxylin and eosin staining. It also significantly reduced inflammatory cytokine levels in pancreas. Pre-treatment with nanocurcumin decreased 8-oxo-20 -deoxyguanosine, a sensitive biomarker of reactive oxygen species (ROS)-induced DNA damage, in pancreas. Thus, this

nanocurcumin exerted diminishing effects of inflammation and apoptosis in pancreatic β-cells of type 1 diabetes mellitus [60].

5.2 Curcumin Nanoformulations for Diabetes Mellitus Associated Complication's Treatment

5.2.1 Effects on Diabetic Cardiomyopathy

Nanocarrier constituted with poly-(γ-benzyl l-glutamate), poly-(ethylene glycol) and poly-(γ-benzyl 1-glutamate) has been shown to improve the bioactivity and water solubility of curcumin [61]. The design of this nanocarrier provided gradual release, high loading capacity and low cytotoxicity [61]. Curcumin-encapsulated multi-polymeric nanocarrier achieved better pharmacological effects in cross-regulation of Ca2+/calmodulin, calcium-sensing receptor gene and endogenous cystathionine γ-lyase/H2S over conventional curcumin formulations in the management of diabetic cardiomyopathy in rats [61]. Nano-curcumin suspension decreased the serum levels of triglycerides, creatine kinase-isoenzyme (CK- MB), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). Histopathological analysis revealed restoration of structural integrity of the myocytes towards normalization. This reveals therapeutic potential of nanocurcumin in the treatment of diabetic cardiomyopathy [62]. Chitosan encapsulated curcumin treatments downregulated the blood sugar and total cholesterol level and upregulated insulin secretion. Histochemical analysis showed that chitosan-encapsulated curcumin ameliorated cell hypertrophy and nucleus enlargement in the left ventricular of heart and reduced fibrosis in the kidney of type-1 diabetic mice [63].

5.2.2 Effects on Diabetic Neuropathy-

Self-nanoemulsification of curcumin has been revealed to improve oral bioavailability and prolong plasma existence of curcumin [64]. (Joshi *et al*., 2013). Thus, in experimentally induced diabetic neuropathy, self-nanoemulsified curcumin formulation exhibited better therapeutic effect than native curcumin in terms of reversing behavioral, biochemical and functional inadequacy in rats [64]. An amphiphilic polymer prepared by the polymerization of methylaluminoxane (MAO), poly-(ethylene glycol) methacrylate (PEGMA) and 2-(dimethylamino) ethyl methacrylate (DMAEMA) constituted as a potential nanocarrier for encapsulating curcumin [65]. The curcumin-loaded nanoparticles were found to impair diabetic neuropathy via suppressing connexin43, interleukin-1β (IL-1β) and phosphorylated protein kinase B (Akt) expressions in dorsal root ganglia and downregulating purinergic 2 (P2) Y12 receptor mRNA expression in satellite glial cells of diabetic rats [65].

5.2.3 Effects on Diabetic Retinopathy-

Curcumin-entrapped PLGA-PVA (polyvinyl alcohol) nanoparticles were found to improve oral bioavailability of curcumin and thus, achieved improved therapeutic effect over free curcumin in delaying cataract formation in diabetic rats [66]. This nanoformulation of curcumin showed superior ability to interfere with the pathological events in the diabetes-mediated cataract formation such as oxidative stress, protein insolubilization, protein glycation, polyol pathway and crystallin distribution [66].

5.2.4 Effects on Diabetic Nephropathy-

Chitosan-encapsulated curcumin treatment reduced fibrosis in the kidney of type-1 diabetic mice showed by histochemical analysis [63].

5.2.5 Effects on Diabetic Wound Healing-

The curcumin-nanoparticle-loaded topical hydrogel discovered the improved water solubility and skin permeability of curcumin [67]. Thus, the nanocurcumin hydrogel improved the wound healing process in diabetic skin of type 1 diabetic rats as compared to that of normal curcumin hydrogel [67]. The ability of developed formulation of curcumin demonstrated reduction in elevated blood glucose level and lipid profile (total cholesterol, triglycerides). It maintained the body-weight, High-Density Lipoprotein (HDL), cholesterol level, other biochemical parameters and triggered the wound healing process upon the treatment of these curcumin-based formulations. The treatment of curcumin loaded mixed polymeric formulations accredited a favorable inhibitory effect to histopathological changes of liver, kidney and pancreas [68]. Self-assembled curcumin nanoparticles were encapsulated within gelatine microspheres to respond to matrix metalloproteinase 9 (MMP-9) which is commonly over-expressed at diabetic wound sides [69]. Thermosensitive hydrogel in the structure of curcumin-assembled gelatine microspheres was found to improve the capacity of drug release in a sustained manner to the diabetic wound and reduced redox stress and reversed MMP-9 (Matrix metalloproteinase-9)-provoked inhibition of cell migration in diabetic mice promoting wound healing [69]. Curcumin-loaded chitosan nanoparticles encapsulated into collagen-alginate complex have been designed for the treatment of diabetic wounds. This nanohybrid scaffold provided improvement in sustainability, stability, biocompatibility, porosity and tissue-regenerating ability to achieve a potential therapeutic option in the management of diabetic wounds [70]. Intranasal Polyvinyl Caprolactam- Polyvinyl Acetate-Polyethylene Glycol (PVCL-PVA-PEG)-based nanomicelles of curcumin treatment effectively reduced accumulation of reactive oxygen species, enhanced free radical scavengers, decreased mRNA expressions of inflammatory cytokines and increased mRNA expressions of neurotrophic factors in the cornea and trigeminal ganglion neuron promoting diabetic corneal epithelial/nerve wound healing [71].

5.3 Clinical Trials of Curcumin Nanoformulations for Diabetes Mellitus and

Associated Complication's Treatment

Several clinical trials have reported that nanoformulations of curcumin exhibit improved bioavailability and pharmacokinetic attributes and offered a strong reasoning for therapeutic applications of nanocurcumin [72]. In a double-blind randomized clinical trial, type 2 diabetic patients (n = 35) receiving curcumin nanomicelles (80 mg/day) for three months showed a significant decrease in the fasting blood glucose levels, glycosylated haemoglobin levels, low-density lipoprotein (LDL)-cholesterol, triglycerides and body mass index when compared with placebo control patients [73]. In another, double-blind randomized, placebo-controlled clinical trial, 80 diabetic patients were allocated randomly to the intervention (n=40) and the control group (n=40) receiving 80 mg of nanocurcumin or placebo capsules for 8 weeks showed significant reduction in glycated haemoglobin (HbA1c), Fast Blood Sugar (FBS), total score of neuropathy, total reflex score (p=0.04) and temperature as compared to placebo group [74]. Similarly, 80 diabetic patients volunteered in double- blind, randomized, placebo-controlled clinical trial were allocated randomly to the intervention (n = 40) and control (n = 40) groups, received 80 mg of nano-curcumin or placebo capsules daily for 8 weeks. After intervention, there was a significant decrease in the mean score of depression, mean score of anxiety in the nano-curcumin group as compared to placebo group suggested that nano-curcumin supplementation for 8 weeks was effective in reducing depression and anxiety scores in patients with diabetic polyneuropathy [75].

Table.1: Different Nanoformulations of curcumin and their effects in diabetes mellitus and associated complications

[↑ - increased, ↓- decreased; i.p. –intraperitoneal]

Nanoformulations of Curcumin		**Dose and Route**	**Study Duration**	**Outcomes**	**References**
Nanocarrier + Curcumin	**Components of Drug Delivery System**				
Nanoparticles of curcumin	--	15 mg/5 ml/kg, orally	3 weeks	Blood glucose lowering effects↑, Gene expression of insulin and insulin receptor↑	[52]

Nanoparticles of curcumin	--	10 mg/ kg 50 mg/ kg, orally	6 weeks	Oxidative stress↓ , Antioxidant effects↑, Anti-inflammation effects↑, Anti-diabetic effects↑	[54]
Nanoparticles of curcumin	Tween 60	30 mg/ml and 60 mg/ml (0.2 ml/kg), orally	30 days	Blood glucose lowering effects↑, Insulin level↑, Antioxidant effects↑,	[53]
Basic Nanoparticles of Curcumin	Sodium Bicarbonate Buffer	300 mg/kg, orally	56 days	Diabetic Cardiomyopathy↓	[62]
Polymeric Nanoparticles of Curcumin	Chitosan-alginate colloid	50 mg/kg	Once a day for 7 days	Blood glucose lowering effects↑, Hepatic glycogen↑, Deposition of curcumin in the liver↑	[48]
Polymeric Nanoparticles of Curcumin	PLA–PEG Copolymer	20 mg/kg/day, orally	60 days	Blood glucose lowering effects↑, Plasma insulin level↑, Anti-inflammation effects↑	[49]
Polymeric Nanoparticles of Curcumin	PLGA-PVA	100 mg/kg/day, orally	15 days	Anti-inflammation effects↑, Antihyperlipidemic effects↑	[56]

Polymeric Nanoparticles of Curcumin	Chitosan	25 µM (in vitro)	16 hours	Anti-hyperglycemic effects↑, Anti-inflammation effects↑	[57]
Polymeric Nanoparticles of Curcumin	PLGA-PVA	150 mg/kg/day, i.p.	20 days	Superoxide dismutase↑, Catalase↑, Glutathione peroxidase ↑	[59]
Polymeric Nanoparticles of Curcumin	PLGA-PVA	25 mg/kg/day, 50 mg/kg/day, 100 mg/kg/day, orally	28 days	fasting blood glucose levels ↓, Islets/β-cells death ↓, 8-oxo-dG ↓, Infammation and apoptosis in pancreatic β-cells↓	[60]
Polymeric Nanoparticles of Curcumin	BLG-NCA, H_2 N-PEG-NH_2	20 mg/kg/3 days, hypodermic injection	8 weeks	Diabetic Cardiomyopathy↓	[61]
Polymeric Nanoparticles of Curcumin	Chitosan	150 mg/kg	4 weeks	Diabetic Cardiomyopathy ↓, Diabetic Nephropathy ↓	[63]
Polymeric Nanoparticles of Curcumin	PLGA-PVA	2 mg/day, orally	70 days	Diabetic Cataract↓	[66]
Polymeric Nanoparticles of Curcumin	PEGMA-DMAEMA-MAO Microspheres	16 mg/kg - in sublingual vein	2 injections in 7^{th} and 8^{th} week	Diabetic Neuropathic Pain ↓	[65]
Nanomicelles of Curcumin	Pluronic F-108	100 mg/kg/day, orally	14 days	Anti-diabetic effects↑ (by ↑ Pdx-1, NKx6.1,	[51]

				insulin gene expression), Oxidative stress↓ , Antioxidant effects↑	
Nanomicelles of Curcumin	PVCL-PVA-PEG	4.5 mg/ml/day , intranasal and/or ocular topical	7 days	Corneal epithelial wound healing ↑	[71]
Nanomicelles of Curcumin	--	80 mg/day, orally (Double-Blind Placebo-controlled Clinical Trial)	3 months	Blood glucose lowering effects↑, HbA1c↓, Triglecerides ↓, serum LDL-C and BMI ↓	[73]
Polymeric Micelles of Curcumin	Chitosan, Alginate, maltodextrin, pluronic	100 µgcurcumin + carrier/rat/ day, topically	14 days	Blood glucose lowering effects↑, Antihyperlipidemic effects↑, Prevention of damage in kidney, liver and pancreas; β-cell regeneration↑, Wound healing effects↑	[68]
Curcumin-ZnO-Nanoparticles	--	10 mg/ kg	21 days	Blood glucose lowering effects↑, insulin level↑, expression of GK and	[55]

				GLUT2 in pancreas and liver↑	
Nanoemulsion of Curcumin (SNEDDS)	SNEDDS of Polypeptide-k, Labrafil M1944 CS, Tween-80 , Transcutol P, Aerosil-200 (A-200)	Polypeptidek-400 mg/kg and Curcumin-40 mg/ kg ; Polypeptidek-200 mg/kg and Curcumin-20 mg/kg	28 days	Blood glucose lowering effects↑, Antihyperlipidemic effects↑, Antioxidant effects↑, Regeneration of pancreatic tissue	[50]
Nanoemulsion of Curcumin (SNEDDS)	Gelucire, Vit. E TPGS, Labrasol, PEG 400, HPMC E5	30 mg/kg, 100 mg/kg, 300 mg/kg, orally	2 weeks	Diabetic Neuropathy ↓	[64]
Curcumin Nanoparticles-hydrogels	N,O-carboxymethyl CS/oxidized alginate hydrogel	7.5 mg/ml, topically	Once daily for 2 weeks	Type-1 Diabetic Wound healing ↑	[67]
Nanoparticles of Curcumin encapsulated in Gelatin Microspheres	Gelatin, glutaraldehyde	200 µl, topically	--	Diabetic Wound healing ↑	[69]
Polymeric Nanoparticles of Curcumin loaded nanohybrid scaffold	Chitosan, Collagen-Alginate Scaffold	--	--	Diabetic Wound healing ↑	[70]
Nanocurcumin	--	15 mg/kg/day	30 days	fasting blood glucose levels↓, insulin level↑, liver	[58]

				glucokinase↑, glycogen synthase↑, mRNA expression of insulin↑, insulin receptor A↑, glucokinase↑, glucose transporter 2↑	
Nanocurcumin	--	80 mg/day, orally (Double-Blind Placebo-controlled Clinical Trial)	8 weeks	Fasting Blood Sugar↓, HbA1c ↓, total score of neuropathy↓, total reflex score↓	[74]
Nanocurcumin	--	80 mg/day, orally (Double-Blind Placebo-controlled Clinical Trial)	8 weeks	mean score of depression↓, mean score of anxiety↓	[75]

6. CONCLUSION

Nanotechnology based formulations of curcumin plays a significant role in transforming curcumin from age-old medicine to modern era key solution with better remedy for the treatment of diabetes mellitus and associated complications. These nanoformulations of curcumin like it's nanoparticles, polymeric nanoparticles, nanomicelles, liposomes, SNEDDS, metallic nanoparticles, hydrogels, nanohybrid scaffold, etc leads to enhancement of bioactivity and bioavailability of curcumin by decreasing it's particle size. In this review, various nanoformulations of curcumin have discussed showing prominent results in the treatment of diabetes mellitus and associated complications than curcumin alone.

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Impact of Gut Microbiota on the Functionality of the Gastrointestinal Tract and Digestion

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ABSTRACT
Gut microbiota has been in limelight over the last fifteen years. Gut microbiota is known to exert a broad range of repercussions from beneficial to detrimental effects, depending on its composition. The current review focuses on the supremacy of gut microbiota on the gastrointestinal tract, how it helps in the digestion of fibers, how its dysbiosis initiates a range of gut-related disorders, and how we can countermand this condition, marking gut a potential site for drug targeting.

KEYWORDS
Digestion, Dysbiosis, Gut microbiota, Prebiotics, Probiotics.

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1. INTRODUCTION

At the time of birth, as the baby travels through the birth canal it is exposed to the billions of bacteria from the mother's vaginal tract which covers every part of the new-born's body [41]. These bacteria try to colonize within the host body very rapidly. The species which cannot colonize strive to death, whereas, bacterial species which are able to colonize, reproduce and form major communities in the skin, gut, urogenital tract etc. which in turn form the culminating communities in adults [19]. These bacterial communities form an essential part of the host's normal bacterial flora. The microflora helps the host in symbiotic fashion, e.g., human beings cannot digest the dietary fibers due to lack of genes that could help the host digest dietary fibers [44]. However, these indigestible dietary fibers serve as a source of food and energy for the gut microbes as the latter can digest the fibers into Short Chain Fatty Acids (SCFAs) like propionate, butyrate and acetate which show numerous health benefits in the host [26]. The types of bacteria in our gastrointestinal (GI) tract fall into five main phyla which include Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia [45]. Generally, in the healthy gut majority of bacteria belong to phyla Bacteroidetes and Firmicutes [5]. However, during the normal process of ageing, dysbiosis of gut microbiota is seen where the composition of gut bacteria changes drastically. During dysbiosis, there is a decrease in the population of beneficial bacteria like *Bifidobacterium* and *Lactobacillus* whereas; increase in the population of harmful bacteria of class Clostridia and family

Enterobacteriaceae is seen [51]. This drastic transition in a number of different bacteria results into catastrophic health events and age-related impairments [58].

2. COLONIZATION OF EARLY GUT BY BACTERIA

During birth through vaginal delivery, the baby gets exposed to the microbes from the vaginal tract and external genitalia of the mother. In addition, there is every possibility of harbouring the bacteria from the immediate surroundings like labour room. [14]. These microbes strive to occupy and populate the host. Many of these microbes disappear from the neonatal tract as they fail to colonize, however some bacterial species which can colonize will reproduce and eventually form the climax communities in adults [48]. Hooper, (2001) studied that the host's immune function, nutrient processing, and many important activities are influenced by the commensals. A large number of microbes reside within the distal gut where they ferment otherwise indigestible fibers and synthesize essential amino acids and vitamins [22]. The bacteria that live inside the host, outnumber the somatic and germ cells by ten times and their collective genome provides the host with traits that the host had not had evolved with [56]. The intestinal microbiota in an adult individual houses several thousand, mostly anaerobic bacterial species, which is the result of the successful establishment of the broad range of bacteria during infancy and early childhood [1].

3. GUT IMMUNITY

Microbes and pathogens enter the gastrointestinal tract through food and water. The Gut Associated Lymphoid Tissue (GALT) is meant for imparting protection against food and water-borne infections [27]. It consists of lymphoid follicles which are located in the lamina propria and sub-mucosa of the distal portion of the small intestine [39]. The GALT comprises of two sites, inductive site and effector site which are able to distinguish between harmful and harmless antigens. Peyer's patches (PP) act as inductive sites which are aggregations of lymphoid follicles, secreting IgA antibody to fight against pathogens. Whereas, effector sites (e.g., mucosal mast cells) are found to be dispersed in lamina propria [37].

Along with GALT, epithelial integrity of the intestine is another prime factor responsible for the protection of the intestine from the intrusion of harmful pathogens [18]. Kohler *et al*., (2003) studied that, commensal gut microbiota act as gatekeepers to protect intestinal epithelial cell integrity from penetration and diseases caused by pathogens and a broad range of antigens. This epithelial cell integrity is maintained with the help of communication between the gut bacteria and intestinal epithelial cells [47]. Some species of bacteria help the host by improving the IgA antibody secretion in the Peyer's patches. Yanagibashi *et al.*, (2009) studied that, the differentiation of B cells into IgA-producing plasma cells is induced by a commensal bacterium of the species *Bacteroides.* Later the IgA secreting plasma cells get dispersed in the lamina propria of intestinal villi.

4. DYSBIOSIS

After birth, as microbes colonize the GI tract rapidly, Actinobacteria and Proteobacteria are seen dominating the early stages of development, whereas, the microbe population of other phyla is generally seen low [6]. As an individual grows, there is shift in the food habit from milk to solid food. During the teething period, babies put most of the things in their mouths called as baby mouthing, which could be a source of entry of bacteria into the GI tract [16]. This is how, as an individual grows, there is a change in the gut microbiota. During infectious illness, antibiotics although prescribed to control and nullify the infection, also kill the normal gut microbiota [31, 46]. This disturbs the gastrointestinal functions, e.g., dysentery caused due to the treatment of antibiotics [52].This is controlled by providing probiotics like Lactobacilli, curd, yogurt etc. [2]. Many intrinsic and extrinsic factors are responsible for imbalance of normal gut microbiota. Small Intestinal Bacterial Overgrowth (SIBO) is one example where, there is an uncommon increase in the overall bacterial population in small intestine leading to intestinal diseases [20]. Dysbiosis is connected with range of chronic gastrointestinal disorders like irritable bowel syndrome (IBS), functional dyspepsia (FD) and inflammatory bowel diseases (Crohn's disease and ulcerative colitis) [7, 21, 42, 43]. A recent study also showed how duodenal microbial dysbiosis is connected with enteropathy [9].

5. AGE RELATED IMPAIRMENTS DUE TO DYSBIOSIS

During ageing, due to dysbiosis, there is chronic low-grade inflammation of the intestinal mucosa, called as 'Inflammaging' [28]. During Inflammaging, innate immune response gets triggered causing tissue degeneration without any infection [3, 36]. Thus, Inflammaging is a characteristic feature of ageing due to dysbiosis [8]. Dominance of different bacteria of phyla Bacteroidetes and Firmicutes changes with age of an individual e.g., the elderly having a higher proportion of Bacteroidetes, while in young adults the Firmicutes predominate. [38]. Moreover, production of anti-inflammatory factors like butyrate is reduced during ageing increasing the susceptibility to Inflammaging. Pathogenic bacterial growth during dysbiosis, may release enterotoxins which increase the intestinal permeability leading to the inflammation of the intestine [11].

6. TARGETING GUT WITH PROBIOTICS

Probiotics are live microorganisms administered into an individual to improve their gut's bacterial balance [15]. Natividad and Verdu, (2013) studied that the beneficial bacteria in gut, helps the host to strengthen the intestinal epithelium and reinforce the gut integrity. Dietary intervention with probiotics, targeted into patient suffering from dysbiosis, showed remarkable improvement in their condition [4]. Factors like unhealthy lifestyle, improper diet and intake of antibiotics may alter the composition of gut bacteria leading to gastro-intestinal disorders. To revert this condition and

fortify intestinal immunity, probiotics play a prime role [29]. The bacteria of genus *Bifidobacterium* predominates in the breastfed infants, where it aids in the acceleration of immune response, improvising balance of immune system, suppression of inflammation, empowerment of intestinal barrier and increasing acetate production [10].

7. DIGESTION OF PREBIOTICS BY GUT BACTERIA

The term "prebiotic" refers to the food ingredients that are normally indigestible by host, but are digested by gut bacteria showing range of beneficial effects on the host [54]. Humans lack the enzymes that can degrade bulk of dietary fibers. However, these dietary fibers which remain undigested in the upper gastrointestinal tract are digested by the anaerobic, cecal and colonic bacteria in the cecum and large intestine [13]. For example, human milk which contains fucosylated oligosaccharides, can be utilized by bacterial species like *Bifidobacterium longum* and several species of genus *Bacteroides* [60].Feeding habits can greatly affect the gut bacterial composition of an individual. Thus, aiming an individual's gut with synbiotics is an emerging and promising technique used in today's comprehensive nutrition. [50].

After metabolization of the fibers, bacteria in gut consequently increase the concentration of circulating Short-Chain Fatty Acids (SCFAs) like butyrate, acetate, and propionate [55]. This production of SCFAs is responsible for the stimulation of ileal propulsive contractions, the release of neuroendocrine factors, and acidification of the intra-colonic pH [25].

8. DEGRADATION OF DIETARY CARBOHYDRATES BY GUT BACTERIA

Cummings and Macfarlane, (1991) studied that, around 20g-60g of carbohydrates derived from the daily diet escapes without getting digested and reaches colon. These escaped carbohydrates are the indigestible resistant starches, polysaccharides of plant cell wall and several oligosaccharides [53]. Even though small intestine tries to digest dietary starches, a small portion is left behind and reaches large intestine, where it is called as 'resistant starch'. [34]. This resistant starch can be digested by bacteria of phyla Firmicutes [35]. Bacteria of genus *Ruminococcus, Clostridium,Eubacterium*, *Bacteroides* isolated from the human feces, turned out to be the main cellulolytic strains.

9. DIGESTION OF HOST-DERIVED GLYCANS BY GUT BACTERIA

Human breast milk consists of D-glucose, D-galactose or L-fucose residues at concentrations around 10g/ L. *Bifidobacterium* species have potential to utilize these oligosaccharides in the milk, thus, they are seen predominating in feces of breast-fed infants [23]. In a study it was found that more than 8% of genes in bifidobacterial

genomes were involved in metabolism of carbohydrate. This is more than 30%of contribution compared to any other gut microbes, in carbohydrate metabolism [30, 33, 49, 57].

10. CONCLUSION

Gut of living organisms house vast communities of bacteria which form an essential segment of the host's gut health. Bacterial species like *Bifidobacterium bifidum*, *Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus rhamnosus* contribute to the homeostasis of intestinal flora and curtailment of the growth of harmful bacteria. Dysbiosis is a condition marked by a decrease in the population of beneficial bacteria and an increase in the population of harmful bacteria. Gut-related disorders are often accompanied with ageing due to dysbiosis. This condition can be reverted by using probiotics, prebiotics, or synbiotics, resulting in the fortification of gut immunity. Gut bacteria thus has a paramount role in the digestion of the dietary carbohydrates and host-derived glycans.

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Age and Longevity of Indian Garden Lizard, *Calotes versicolor* by Skeletochronology

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ABSTRACT

In this study, age structure and longevity of 40 individuals (22 males and 18 females) of Indian garden lizard Calotes versicolor was determined by skeletochronology. This is a medium-sized, arboreal lizard with oval head and laterally compressed body. They are commonly found among the undergrowth in open habitats including highly urban areas. Phalangeal bones were cross-sectioned on a rotary microtome, then sections stained with Harris hematoxylin for 10-15 min. Sections from the mid-diaphysis were selected and mounted in glycerin after rinsing with tap water and observed with light microscopy and enumerated the number of LAGs. It's average snout vent length (SVL) was 9.49 ± 1.74 cm and 8.35 ± 1.07 cm, whereas the median age was 2.77 ± 1.31 (range = 2 - 5) for males and 2.32 years (SD = 1.04, range = 2 - 4) for females, respectively. No statistically significant differences were noticed in body mass and SVL between the sexes. However, there was a significant positive correlation between body mass and SVL (r = 0.86). Based on this study, the maximum longevity of this lizard is from 5 years for females to 6 years for males in a natural population.

KEYWORDS

Age structure, Garden lizard, Skeletochronology, Tropics.

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1. INTRODUCTION

India, being a mega-diverse country, harbors more than 518 species of reptiles [1]. Among these reptiles, the Indian garden lizard *C. versicolor* (Daudin, 1802) is a largely widespread tropical lizard and found in the Indian subcontinent [2]. However, it ranges from South-eastern Iran, Afghanistan, Bangladesh, Pakistan, Nepal, Bhutan, Sri Lanka, Myanmar, Thailand, Western Malaysia, Maldives, Vietnam, Cambodia, South China, Indonesia and Mauritius [3]. Recent studies confirm its distribution to Oman, Singapore and United States [4]. They are commonly found among the undergrowth in open habitats including highly urban areas. Mainly they feed on insects and small vertebrates, including rodents and other lizards. Body colour is light brown and grayish on dorsal side with transverse spots on back and sides [5].

This species shows high degree of sexual dimorphism. The males have a larger relative head size and longer relative limb lengths; while female exhibit a longer relative trunk length [6]. Scales on head and dorsal line are more prominent in males than in females [7]. The lizards reach sexual maturity within a year and has longer breeding season coinciding with the South West monsoon [4]. Both the sexes develop red color on anterio-dorsal region during breeding season, a sign of onset of sexual maturity [8-10]. A lot of studies have been conducted on the garden lizard *C. versicolor* inhabiting tropical region for instances, studies on systematic, distribution and attainment of sexual maturity, feeding, growth, sexual dimorphism, gametogenic cycles and reproductive strategies [4, 11-14]. However, there is no age structure studies on the tropical garden lizard *C. versicolor.* The present investigation is an attempt to determine age, longevity and relationship between body mass and size among the sexes in *C. versicolor* inhabiting the tropical region.

2. MATERIALS AND METHODS

Indian garden lizard *C.versicolor* were collected from the forest area of Dr. Yashvantrao Chavan Sagareshwar Wildlife Sanctuary, Devrashtre (17^0 09' N and 74^0 45' E), Sangli District, India in the month of April. Sanctuary has an area of 10.87 Sq. km with high peaks and very deep valleys. The forest is dry mixed deciduous and southern thorn type. Hill slops are covered with a grass which supports to many grazing mammals, lizards, and insects. Study area receives an annual rainfall about 300–500mm during monsoon (June–October) and frequently undergoes drought condition. The maximum recorded temperature was up to 41°C during summer and a minimum temperature of 10°C in winter. In the study area *C. versicolor* population is very prosperous due to availability of food sources around the year. Collected lizards were brought to the laboratory where the sex of each individual was assessed from their breeding behavior and cloacal morphology. Males have an elongated cloaca while that for females is swollen and round. Base of the tail is swollen in males compared to that of females. Simultaneously the body size (snout-vent-length, SVL) was measured to the nearest 0.1 mm with a digital caliper and body mass was measured to the nearest 0.1 g with a single pan balance, the 4^{th}toe of right limb was chopped off under light ether anesthesia [13, 15] and fixed in 10% formalin for histological process. The lizards were kept under observation until the recovery and then allowed to release at the site of collection. The formaldehyde fixed toes were washed in running water for 24 hrs, decalcified in 5% Nitric acid and then washed in running water for 24 hrs. The resulting phalangeal bones were then cross-sectioned (10μ thick) on a rotary microtome, then sections stained with Harris hematoxylin for 10-15 min. Sections from the mid-diaphysial were selected and mounted in glycerin after rinsing with tap water and observed with light microscopy and enumerated the number of LAGs. For each individual, at least 5 sections were scored to check for

consistent reproducibility and reduce the risk of errors due to localized breakage of LAGs by endosteal resorption. Photomicrographs of representative sections were taken with a digital camera. Students-*t* test was carried out to determine the significant difference between the sexes in body mass, body size (SVL) and LAGs. Furthermore, relationships between body mass vs body size and body size vs LAGs were calculated by Karl Pearson's correlation coefficient 'r'. Statistical analyses were performed by SPSS (Version 10.0).

3. RESULTS

Among 40 individuals with sex ratio of 22 males and 18 females, the mean SVL was 9.49 ± 1.74cm and 8.35 ± 1.07cm for males and females, respectively (Table 1). The median age was calculated as 2.77 years (SD = 1.31, range = 2-5) for males and 2.32 years (SD = 1.04, range = 2-4) for females (Table 1). The hematoxylin-stained phalangeal cross sections consisted of, a light wide zone representing a season of rapid growth; and a thin, dark zone representing a season of slow growth, making up a single year's growth. Zero to five LAGs were observed in the cross sections of phalanges of different sized individuals (Figure 1 A-D). The maximum age found was 5 years for females and 6 years for males (Table 2; Figure 2). The LAGs were closer together near the margin of the bone opposite from the bone marrow cavity and so the distance between inner was much larger than that for outer periosteal layer in both sexes (Figure 1D). In 5 individuals (12.5%) cross sections, the first LAG was partially resorbed by the endosteal bone but did not disappear completely (Fig. 1C). There was a positive correlation between body mass and SVL in male (r = 0.61) and female (r=0.86). Males were larger than females but there was no significant difference between body mass ($t = 2.3761$, $df = 38$, $P < 0.0226$) and SVL ($t = 2.5262$, $df = 38$, $P < 0.0158$) between the sexes (Table 1).

4. DISCUSSION

Determining the age of individual animal is extremely important for life history and population dynamics studies in amphibians and reptiles [11]. Various methods like; body size analysis, testis lobation, mark-release-recapture and skeletochronology are employed for estimating age in reptiles. Mark-release-recapture is most useful method among the above; however, this method is time consuming and requires an important amount of field hours to reach the results [11, 12, 16]. Alternatively, skeletochronology is widely used method for age estimation in amphibians and reptiles [11, 17-19]. Moreover, skeletochronology is proved to be reliable and accurate method for assessment of age in temperate as well as tropical reptilian species [13]. In India, skeletochronological studies are more concentrated on anuran species [20-24], few studies have employed this technique in Indian reptilian species, such as, South Indian rock agama, *Psammophilus dorsalis* [24], male garden lizard,

C. versicolor [25], Fan-throated lizard, *Sitana ponticeriana* [26, 27], common house Gecko, *Hemidactylus brooki* [28], Indian skink, *Mabuya carinata* [29],

The results of the present study demonstrate that growth marks are detectable in the cross sections of phalanges of south Indian garden lizard *C. versicolor* comparable to those reported in reptilian species inhabiting North-East region of India. The fact that many of the Indian reptilian species were exhibit marked seasonality in the reproductive activity and abdominal fat body mass [8, 12], suggests that bone growth is a cyclical phenomenon in these animals. However, both body and fat-body mass of the garden lizard *C. versicolor* were very lower in breeding phase (June –August), which coincides with the onset of monsoon rains; from September onwards, there is an increase in body and fat-body mass, and they attain their maximal values between December and April [12]. Therefore, in this lizard, the LAG(s) may be laid down between May - August when the body growth and food reserves almost ceased coinciding with the wet season of the year. From September onwards when body and fat-body masses start to restore, the next osteogenic cycle begin.

Differences in life expectancy of *C. versicolor* have been found in two populations. The estimated longevity is 5 years in females and 6 years in males in the present study area and maximum recorded longevity was 4 years in male garden lizard *C. versicolor* population of Bhuvaneshwar, Odisha, North-Eastern region of India [25]. Present result reveals that male lizard population of southern region of India has sharply increased longevity than that of male population of the north east region [34]. Previous skeletochronological studies have showed more or less similar longevity (ranges from 4 to 5 years) in Indian reptilian species [28-30] found that age ranged from 2 to 4 years in males and from 2 to 3 years in females for a population of *Lacerta agilis* from Italy. However, in *L. agilis* living in Russia the maximum longevity observed was 6–7 years for males and 5–6 years for females, depending upon altitudes. Life expectancy of *C. versicolor* is similar in some tropical lizard species such as, *Agama impalearis, Phrynocephalus melanurus, Phrynocephalus horvathi* [31-33]. Maximum recorded longevity was 9-10 years in *Laudakia stoliczkana*, 12-14 years in *Varanus griseus* and 12-13 years in *Laudakia caucasia* [32-36].

One of the problems generally associated with skeletochronological age estimation is the phenomenon of bone resorption [14, 15]. In the present study the rate of endosteal resorption is assessed based on the comparison between the phalangeal sections at the same magnification from different individuals; the first innermost LAG is partially eroded in five (12.5%) individuals. There is no loss of complete LAG due to resorption unlike, earlier studied southern Indian species *H. brooki* [28] and *M. carinata* [29].

There is a strong positive correlation between the body mass and SVL in males (r = 0.61) and females (r = 0.86). This result suggests that body mass and size may be a

reliable criterion for aging in this species**.** Present data shows that males are larger than females but there is no significant difference in body mass and body size between sexes. Similarly, the males of *Agama agama, Phrynocephalus interscapularis, Anolis opalinus* and *Cophosaurus texanusscitulus* grow larger than the females [33-36]. There are some reports comparing growth rates between the sexes in lizards based on the wild populations, mark-recapture studies and maintained in outdoor terraria [8, 35-38]. Generally, differences in body size between male and female is caused by differences in age structure, growth rate and timing of growth deceleration [34-38].

5. CONCLUSION

The results of the present study reveal that skeletochronology is applicable to tropical lizard *C. versicolor*. Animals of different body size exhibit a range from zero to five LAGs in phalanges. Moreover, there is no significant difference between body mass and SVL between the sexes for this species.

ACKNOWLEDGMENT

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Table 1: Body mass, snout-vent length (SVL) and number of LAGs in male and female *Calotes versicolor* (Values in Mean ± SD)

Sex	Body Mass (g)	SVL (cm)	LAGs
Male	38.88 ± 17.44	9.49 ± 1.74	2.77 ± 1.31
Female	22.41 ± 9.46	8.35 ± 1.07	2.32 ± 1.04
T-value	-1.0859	1.283	- 1.0375

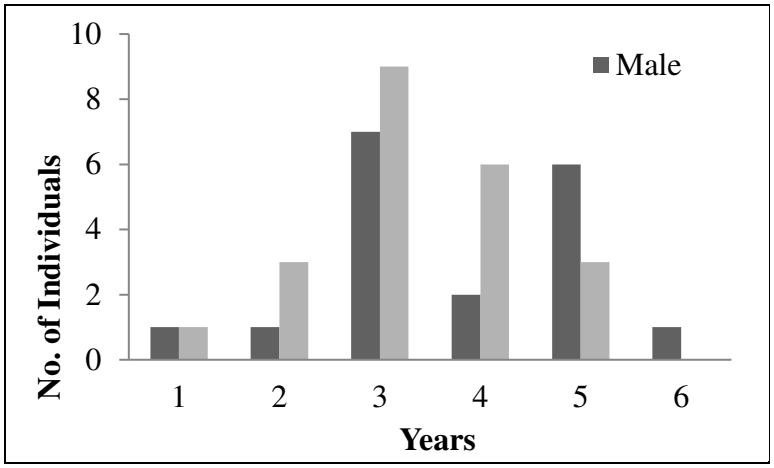
Table 2: Age, number of individuals, mean body size (cm) And range of male and female *Calotes versicolor*

Sex	Age	N	SVL		
			Mean	SD	Range
Female	I	01	8.4	-	-
	II	03	7.53	1.30	6.5-9.0
	III	09	8.43	0.88	7.3-10.1
	IV	06	8.26	1.02	6.5-9.5
	V	03	9.13	1.67	7.2-10.2
	Total	**22**			
Male	I	01	8.1	-	-
	II	01	11.0	-	-
	III	07	8.91	1.68	6.5-12.0
	IV	02	8.0	0.28	7.8-8.2
	V	06	10.65	1.75	8.4-13.5
	VI	01	9.5		
	Total	**18**			

Table 3: Longevity of some tropical lizard species assessed by skeletochronology

Sr. No.	**Genus & Species**	**Authors**	**Longevity in years**
1	*Hemidactylus brooki*	Pancharatna and Kumbar, 2005	4
2	Male*Calotes versicolor*	Patnaik and Behera, 1981	5
3	*Mabuya carinata*	Kumbar, 2010	5
4	*Sitana ponticeriana*	Rath and Pal, 2009	6
5	*Psammophilus dorsalis*	Mahapatro *et al.,* 1989	5
6	*Agama impalearis*	El Mouden *et al.,* 1997	5
7	*Phrynocephalus melanurus*	Smirina and Ananjeva, 2007	5
8	*Phrynocephalu shorvathi*	Cicek *et al.*, 2012	5
9	*Lacerta agilis*	Guarino *et al.*, 2010	3-4
10	*Laudakia stoliczkana*	Ananjeva *et al.*, 2006	9-10
11	*Varanus griseuswas*	Smirina and Tsellarius, 1996	12-14
12	*Laudakia caucasia*	Panov and Zykiva, 2003	12-13

Figure 2: Number of individuals with their respective body size (cm) and number of Growth marks in the phalangeal cross section of male and female *Calotes versicolor*



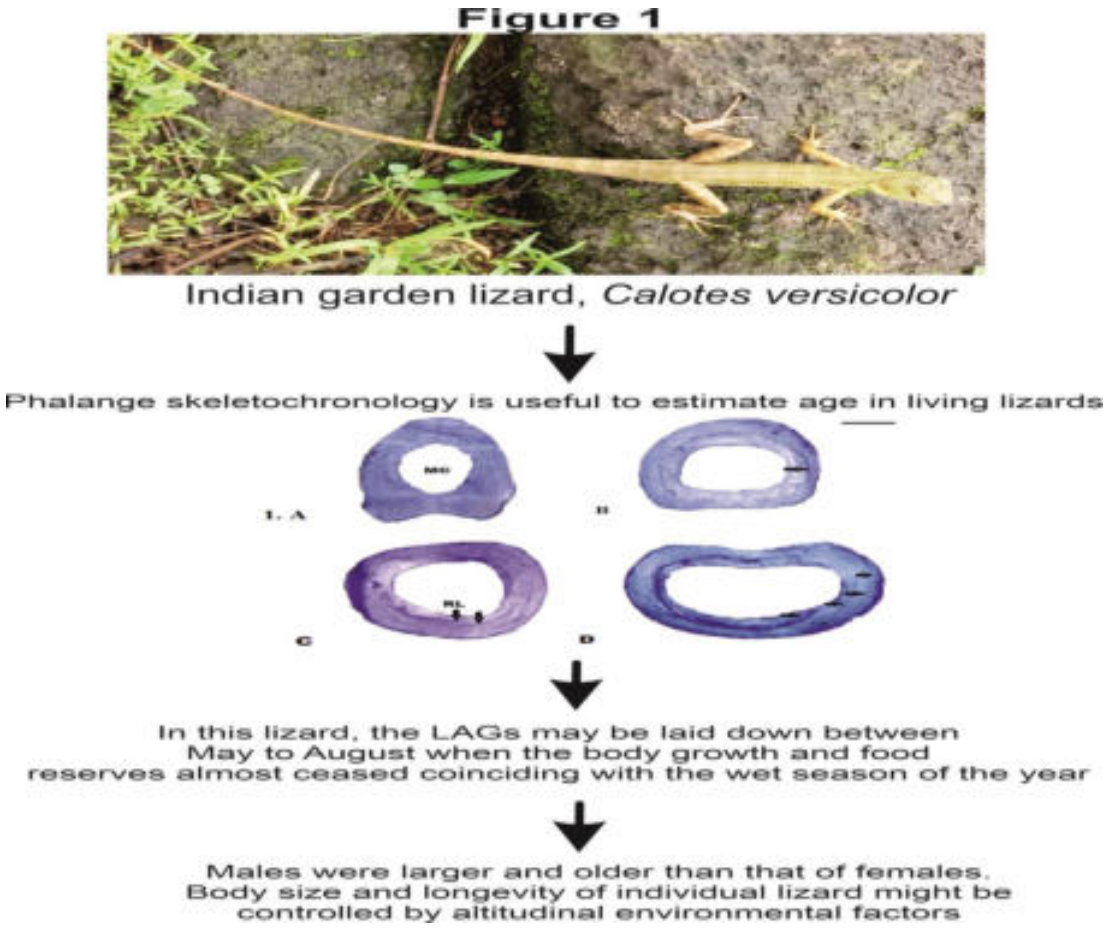


Figure 1A - D: Mid-diaphyseal **c**ross sections of right phalanges of *Calotes versicolor* (Hematoxylin). ***A***, Showing the absence of LAG in a female lizard with SVL 8.4 cm; ***B***, one LAG in the phalange of male lizard with SVL 9 cm; ***C***, two LAGs with resorbed line (RL) in the phalanges of female individual with SVL 8.5 cm; ***D,*** four LAGs (arrows) in the male adult with SVL 10.5 cm; Scale line = 100 µm.
Abbreviations: MC, Marrow cavity; RL, Resorption line; Arrows = Lines of arrested growth (LAGs).

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Alterations In Protein Content in Brain and Liver Tissues of Fingerlings of Freshwater Major Carp *Cirrhinus mrigala* After Exposure to Insecticide Emamectin Benzoate

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ABSTRACT
Emamectin Benzoate (EB) is an insecticide developed to control lepidopteron insects. Insecticides through agricultural runoff and leaching enter in water bodies affecting non-target aquatic animals. Alterations in the biochemical changes give first indication of stress due to insecticide exposure. The Current research is directed to evaluate the toxic effects of EB on protein content in fingerlings of freshwater fish Cirrhinus mrigala. The fingerlings were exposed to predetermined values of LC0 and LC50 concentration and acute toxicity of EB was studied. Brain and Liver tissues were examined for any alterations due to insecticide used. Fingerlings were divided into three groups: Control group, LC0 group and LC50 group. After 96 hours, the protein content was estimated by Lowry method. Results reveal decrease in the protein content in both the tissues in LC0 and LC50 group as compared to the control group.

KEYWORDS
Emamectin benzoate, Protein, Cirrhinus mrigala, Toxicity.

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1. INTRODUCTION

The natural environment has been facing threat due to the different kinds of pollutants. Harmful discharges from industries like distilleries, cotton mills, tanneries, paper mills, jute mills etc. also mining activities, agricultural developments and processing find their way to water[1-2]. Indiscriminate use of insecticides on crops can cause detrimental effects on aquatic and terrestrial non-target organisms. Fish is one of good source of protein for many aquatic life forms and even human beings. Bioaccumulation of pesticides in fish body can cause detrimental effect on human being.

Fish are in close contact with water, any alterations in quality of water will have effects on them. Fish act as important bio-indicators, in that wise, toxic compounds when enter into organs of fishes they modify some physiological and biochemical processes[3-4]. Taking into consideration of

All these facts, we have selected a novel insecticide Emamectin Benzoate (EB) for present study and to evaluate its toxic potential on protein content in *Cirrhinus mrigala*. It is widely being used in a variety of plants- tea, Coffee, Chili, Cabbage, Brinjal, Okra, Grapes etc. to control lepidopteron pests.

2. MATERIALS AND METHODS

Fingerlings of *Cirrhinus mrigala* were collected from fish seed centre Dhom, Satara, Maharashtra. They were brought to the laboratory and treated with 0.1 % $KMnO_4$ solution so as to avoid infections. Fish were then acclimatized to laboratory conditions for 15 days and fed daily with floating fish feed. The physico-chemical parameters of water holding the fingerlings were analyzed as per the standard procedures of APHA (1998) the pesticide was brought from Sat Sri Sai crop protection science private limited, Delhi. Healthy fish measuring 6±2 cm and weighing 6±2 g were selected for present study. Fishes from the control group were not exposed to insecticide EB, whereas the LC_0 group and LC_{50} group were exposed to 0.583 ppm and 0.833 ppm of EB respectively. Ten fish were released in container with twenty liters of dechlorinated tap water. After 96 hours the protein content was estimated by Lowry method (1951)[5]. The data was statistically analyzed by One Way ANOVA method.

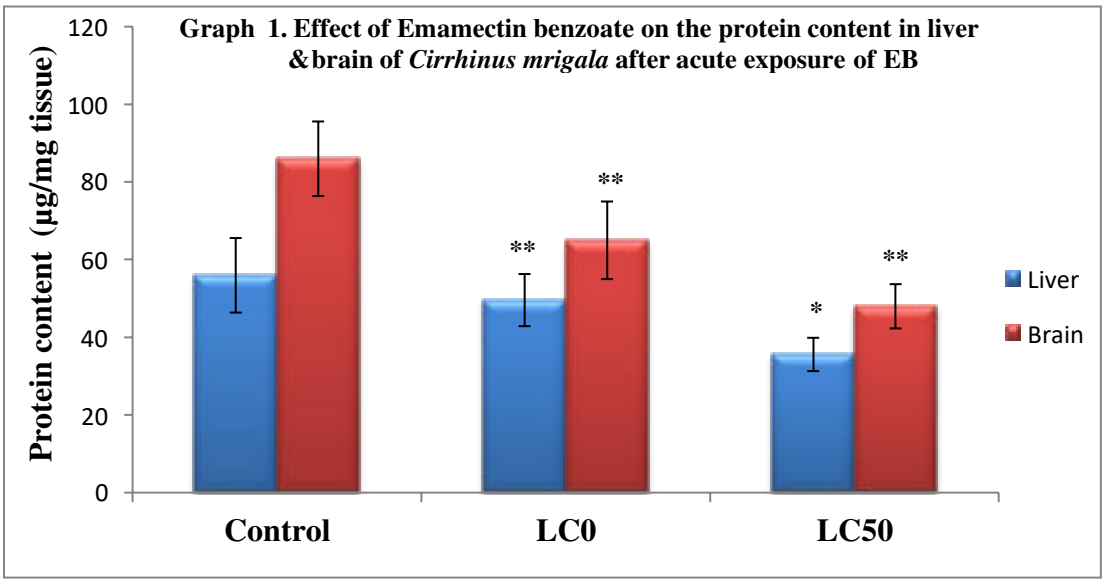
3. RESULTS AND DISCUSSION

The results in the table clearly show that the protein content decreased significantly in LC_0 group and LC_{50} group as compared to the Control group. Decrease in protein content may be because of damage to protein synthesis mechanism or may be due to increase in mechanism of degradation of proteins to amino acids. [6-7]have reported stress is caused due to toxicity which leads to decrease in protein content. Cadmium Chloride reduced protein content in liver of freshwater fish *Catla catla* [8]. Decrease in protein contentcan be due to decrease in the rate of anabolism of proteins[9]. The decreased protein content may be attributed to damage, necrosis of cells and even impairment in protein synthesis machinery

Table 1. Effect of insecticide EB on protein content in liver and brain tissue of *Cirrhinus mrigala* after exposure to insecticide EB.

Sr. No.	Protein(ug/mg wet wt. of tissue)	Experimental Group		
		Control	LC_0	LC_{50}
1.	Liver	56± 9.6	49.6±6.7**	35.6± 4.3*
2.	Brain	86±9.6	67± 6.7**	48±5.7**

(Values expressed as Arithmetic mean of (n=5); ±SD)*=P<0.05;**=P<0.01;***P<0.0001; NS=>0.05)



4. CONCLUSION

The study revealed that the protein content decreased significantly in LC_0 group and LC_{50} group as compared to the Control group. The sub lethal exposure of EB proved moderately toxic to fish which effects on protein levels in vital organs like brain and liver. Therefore, utmost care must be taken to prevent its drainage into the water bodies.

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Effect of Paclobutrazol on Mantle Epidermal Lining of Terrestrial Slug S*emperula maculata*

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ABSTRACT
Paclobutrazol (PBZ) is one of the phytoregulator which also used as fungicide. PBZ biochemically shows low solubility, hence it gets accumulate in the tissue of organism. Excess concentration can be harmful to organisms. Study aimed to observe induced effect of PBZ on the mantle epidermal lining of terrestrial slug S. maculata. The animals were exposed for the dose of 10 ppm and 20 ppm of PBZ for 96 hours. It showed pathological signs and symptoms with lesions, pitting on the mantle epidermal cells of selected experimental animal. Obtained results were interpreted for morphometric and histological changes in epidermal layer of terrestrial slug S. maculata in order to toxicity impact of PBZ.

KEYWORDS
Epidermal lining, Mantle, PBZ, Phytoregulator, Semperula maculate.

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1. INTRODUCTION

In modern agriculture, including pesticides many compounds were known as phytoregulators [1]. Generally, phytoregulators are the chemicals that naturally produced by plants in low concentration to regulate normal growth and development [2]. Among chemicals, Paclobutrazol (PBZ) is the synthetic phytoregulator, it inhibits the biosynthesis of Gibberellin and used to enhance the flowering and growth of the plants [3]. Excess and long-term use of PBZ with high mobility and stability in ecosystem reported as hazardous chemical contaminant for soil and water media [4].

Among invertebrates after the Arthropods, Molluscs are the second largest phylum in the animal kingdom. Approximately 1,10,000 species of molluscs were known, in which 75 % are from class Gastropods [5]. Molluscs are soft bodied, unsegmented animals with a muscular foot, head, visceral mass with most of fleshy organ system covered with shell [6]. Gastropods play vital role in food chain and maintain the Eco-balance. Gupta et. al., reported that among the aquatic organisms, gastropods and bivalves were recognized as a bioindicators of pollution, which can provide early signs and symptoms of contaminations [7].

Selected slug, *S. maculata* is shell-less gastropods. Dorsal surface of the body is entirely covered by the mantle which acts as integuments. It also plays role in respiration, locomotion, osmoregulation and also protects themselves from the predators [8]. Morphologically, mantle is a thick spongy covering and dark black in colour. It encloses and protects the visceral mass with internal organs such as heart, stomach, intestines, and gonads [9].

After consideration of all the above facts and available literature, present investigation was aimed to study effect of PBZ on the selected experimental animal *S. maculata.*

2. MATERIALS AND METHODS

2.1 Selection of animal

Figure- 1 Invertebrate terrestrial molluscan slug *Semperula maculata* was selected as experimental animal. The animals were collected from Panmala, Village Bedag, District Sangli, Maharashtra, India (**Figure- 2).** Animals were carried in aerated plastic trough in the laboratory. Animals were kept in laboratory condition at temperature of $20^0 \pm 2^0$C with photoperiodicity of 12 hours. Acclimatization is done up to 7-8 days. Healthy adult Slugs were selected and kept in a plastic trough containing moistened soil. Animals were fed with cabbage and mulberry leaves regularly.

2.2. Dose preparation:

Paclobutrazol ($C_{15}H_{20}ClN_{3O}$) 95% was purchased from Himedia **Figure- 3 and Figure- 4**. The dose was prepared in distilled water (D/W) by using DMSO as solvent. Daily, fresh working solution was prepared for intoxication.

2.3 Animal intoxication:

The adult slugs with the standard size (6-7 cm length, 1 to 1.2 cm width and 3- 4 gm weight) were selected and separated for experiment. Three groups were prepared including first as a control and remaining two are as experimental groups **Figure- 5 and Figure- 6**. In each group 30 slugs were used having each set with 10 animals. All three groups including control and two experimental groups of 10 ppm and 20 ppm respectively against PBZ upto 96 hours.

After completion of experimental period, all slugs were observed for morphometric and histological study by applying standard protocol.

Plate No. 1

Fig. -1

Fig. - 2

Fig. - 3

Fig. - 4

Fig. - 5

Fig. - 6

Fig. 1- Collection site- Parasala, Fig. 2 Experimental animal – slug, Fig. 3 - Toxicant- Paclobutrazol (PBZ), Fig. 4- Powdered form of PBZ, Fig. 5 - Experimental set- 10 ppm, Fig.- 6 Experimental set- 20 ppm

2.4 Morphometric study:

The length and width of the animals was measured by visual method using meter scale [10,11]. By using electronic weighing balance (**Contech CA-223**), weight of the animals was measured.

2.5 Histological study:

After completion of exposure period animals were sacrificed. mantle was dissected and selected for standard microtomy procedure. The sections of 4 -5 µm were stained by Harris Hematoxylin and Eosin, (1900) staining protocol.

3. RESULTS AND DISCUSSION:

In the present investigation experimental animal *Semperula maculata* was exposed to dose dependent intoxication of PBZ showed various morphometric and histological changes.

3.1 Morphological changes in slug:

In control group of *S. maculata,* the external morphology was normal with thick, spongy blackish covering on the dorsal surface with a yellowish white line. In the experimental group of 10 ppm for 96 hours, white patches with lesions were observed on the body of the animal also length is gets reduced. The pigmentation of the mantle is also reduced. At concentration of 20 ppm for 96 hours, the size and number of lesions on the body was significantly increased as that of 10 ppm concentration. Pitting on the mantle was observed also size of the body is decreased the body of the organism becomes slimier.

3.2 Histological study:

Under histological observations, mantle of *S. maculata* composed of outer layer epidermis and consists of epithelial cells with single layer cuboidal or simple columnar cells resting in basement membrane. Below the epidermis scattered unicellular glands were found, which secrets mucus, primarily called as Mucus Secreting Cells. Dermis found with the pigment cells, blood spaces, muscle fibres, network of loose connective tissue with blood vessels.

Plate-2 In the control group, cytoplasm of epidermal cells was stained pink with blue nucleus. The muscle fiber and connective tissue were stained faint pink, no any alteration was observed in the transverse section of the mantle of *S. maculata.* After the exposure of Paclobutrazol for 10 ppm up to 96 hours, the epithelial lining of the epidermis is disturbed, the nucleus was also disturbed. In group 20 ppm, the prominent damage in epithelial lining of the mantle was observed, it was broken in some of the places, connective tissue was damaged. Mucous secreting cells were ruptured, the blood space is also increased and show prominent damage in the epidermal layer.

The mantle of the slug *S. maculata* found outer covering of the body which has main function of respiration and mucus secretion for the protecting the animal from the mechanical injury and from the predator. Below the epithelial lining there is presence of unicellular mucous glands which are scattered on the dorsal surface of the body, it is important for the locomotion and protect outer surface [12]. The epithelial cells of mantle were fused with lysosomes and were thereby digested [13]. Triebskorn et. al., studied, the effect of metaldehyde on the *D. reticulatum* on the mucocytes of the digestive tract and the skin it showed some alterations in metabolism [14]. The above study shows the morphological and histological alteration after the intoxication of PBZ at different doses of 10 ppm and 20 ppm. The PBZ which is widely used for the growth and flowering of plant from soil it enters in the terrestrial slug and it alters the normal function of the mantle by disturbing the epithelial lining of body surface, mucous secreting glands their quantity and

morphology is disturbed it ultimately affects the respiration and protective function of the animal.

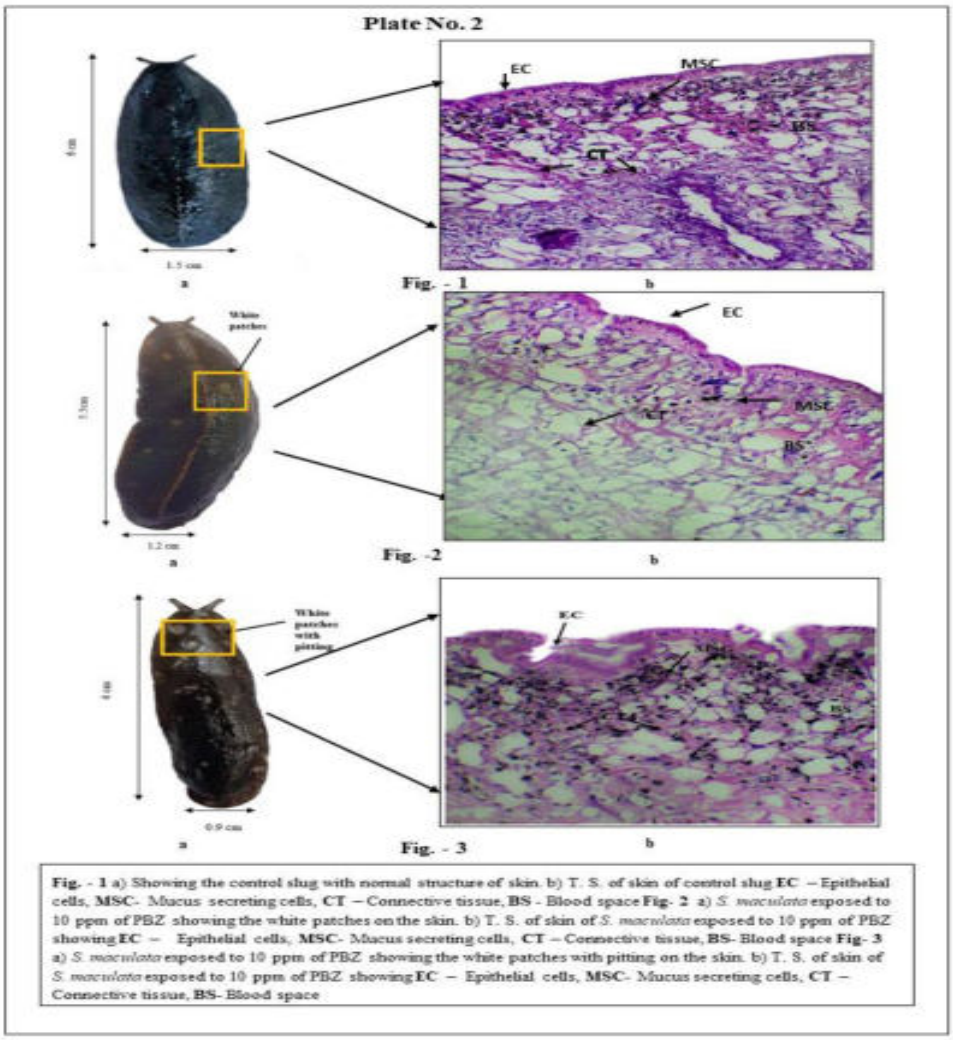

Fig. - 1 a) Showing the control slug with normal structure of skin. b) T. S. of skin of control slug **EC** – Epithelial cells, **MSC**- Mucus secreting cells, **CT** – Connective tissue, **BS** - Blood space **Fig- 2** a) *S. maculata* exposed to 10 ppm of PBZ showing the white patches on the skin. b) T. S. of skin of *S. maculata* exposed to 10 ppm of PBZ showing **EC** – Epithelial cells, **MSC**- Mucus secreting cells, **CT** – Connective tissue, **BS**- Blood space **Fig- 3** a) *S. maculata* exposed to 10 ppm of PBZ showing the white patches with pitting on the skin. b) T. S. of skin of *S. maculata* exposed to 10 ppm of PBZ showing **EC** – Epithelial cells, **MSC**- Mucus secreting cells, **CT** – Connective tissue, **BS**- Blood space

4. CONCLUSION:

Unwanted and excess induction of PBZ against *Semperula maculata* proved toxic were it has changed overall morphometric characters of experimental animals. We found epidermal, pigmental changes with cuticular depressions with respect to varied

dose of 10 ppm and 20 ppm, animals were become more sluggish it was due to pathetic condition of epidermis. We found epidermal cells were more scattered and layer was deteriorated. In 20 ppm of dose, it was irreversible with permanent damage. The above study indicates that PBZ has dose dependent lethal impact against experimental terrestrial slug *Semperula maculata.* PBZ has confirmed its bioaccumulation and biotoxicity in tissue. Present work confirmed the toxicity impact of PBZ.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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Effect of Gut Microbiota on Neuronal Fine Tuning

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ABSTRACT
Gut microbiota consists of a community of microorganisms that inhabit the gastrointestinal tract which helps to maintain gut homeostasis. Gut microbiota communicates with the brain via bidirectional communication through the gut microbiota-brain axis. Gut microbiota produces several metabolites which act as neuromodulators e.g. Ferulic Acid, Short-Chain Fatty Acids, Dopamine, Noradrenaline, and Serotonin. These molecules work through vagal afferents. In addition, after absorption in the blood, they act through their endocrine actions. A decrease in the levels of these gut-microbiota derived metabolites causes improper communication between the brain and the gut. This may cause altered neuronal activity. This may eventually lead to neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, Dementia and Mild Depression Disorder. This article illustrates how the gut microbiota-brain axis works and how manipulation of the gut microbiota with probiotics and prebiotics can modulate neural signaling and finely tune the nervous functions.

KEYWORDS
Enterochromaffin Cells, Gut microbiota, Gut-Microbiota Brain Axis, Neurons, Prebiotic, Probiotic, etc.

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1. GUT-MICROBIOTA

About 30 trillion microbes reside in the human body in various regions like skin, the gastrointestinal tract, respiratory tract, urogenital tract, and mammary gland [68]. The human gastrointestinal tract comprises a broad range of microorganisms which is termed Gut microbiota [62]. Among these, Bacteroidetes and Firmicutes are dominant phyla, whereas, the rest of the bacteria exist in a minor population belonging to the phyla Proteobacteria, Actinobacteria, Fusobacteriota, Spirochaetota, Verrucomicrobiota and Lentisphaerota [56, 58]. These diverse varieties of communities of bacterial species interact symbiotically with gut mucosa and maintain structural integrity, the host metabolism, nutrition, and immune functions

[65]. Some bacterial species are not pathogenic, if they are in small numbers. However, overpopulation of non-pathogenic bacteria can cause disease [1, 55].

2. GUT-MICROBIOTA BRAIN AXIS

The Gut microbiota Brain Axis is the bidirectional communication between gut microbes and the brain, carried through various metabolites like Ferulic Acid, Short-Chain Fatty Acids, Dopamine, Noradrenaline, and Serotonin [59]. The neuroendocrine system consists of three main modes of communication between the gut and the brain namely; [i] neuronal messages carried by vagal afferents, [ii] endocrine messages carried by gut hormones, and [iii] immune messages carried by cytokines [11].

3. DYSBIOSIS

Ageing and environmental factors alter the gut microbiota [7]. According to Kohler *et al.,* (2016), Dinan and Cryan, (2017) age-related neurodegeneration is due to reduced diversity in the gut microbiota. Dysbiosis is a condition, in which there is an increase in the population of harmful bacteria and a decline in the population of beneficial bacteria [73]. Such a decrease in beneficial bacteria results in the reduction of beneficial neurometabolites [52, 53, 57]. During infection, an individual is treated with antibiotics which help to combat the infection and get rid of the disease. However, the antibiotics also kill the resident microbiota causing gastrointestinal disturbances [3]. Prolonged or chronic dysbiosis may cause progression of the neurological disease by influencing low-grade inflammation, increased oxidative stress, imbalance in energy homeostasis, and elevated cellular degeneration [14, 49]. During ageing, the imbalance of gut microbiota may cause a number of neurodegenerative disorders such as stress and depression [82], Autism spectrum disorders [26], Alzheimer's disease [35], Parkinson's disease [32].

4. NEUROMETABOLITES FROM BACTERIAL ORIGIN

For the proper functioning of neurons, neurometabolites play important roles. However, a shortfall of neurometabolites may result in the persistent low-working performance of the neurons [76].

4.1 Vitamins

Gut microbiota is a rich source of vitamins. Genera like Bacteroides, Bifidobacterium, and Enterococcus are known to produce fat soluble vitamin such as vitamin K and water soluble vitamins like folic acid, cobalamin, biotin, riboflavin, and pyridoxine [20, 33].Vitamins like Folic acid, riboflavin cobalamin, pyridoxine, and Biotin able to cross the Blood-Brain Barrier thereby regulating neuronal functions [46, 60, 61, 71]. Pyridoxine is essential to produce neurotransmitters like serotonin, dopamine, and Gamma-amino butyric acid [66]. Vitamins like cobalamin take part in DNA synthesis in oligodendrocytes and are responsible for the

production of myelin [38]. These communications are important in order to maintain functional coordination between the brain and the gastrointestinal tract.

4.2 Short chain fatty acids

After digestion of these fibers by bacteria, there is generation of short chain fatty acids like acetate, propionate, and butyrate which are helpful to the host. These are recognized as prime neuro-metabolites which influence the functioning of Central Nervous System [64]. The Acetate is produced by gut bacteria, like *Prevotella spp., Ruminococcus spp., Bifidobacterium spp., Bacteroides spp.,* [74]. It crosses the Blood-Brain-Barrier where it acts as the source of energy. It also helps as substrate in the production of Gamma-Aminobutyric acid and Glutamate in the brain [25, 70]. Propionate is produced by the bacteria like *Akkermansia municiphilla, Bacteroides uniformis* and *Bacteroides vulgatus* [4, 56, 81] which crosses the Blood-Brain-Barrier and protects the brain from bacterial endotoxins like Lipopolysaccharides (LPS) [31]. High quantities of butyrate are produced by the *genus Roseburia* and *Eubacterium* [50]. The bacteria like *Ruminococcus bromii* produce butyrate by fermenting starch [80]. Butyrate crosses the Blood-Brain-Barrier, exerts anti-inflammatory properties, thereby decreasing the neuroinflammation, and imparting longevity
of the neurons [5, 42]. Moreover, butyrate also modulates the levels of neurotrophic factors and contributes in the biosynthesis of serotonin [64].

4.3 Ferulic acid

Ferulic acid is another beneficial molecule produced by gut microbiota. It exhibits anti-inflammatory and antioxidant properties [29, 63]. Cheng *et al.,* (2008) demonstrated that ferulic acid inhibits the aggregation of Aβ. Tomaro-Duchesneau *et al.,* (2012) found that ferulic acid is synthesized by gut bacteria like *Lactobacillus fermentum* of strain NCIMB 5221 and *Bifidobacterium animalis*. Ferulic acid showed neuroprotection against cerebral ischemia in rats [78].

4.4 Dopamine and Serotonin

Dopamine is an excitatory neurotransmitter produced by the neurons. In addition, it is produced by bacteria of the genus *Bacillus* [15]. More than 50% of dopamine in the human body is synthesized in the gut [2]. It plays a major role in the decision-making functions, motivation and improves learning [22]. Dysregulation of the dopamine system results to several neurological disorders such as schizophrenia and Parkinson's disease [47, 48].

Serotonin is an inhibitory neurotransmitter produced by the neurons. In addition, the gut bacteria such as *Lactobacillus brevis, Lactobacillus plantarum* and *Bifidobacterium bifidum* produce serotonin [54]. Within the mucosal lining of the intestine there are specialized cells called as enterochromaffin cells. Their major role

is gastrointestinal fluid secretion and to maintain intestinal mobility. The spore forming bacteria produces metabolites like α-Tocopherols and Tyramine which trigger the enterochromaffin cells to produce serotonin [77].The serotonin influences the enteric nerves which communicate with the brain through the vagus nerve and influence brain functions [30]. Abnormal expression and function of serotonin in the brain may results into major depressive disorder and anxiety disorders [34].

5. HARMFUL EFFECTS DUE TO DYSBIOSIS

An increase in the population of harmful bacteria causes an increase in bacterial lipopolysaccharides and the bacterial amyloids make the intestinal mucosal barrier leaky [24, 79]. Dysbiosis can influence microglial activation which induces amyloid-beta peptide deposition that results in the progression of Alzheimer disease [37]. Many species from the genus *Clostridium* are harmful, affecting the nervous system such as *Clostridium perfringens* produces epsilon toxin which accumulates in the brain and disrupts the Blood-Brain Barrier [28, 43]. *Clostridium botulinum* produces botulinum toxin which through blood circulation reaches the nerve terminals and gets deposited thereby interfering with neurotransmission [41]. This contributes to increased susceptibility of neurons to neurodegenerative diseases.

6. INTERVENTION BY SUPPLEMENTATION OF PROBIOTICS

The gut microbiota disturbance can be improved by supplementation of probiotics. Probiotics are live, beneficial bacteria that are given through the oral route to improve gut microbial balance and to increase the population of beneficial bacteria in the gut [23, 72]. Several studies have found that if probiotics are consumed in regular amounts; have anti-inflammatory effects [17, 44, 45]. Wang *et al.,* (2016) studied the effects of *Bifidobacterium* and *Lactobacillus* on the functioning of the central nervous system and found that improving psychiatric disorder-related behaviours including anxiety, depression, autism spectrum disorder and memory abilities. The dietary fibers from plants origin are indigestible in the human gut. The gut bacteria play an essential role in the digestion of such fibers which promote the growth of beneficial bacteria [6, 27, 40].

7. INTERVENTION BY SUPPLEMENTATION OF PREBIOTICS

Prebiotics acts as food for bacteria residing in the gut. These are the food items rich in dietary fibers when supplemented as a prophylactic or therapeutic regimen are termed prebiotics [27]. These dietary fibers cannot be digested by the host but are easily digested by the gut bacteria. Kruse *et al.,* (1999) and Bouhnik *et al.,* (1999) demonstrated that the administration of different prebiotics like inulin, fructo-oligosaccharides responsible for increasing the number of Bifidobacteria and lactic acid bacteria. Inulin is a type of 'fructan' which is an excellent source of prebiotics [39]. Both in vivo and in vitro studies brought into notice that flavanols could stimulate lactic acid bacteria [67]. The administration of fructo-oligosaccharides

effectively alleviates Alzheimer's Diseases by modulating the gut microbiota [13]. Another study further supported the benefits of fructo-oligosaccharides in spatial memory function [21]. Fructo-oligosaccharides and galacto-oligosaccharides serve as a major source of prebiotics in the food [18].

8. NEURONAL FINE-TUNING

Interventions with probiotics and prebiotics have shown remarkable effects on the dysbiosis. *Lactobacillus* and *Bifidobacterium* when administered to the host showed increased Gamma Amino-Butyric Acid, acetylcholine, which play important roles in controlling the neural excitatory-inhibitory balance, mood, cognitive functions [8,54] and secretion of important neurotransmitters such as dopamine and serotonin which help to reduces the stress [16]. Whereas, *Bifidobacterium longum* alone increases the expression of Brain-Derived Neurotropic Factors that play an important role in enhancing learning and memory [10]. *Lactobacillus* increases cognition activity by enhancing the secretion of memory-based neurotransmitters like acetylcholine and glutamate [51]. Fine-tuning refers to achieving the best or the desired performance by making fine adjustments. Various neuroregulatory molecules produced by the gut microbiota as exemplified earlier, cross the Blood-Brain Barrier and finely regulate the brain functions.

9. CONCLUSION

Gut microbiota consists of beneficial bacteria and harmful bacteria. The dominance of these bacteria either beneficial or harmful decides the fate of gut homeostasis. The beneficial bacteria like *Lactobacillus* and *Bifidobacterium* produce essential neurometabolites which play important role in neuronal fine-tuning. During ageing, the population of beneficial bacteria in the gut decreases. This results in decreased production of neurometabolites. While, there is increase in the population of harmful bacteria that causes increased production of toxic metabolites that adversely affects the functioning of Central Nervous System. By using a combination of probiotics and prebiotics we can increase the population of beneficial bacteria and reverse the dysbiosis to maintain the gut microbiota to its normal form and functions.

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Assessment of Kidney and Liver Damage Against Ethylene Glycol Induced Renalcalculi in Vertebrate Experiment Model: *RATTUS NORVEGICUS*

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ABSTRACT

Present investigation aimed to the study nephrocytes and hepatocytes against experimentally induced effects of Ethylene glycol (EG). Also focused on antiurolithiactic activity of herbal medicine Cystone (CS) on the same protocol. For Oxalate urolithiasis, female wistar rats Rattus norvegicus were used. Experimental animals grouped into 3 with 6 animals in each. Group A: Control, Distilled Water, Group B: 1 % Ethylene Glycol, Groups C: 1 % Ethylene Glycol + Cystone (750 mg /Kg Body weight). All the dose were supplemented orally for 30 days. The selected animals were sacrificed by cervical dislocation. Targeted tissues like kidney and liver were selected for histopathology and lipid peroxidation study. Blood sample were collected for hematological study, kidney, and liver function test. Results revealed that the EG induced damages was recovered by administration of CS. Obtained results were interpreted for histopathological and biochemical alteration in experimental animals.

KEYWORDS

Ethylene Glycol (EG), Kidney, Liver, Cystone (CS), Rattus norvegicus.

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1. INTRODUCTION

Nephrolithiasis is common urological pathology affecting near about 12 % of world population and reported one of the disorders found in mankind from 4000 BC [1]. Due to the anatomical similarities and easy access, rodents are the most widely used experimental model for the kidney stone [2]. Ethylene glycol (EG), (CAS No.101-21-01) is colorless, odorless, having sweet taste, non-volatile compound and have complete miscibility in water.EG widely used in automobile industries for permanent anti freezing agent, in paint industry and also considered as industrial solvent [3]. Toxicological study showed that the toxicity of EG can cause by ingestion, absorption of skin, inhalation etc which mainly effects on Central Nervous System (CNS), cardiopulmonary system and excretory system i.e., kidney and metabolically alters various processes like Electron Transport Chain (ETC), oxidative

phosphorylation, cellular respiration, glucose metabolism and also interfere process of DNA replication[3]. As per study there are several chemical compounds available for urolithiasis in experimental models. For artificial kidney stone, it can be induced intraperitoneal, subcutaneous, osmatic minipump or oral administration. Against all these, traditional Indian medicines, Cystone mostly recommended because of its antiurolithiactic property including antinephrolithiasis, prevention of supersaturation of lithogenic substances, inhibition of stone forming substances like oxalic acid and calcium hydroprolin. Along with that it is widely useful in treatment in anti-inflammatory activity in ureter and painful sign and symptoms of micturition [4].

Considering present scenario and scientific focus about assessment of dose dependent pathological effect of EG and roll of CS against scanty literature found in the research and investigation and need more attention regarding pathophysiological and phytoremedial study. With this intention present investigation was carried out in worldwide recommended vertebrate model *Rattus norvegicus.*

2. MATERIALS AND METHODS

a) Experimental animal:

For the present investigation, female wistar rats *Rattus norvegicus* were used. The investigation was carried out with permission of authorized CPCSEA approval for animal experiment. Animals were housed individually in mesh cages. Animals were maintained in accordance with the guidelines as per the care and use of laboratory animals. By applying standard protocol, animals were reared in animal house of Department of Zoology, Shivaji University, Kolhapur (1825/PO/EReBi/S/15/CPCSEA). Animals were kept at optimum room temperature, ranged from 71°F to 72 °F (21.9 °C to 22.4 °C) with relative humidity ranged from 38% to 50% during the study. During the experiment animals were exposed to 12-hour light/12-hour dark photoperiod. Animal were fed on regular recommended food and provided Reverse Osmosis (RO) water by bottles as per need and experimental protocol.

b) Dose administration:

For the experimental study,18 animal of an average 95-100 days old, body weight about 350 - 400 gm were selected. The animals were grouped into 3 different groups which consist 6animals in each. Group A includes control animal fed with RO water. Group B fed with 1 % Ethylene Glycol. Group C fed with 1 % Ethylene Glycol + Cystone (750 mg /Kg Body weight). All the animal exposed to the respective dose for 30days.

Table No. 1: Experimental design

Sr.		Group A	Group B	Group C

No.				
1	Dose	Control with distilled water	1 % EG	1 % EG + Cystone(750 mg /Kg Body weight)
2	Duration	30 Days	30 Days	30 Days

c) Histopathology:

For histopathological study, experimental rats were sacrificed after the completion of exposure period as per the protocol. Interested and targeted organs, kidneys and liver were harvested and morphometrically observed. Tissue was subjected to micro technique. The paraffin embedded tissue was trimmed and used for sectioning on the microtome at 4-5 µ. Sections were stained by Haematoxylin-Eosin (HE) stain. Stained slides observed under the microscope at 400 X magnification.

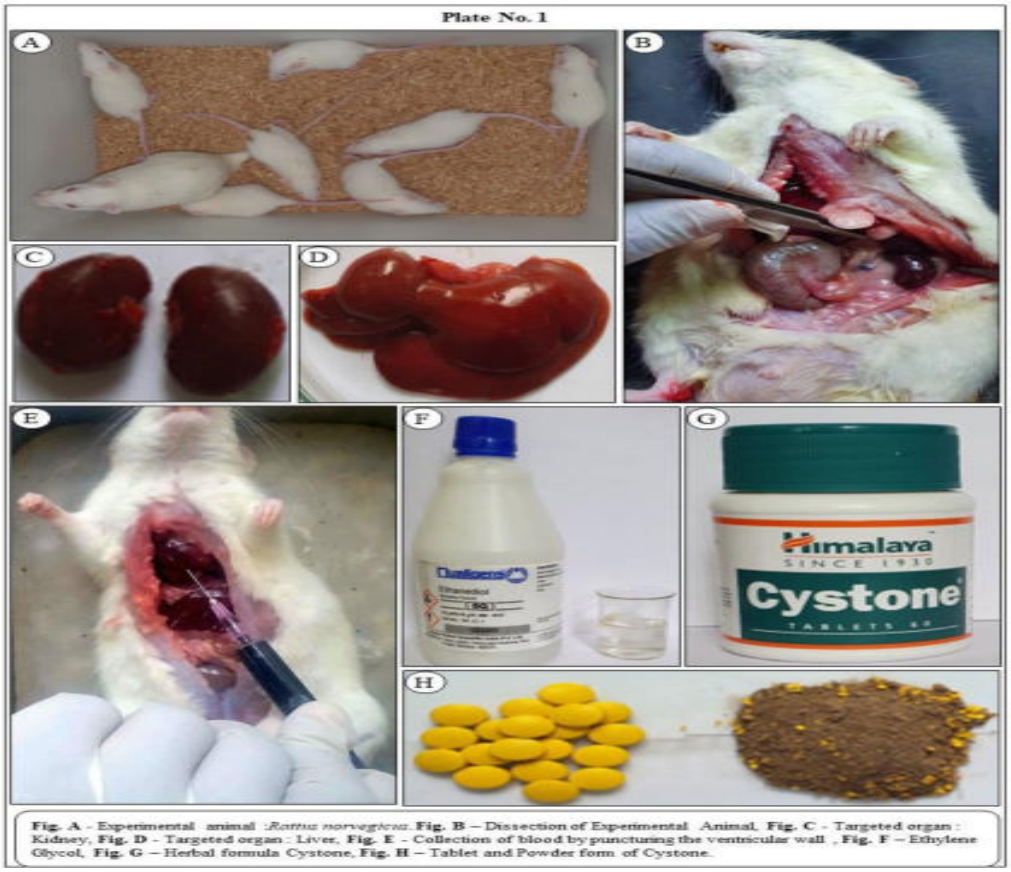


Fig. A - Experimental animal :*Rattus norvegicus*. **Fig. B** – Dissection of Experimental Animal, **Fig. C** - Targeted organ : Kidney, **Fig. D** - Targeted organ : Liver, **Fig. E** - Collection of blood by puncturing the ventricular wall , **Fig. F** – Ethylene Glycol, **Fig. G** – Herbal formula Cystone, **Fig. H** – Tablet and Powder form of Cystone.

d) Blood Analysis:

After scarification, the blood sample were collected from ventricles by using syringe. The blood sample were collected into the CBC tube containing EDTA and clot activators were analyzed using Sysmex automatic hematology analyzer. The following blood parameter were determined Hemoglobin (Hb), Red blood cells (RBC), Peak Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Blood Cells Distribution width (RDW). White blood Cells (WBC), Neutrophil, Lymphocytes, Eosinophil, Monocytes, Basophils, Platelet count, Procalcitonin (PCT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW). Using syringe blood was collected. After centrifugation at 3000 rpm for 10 minute the samples were kept in refrigerator (2ºC to 8ºC) further used for kidney and liver function test [5].

e) Kidney function test:

The serum was collected by centrifugation at 3000 rpm and following parameters were determined, random blood sugar was determined by GOD-POD method using Agappe enzymatic kit, blood urea and blood urea nitrogen was determined by UV-GLDH methods by using Path Zyme enzymatickit,creatinine concentration in serum was measured using Erba- Liquixx Creating Cummersial kit by Jaffe's Method, serum uric acids was determined by modified Uricase – PAP method by using Yucca Diagnostics enzymatic kit, serum calcium determined by Modified Arsenazo III method by Yucca diagnostics enzymatic kit, sodium electrolyte concentration was determined by ISE Analyzer method (Sensa core ST 200) [6].

f) Liver function test:

AST (Aspartate aminotransferase) or SGOT and ALT (Alanine aminotransferase) or SGPT are the two important bioindicators present in excess amount when there is a damage in the tissue especially in heart, kidney, liver, pancreas, spleen, skeletal muscle and lung. The amount of AST or SGOT and ALT or SGPT was determined by standard IFCC method, Kinetic by using Erba enzymatic kit [7].

g) Lipid peroxidation:

3-Carbon compound Malonaldehyde is a biproduct formed during the oxidation of polyunsaturated fatty acid which is a major component of plasma membrane of the cell. The activity of lipid peroxidation was done by Wills method (1966). Tissue homogenate (2mg/ml) were prepared in chilled mortar and pestle using 75mM potassium phosphate buffer (pH 7.0). The end product Malondialdehyde (MDA) reacts with Thio barbituric Acid (TBA)gives pink colored complex. The Optical Density (OD) was measured at 532 nm. The concentration of MDA was expressed as nMol MDA/mg wet tissue.

3. Results and Discussion

The present work is carried out by considering the scenario for to cure the damage of liver and kidney. Number of metabolic activities associated with development and deposition of calculi which can disturb the normal functioning of liver and kidney.There are number of experimental protocols has been carried out in the assessment of liver and kidney damage against ethylene glycol. By considering the available literature regarding the effect of ethylene glycol and as a part of assessment of kidney and liver damage against ethylene glycol, Cystone against ethylene glycol is used. Following are the parameters that has been recorded for all the three groups are as below -

a. Hemogram

b. Differential count

c. Kidney function test

d. Liver function test

e. Lipid peroxidation

Table No. 2: HAEMOGRAM

Sr.No.	Parameters	Group A Control	Group B 1 % EG	Group C 1 % EG + Cystone
1	**Hemoglobin (gm)**	15.9 ±0.45	14.1±1.97	15.43±2.65
2	**RBC Count (millions/mm^3)**	9.61±0.59	8.36±1.09	9.60±1.62
3	**Peak Cell Volume (%)**	54.53±3.18	46.55±3.88	50.96±8.02
4	**MCV (fL)**	54.03±2.90	55.8±2.68	53.1±0.7
5	**MCH (Pg)**	17.76±1.40	16.9±0.28	16.06±0.05
6	**MCHC (g/dl)**	31.23±3.74	30.3±1.83	30.23±0.46
7	**RDW CV (%)**	17±1.57	13.85±1.48	15.46±2.67

Table No. 3: DIFFERENTIAL COUNT

Sr. No.	Parameters	Group A Control	Group B 1 % EG	Group C 1 % EG + Cystone
1	**WBC Count (Cells/mm^3)**	8500±2300	6366±2581	5000±1352
2	**Neutrophils (%)**	45±5	35±2	31±7.93
3	**Lymphocytes (%)**	60.66±6.65	58±1.6	63.33±7.63
4	**Eosinophils (%)**	3.33±2.1	4±1	2.33±0.57
5	**Monocytes (%)**	3±1	4±1	3±1
6	**Basophils (%)**	---	---	---

Table No. 4: PLATELEST COUNT

Sr. No.	Parameters	Group A Control	Group B 1 % EG	Group C 1 % EG + Cystone
1	**Platelet count (Cells/ mm^3)**	948000±25244	887500±23334	1130333±132613.5
2.	**PCT (%)**	0.80±0.14	0.65±0.02	0.74±0.07
3	**MPV (fL)**	8.1±1.429	7.3±0.14	6.63±0.11
4	**PDW (%)**	8.7±1.13	8.7±0.2	7.53±0.32

Table No. 5: KIDNEY FUNCTION TEST

Sr. No.	Parameters	Group A Control	Group B 1 % EG	Group C 1 % EG + Cystone
1	**Random Blood Sugar (mg/dl)**	52.26±3.70	170.43±5.02	220.2±18.66
2.	**Blood Urea (mg/dl)**	25.26 ±3.45	33.5±1.27	38.63±5.65
3.	**Blood Urea Nitrogen (mg/dl)**	11.07±1.80	16.12±0.94	18.03±2.61
4	**Serum Creatinine (mg/dl)**	0.8±0.1	1.06±0.35	0.83±0.05
5.	**Serum Uric Acid (mg/dl)**	7.8±1.36	10.8±1.11	6.9±0.52

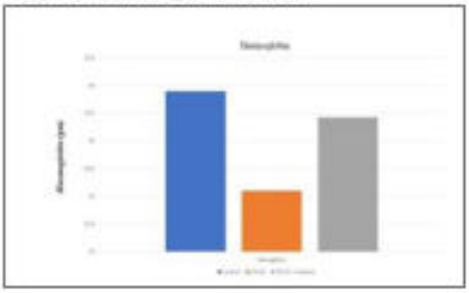
6.	**Serum Calcium (mg/dl)**	9.53±1.20	10.1±1.20	10.26±0.47
7.	**Sodium (mmol/ l)**	141.93±3.80	148±2.45	142±4.31

Table No. 7: LIPID PEROXIDATION

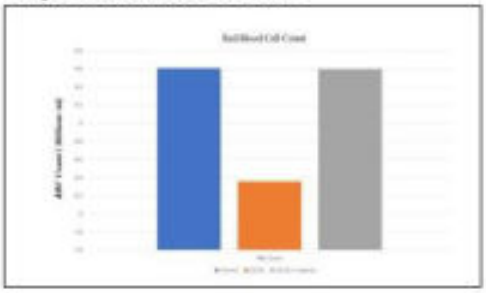
Sr. No.	Group A Control			Group B 1 % EG			Group C 1 % EG + Cystone		
	Kidney		Liver	Kidney		Liver	Kidney		Liver
	Right Kidney	Left Kidney		Right Kidney	Left Kidney		Right Kidney	Left Kidney	
1. Lipid Peroxidation MDA/mg tissue	11.67± 1.05	11.82± 0.51	10.48± 1.17	40.56± 1.17	49.8± 2.60	38.30± 1.799	25.70± 0.70	26.31± 0.72	25.69± 0.78

Table No. 6: LIVER FUNCTION TEST				
Sr. No.	**Parameters**	**Group A Control**	**Group B 1 % EG**	**Group C 1 % EG + Cystone**
1	**S.G.O.T. (IU/L)**	78.5±2.26	75.5±3.79	164.1±3.99
2.	**S.G.P.T. (IU/L)**	52.8±1.03	85.8±4.21	146.2±4.76

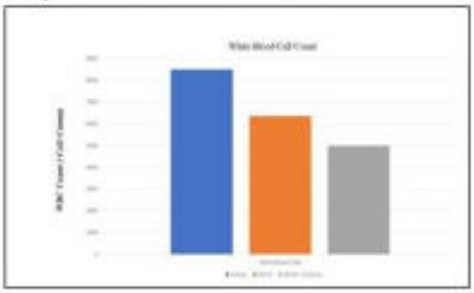
Graph 1 : Haemoglobin content



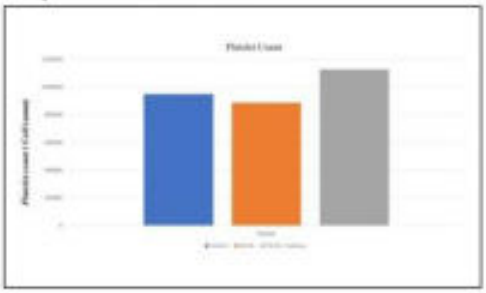
Graph 2 : Red Blood Cell Count



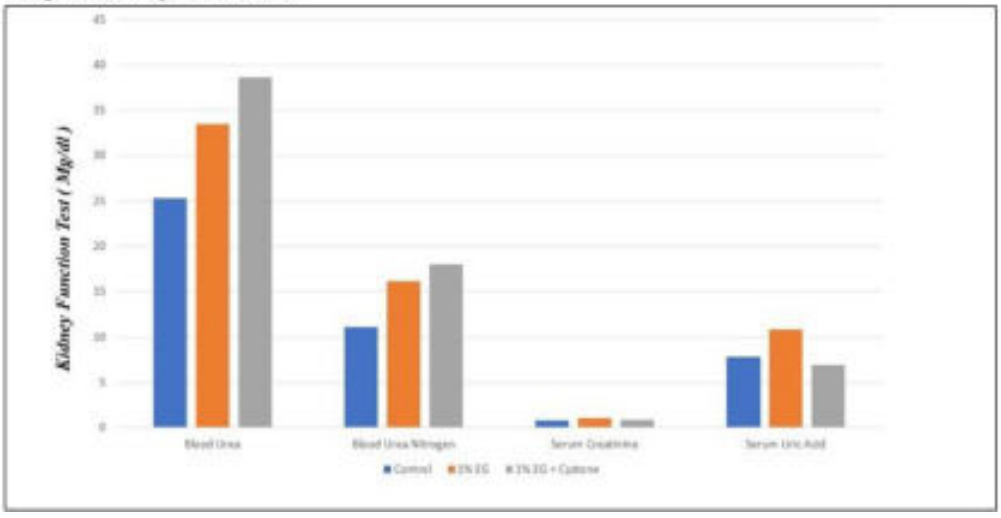
Graph 3 : White Blood Cell Count



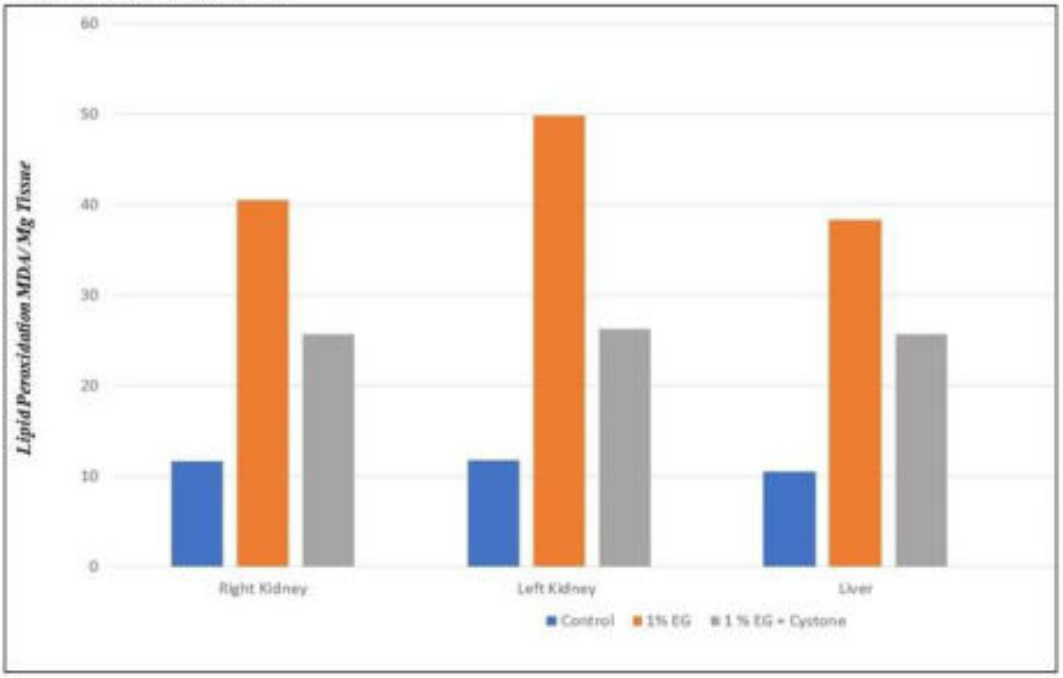
Graph 4 : Platelet Count



Graph 5 : Kidney Function Test



Graph 7 : Lipid Peroxidation



Graph 6 : Liver Function Test

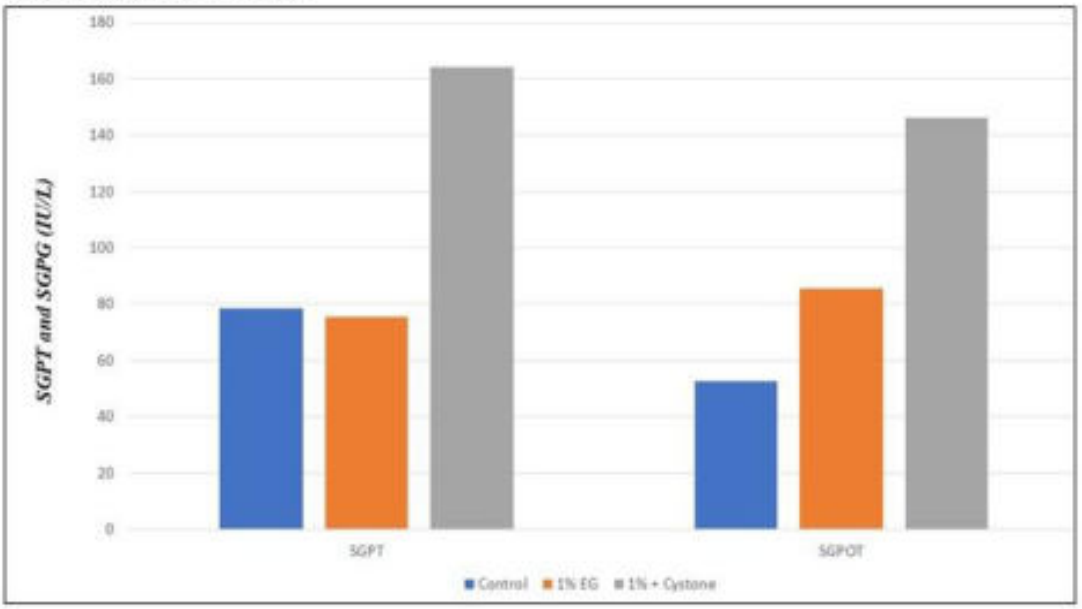


Plate No. 2

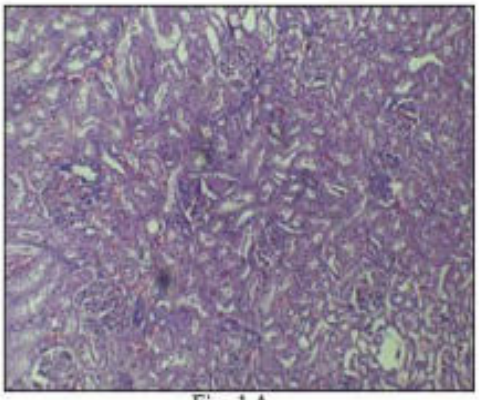


Fig. 1 A

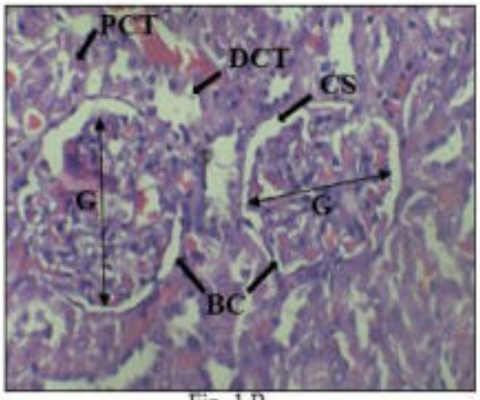


Fig. 1 B

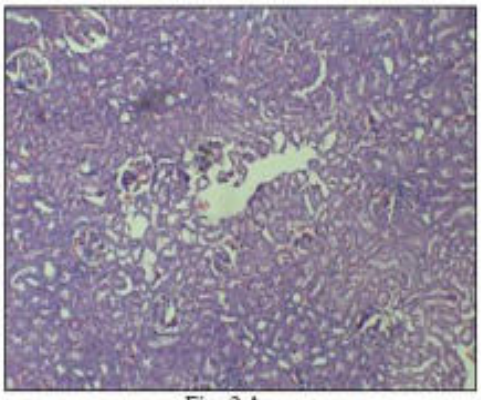


Fig. 2 A

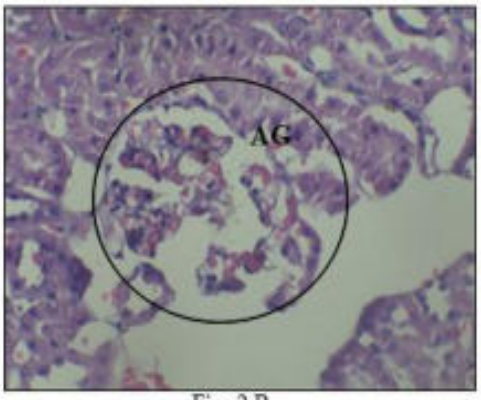


Fig. 2 B

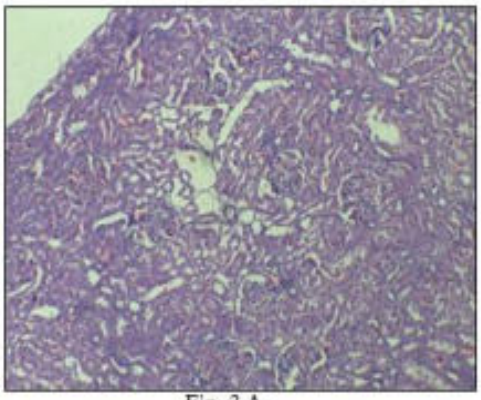


Fig. 3 A

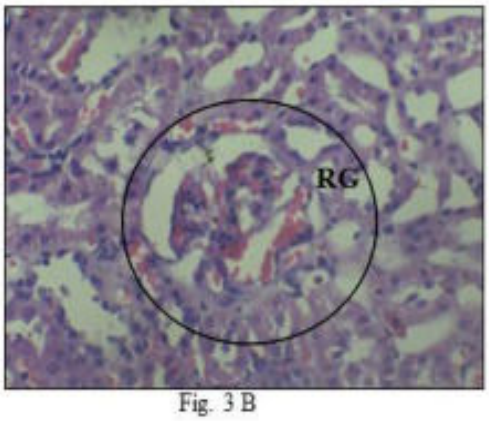


Fig. 3 B

Fig. 1 A – Rat Kidney Control 10 X , **Fig. 1 B** – Rat Kidney Control 40 X , **Glomerulus (G), Bowman's capsule (BC) , Proximal convoluted tubules (PCT) , Distal convoluted tubules (DCT), Capsular space (CS), Fig. 2 A** – Rat Kidney 1 % EG 10 X **Fig. 2 B** – Rat Kidney 1 % EG, **Altered Glomerulus (AG)** 40 X , **Fig. 3 A** – Rat Kidney 1 % EG + Cystone 10 X, **Fig. 3 B** – Rat Kidney 1 % EG + Cystone **Recovered Glomerulus (RG)** 40 X.

Plate No. 3

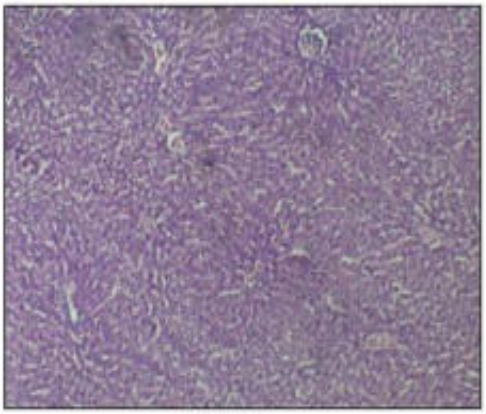


Fig. 1 A

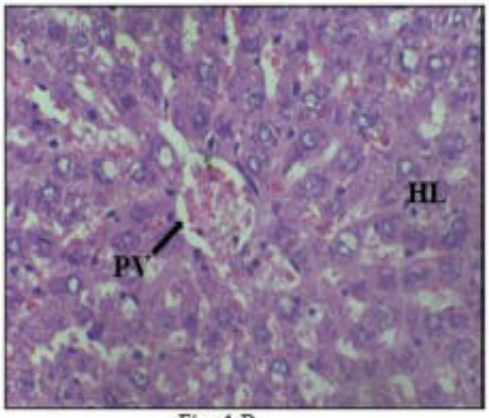


Fig. 1 B

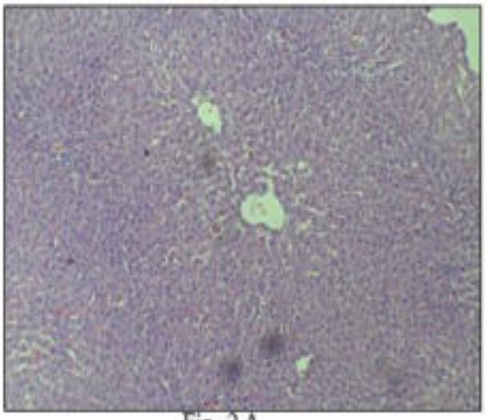


Fig. 2 A

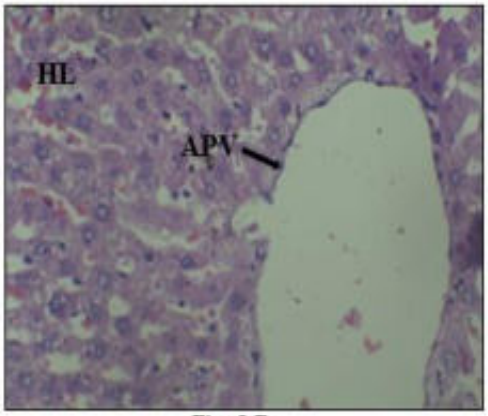


Fig. 2 B

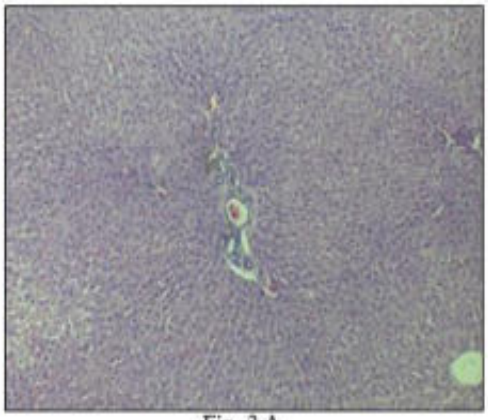


Fig. 3 A

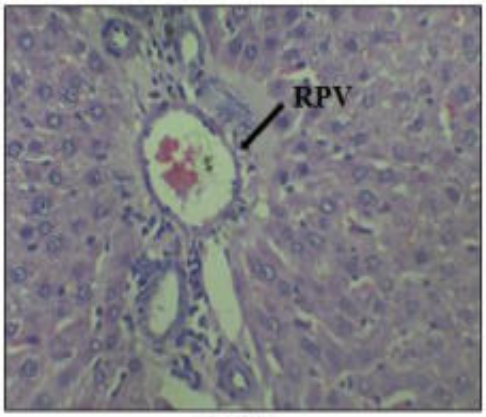


Fig.3 B

Fig. 1 A – Rat Liver Control 10 X , **Fig. 1 B** – Rat Liver Control, **Portal vein (PV), Hepatic lobules (HL)** 40 X , **Fig. 2 A** – Rat Liver 1 % EG 10 X **Fig. 2 B** – Rat Liver 1 % EG, **Altered Portal vein (APV),** 40 X , **Fig. 3 A** – Rat Liver 1 % EG + Cystone 10 X, **Fig. 3 B** – Rat Liver 1 % EG + Cystone, **Recovered Portal vein (RPV),** 40 X.

A) Histological assessments of Kidney and Liver:

The histology of normal kidney showing numerous uriniferous tubules cut in various planes. Each uriniferous tubule or nephron is distinguished into Malpighian body, Proximal convoluted tubules, thin and thick limbs of loop of Henle, distal convoluted tubules and collecting ducts. The Histological examination of kidney after the exposure of Group B, 1 % EG for 30 days dose cycle showed alteration in structural architecture of the cortex region of the kidney. The cortex region destructed in some extend severely. The 10 X and 40X magnifying images shows that there is loss of renal tubules and Bowman's capsule. Recovery group C i.e., Cystone + 1% Ethylene Glycol showed less disturbance in the structural arrangement of kidney as compare to group B. In the pathological assessment of liver in control group showed normal architecture of the liver consisting functional units, hepatic lobules and central portal veins. The group B i.e. exposed with 1 % EG for 30 days dose cycle showed elongation and alteration in hepatic portal vein size. As compare to Group B, Group C i.e., Cystone + 1% Ethylene glycol shows less alteration and retains the its structure.

B) Hemogram

1. **Hemoglobin (gm/ dl):** The Hb concentration recorded in Group A i.e control animals it was 15.9 ± 0.45, in group B i.e., 1% EG recorded 14.1± 1.97 and in group C i.e., 1% EG + Cystone 15.43 ± 2.65 was observed.

2. **RBC count (million /mm^3):** In Group A i.e., control animals it was 9.61 ± 0.59, in group B i.e. 1% EG animal recorded 8.36 ± 1.09, in group C i.e. 1% EG + Cystone 9.60 ± 1.62. As compared to group A and group C, group B count was decreased.

3. **Peak cell volume (%):** Group A i.e., Control group the PCV was 54.53 ± 3.18, in group B i.e., 1% EG, the PCV was 46.55 ± 3.88, in group C i.e. 1% EG + Cystone, the PCV was 50.96 ± 8.02. The PCV was observed higher in group A and lower in group B. Group A (PCV) > Group C (PCV) > Group B (PCV).

4. **MCV (fL):** In Group A i.e., Control animal, the MCV reported 54.03, in group B i.e.,1% EG animal 55.8 ± 2.68, in group C i.e. 1% EG + Cystone 53.1 ± 0.7. There was increased MCV in group B than group A and group C.

5. **MCH (pg):** In Group A i.e. Control animal the MCH was 17.76 ± 1.40, in group B i.e. 1% EG animals the MCH is 16.9 ± 0.28, in group C i.e. 1% EG + Cystone 16.06 ± 0.05. In group B and group CMCH was lowered than group A.

6. **MCHC (gm/dl):** In Group A i.e., Control animal the MCHC was 31.23 ± 3.74, in group B i.e. 1% EG animal the MCHC was 30.3 ± 1.83, in group C i.e. 1% EG + Cystone 30.23 ± 0.46. MCHC value was higher in group A and as compared to group B and group C.

7. RDW CV %: In Group A i.e., Control animal the RDW CV was 17 ± 1.57, in group B i.e., 1% EG animal the RDW CV was 13.85 ± 1.48, in group C i.e. 1% EG + Cystone 15.46 ± 2.67.RDW CV was elevated in group A then group C then group B.

1. WBC Count: Group A i.e., control animals the WBC count was 8500 ± 2300, in group B i.e. 1% EG animals was recorded 6366 ± 2581 and in group C i.e. 1% EG + Cystone was 5000 ± 1352. As compare to group A and group B; group C value was decreased.

2. Neutrophils: In group A i.e., control animal was 45 ± 5, in group B i.e., 1% EG animal it was 35 ± 2 and in group C i.e. 1% EG Cystone it was reported 31 ± 7.93. As compare to group A and group B; group C values was decreased.

3. Lymphocytes: In group A i.e., control animals the lymphocyte count was reported 60.66 ± 6.65, in group B i.e., 1% EG it was reported 58 + 1.6 and in group C i.e. 1% EG + cystone the lymphocyte count was 63.33 ± 7.63. As compare to group A and group C the group B was decreased.

4. Eosinophils: In group A i.e., Control animal, the eosinophil count was reported 3.33± 2.1, in group B i.e. 1% EG the eosinophil count was 4±1 and group C i.e. 1% EG + Cystone it was 2.33 ± 0.57. As compare to group A and group B; the group C was increased.

5. Monocytes: In group A i.e. control animal, the Monocytes count was recorded 3 ±1, in group B i.e. 1% EG the Monocytes count was 4±1 and in group C i.e. 1% EG +Cystone the Monocytes count was 3±1. As compare to group A and group C; the group B value was increased.

6.Platelet count (cells/mm^3): In group A i.e., control animal, the platelet count was reported 948000 ± 25244, in group B i.e., 1% EG; the platelet count was 887500 ±23334 and in group C i.e. 1% EG + Cystone it was 1130333 ± 132613.5. As compare to group A and group B the group C value was increased.

7. PCT: In group A i.e. control animal, the PCT value was reported 0.80 ± 0.14, in group B i.e. 1% EG the PCT value was 0.65 ± 0.02 and group C i.e. 1% EG + Cystone the PCT value was reported 0.74 ±0.07.As compared to group A and group C the group B value was increased.

8. MPV (fL): In group A i.e., control animal the MPV value was reported 8.1 ±1.429, in group B i.e., 1 % EG the MPV value was 7.3 ± 0.14 and in group C i.e. 1 % EG + Cystone the MPV value was 6.63 ± 0.11.

9. PDW (%): In group A i.e., control animal the PDW value was reported 8.7 ± 1.13, in group B i.e. 1% EG the PDW was 8.7 ± 0.2 and in group C i.e. 1% EG

+Cystone the PDW value was 7.53 ± 0.32. As compare to group A and group B the group C value was decreased.

D) Kidney function test: Kidney function test was determined by following parameters.

1. Random Blood Sugar(mg/dl): In group A i.e., Control animal random blood sugar level observed 52.26 ± 3.70, In Group B i.e.,1% EG animal random blood sugar level was 170.43±5.02 and In Group C i.e., 1% EG + Cystone it was220.2 ± 18.66. Amongst three Groups; Group C has maximum value.

2. Blood urea (mg/dl): In group A i.e., Control animal blood urea was 25.26 ± 3.45, In Group B i.e.,1% EG Rat the blood urea was 33.5 ± 1.27, In Group C i.e. 1% EG+ Cystone it was38.63 ± 5.65. As compared to three groups blood urea was increased in Group C.

3. Blood Urea Nitrogen (mg/dl): In group A i.e. Control animal blood Urea Nitrogen was11.07 ± 1.80, In Group B i.e.1% EG Rat the blood urea nitrogen was 16.12 ± 0.94, In Group C i.e. 1% EG+ Cystone it was 18.03 ± 2.61. As compared to three groups blood urea nitrogen was increased in Group C.

4. Serum Creatinine (mg/dl): In group A i.e. Control animal serum creatinine was 0.8± 0.1, In Group B i.e.1% EG Rat Serum Creatinine was 1.06 ± 0.35, In Group C i.e. 1% EG+ Cystone was 0.83 ± 0.05. Group B has elevated level of serum creatinine as compared to other two groups.

5. Serum Uric acid (mg/dl): In group A i.e., Control animal serum uric acid was 7.8± 1.36, In Group B i.e.1% EG animal serum uric acid is 10.8 ± 1.11, In Group C i.e. 1% EG+ Cystone it was 6.9 ± 0.52. Serum Uric Acid was higher in Group B than Group A and Group C.

6. Serum Calcium (mg/dl): In group A i.e., Control animal serum calcium was 9.53 ± 1.20, In Group B i.e.1% EG animal serum calcium was 10.1 ± 1.20, In Group C i.e. 1% EG+ Cystone it was 10.26 ± 0.47. Serum calcium levels was slightly increased in Group C than Group B.

7. Sodium (mMol/L): In group A i.e. Control animal the sodium was 141.93 ± 3.80, In Group B i.e.1% EG animal sodium was 148 ± 2.45, In Group C i.e. 1% EG+ Cystone it was 142 ± 4.31. sodium was higher in Group B than Group C and Group A.

E) Liver Function Test: Liver function test measure the different enzymes, proteins and other substances produced by liver. These tests check the overall health of liver.

S.G.O.T. (IU/L): In group A i.e., control animal; the SGOT value was reported 78.5±2.26, in group B i.e., 1% EG the SGOT value was 75.5±3.79and in group

C i.e. 1% EG + Cystone the SGOT value was 164.1±3.99. As compare to group A and group B the group C value was increased.

S.G.P.T. (IU/L): In group A i.e., in control rat the SGPT value was reported 52.8±1.03, in group B i.e., 1% EG the SGPT value was reported 85.8±4.21and in group C i.e., 1% EG + Cystone the SGPT value was reported 146.2±4.76. As compared to group A and group B the group C value was increased.

F) Lipid Peroxidation: In group A i.e. control rat the lipid peroxidation value in right kidney was reported 11.67 ± 1.05 and in left kidney it was 11.82 ± 0.51 while in liver the value of lipid peroxidation was 10.48 ± 1.17.In group B i.e. 1% EG the lipid peroxidation value in right kidney was 40.56 ± 1.17 and in left kidney it was 49.56 ± 1.17.While in liver the lipid peroxidation value was 38.30 ± 1.799.In group C i.e. control rat the lipid peroxidation value in right kidney was reported 25.70 ± 0.70 and in left kidney it was 26.31 ± 0.72 while in liver the value of lipid peroxidation was 25.69 ± 0.78.

4. DISCUSSION

In clinical physiology term urolithiasis or nephrolithiasis is nothing but a formation of kidney stone or renal calculi or renal crystals [8]. It is the most common, serious and life-threatening disorder worldwide where younger population is affecting with the high rate [9]. Newly formed kidney stone crystals are generally made up of calcium oxalate salt. The amount of calcium oxalate ions concentration increased and associatory ions concentration like hydrogen, sodium, uric acid present in the composition that condition is known to be as supersaturation and it is the primary step for the formation of kidney stone [10].

Chemically stone contains calcium, magnesium, ammonium phosphate, uric acid, cysteine in which almost 70-75 % of urinary stone are calcium containing stone [11]. Kidney stone exist in two forms i.e., Calcium oxalate and Calcium Phosphate. Sometime mixture of both can be occur [12]. The high concentration of calcium carbonate in water and low nutrients diet are the major factors for the calcium dependent stone formation [13]. In calcium oxalate crystals calcium oxalate monohydrate crystals (COM) is oxalate dependent whereas calcium oxalate dehydrate crystals (COD) is calcium dependent [14].

The selection of Wister rat *R. norvgicus* for present investigation was due to anatomical similarities and pattern of biosynthesis of formation of renal calculi in experimental vertebrate model is somewhat identical to human along with that the it is most susceptible model for calculi study.

The toxicity of EG can be spread via different modes like ingestion, absorption and also by means of inhalation exposure. With the several metabolic processes EG converted into toxic metabolites. The newly formed toxic metabolites can affect the

major systems like nervous system, cardiopulmonary and renal failure [15]. EG is bioprecursor for oxalate which converts into its metabolite as glycolaldehyde glycolic acid, glycolic acid and oxalic acid which binds with calcium and forms calcium oxalate and deposited in renal tubeless [16].

In herbal therapy the extraction of plant materials gives diuretic activity, crystallization inhibitor activity, lithotrophic activity, anti-inflammatory activity and increase renal functions [17]. In traditional Indian medicines, Cystone is recommended because of its antiurolithiactic property including nephrolithiasis, prevention of supersaturation of lithogenic substances, Inhibition of stone forming substances like Oxalic acid calcium hydroprolin etc. along with that its widely useful in treatment in anti-inflammatory activity in ureter and painful sign and symptoms of micturition [18].

Electrolytes are positively and negatively charged ions that are found within cells and extracellular fluids, including intestinal fluid, blood and plasma. A test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate. These ions are measured to assess renal (kidney), endocrine (glandular) and acid-base function, and are components of both renal function and comprehensive metabolic biochemistry profiles [19].

The present research work undertaken to the study of kidney and liver damage against experimentally induced kidney stone by chemical Ethylene glycol and its Antiurolithiatic activity against Herbal medicinal formula Cystone. For Oxalate urolithiasis in female wistar rats, *Rattus norvegicus* were used. Total 18 animals were distributed into 3 groups, 6 animals in each group. Group A: Control, Distilled Water, Group B: 1 % Ethylene Glycol, Groups C: 1% Ethylene Glycol + Cystone. All the dose were supplemented orally for 30 days. The animals were sacrificed by cervical dislocation. The biopsy of Kidney and liver were taken for Histopathology and Lipid peroxidation, blood sample were collected for hematological study, Kidney function Test and Liver function test. The plate no. 2 and 3represent histopathological assessment of kidney and liver respectively. Our study reveals that nephrocytic and hepatocytic damage of administration cystone is significantly reduced as compared to administration of EG. An account related with kidney function test, the previous study revealed that EG administration elevate level of urea, creatine and uric acid in tissue of experimental animals [19]. Our study reveals that the administration of ethylene glycol in experimental model the level of urea, creatinine and uric acid also increases significantly on the other hand in cystone administration group this level is significantly decreases. In contrast the blood glucose level were increased in experimental group. Hematological study shows that no significant difference between control, EG and cystone group for parameters like RBC, WBC, Platelets and hemoglobin. The study of liver function test revels that administration of EG increases SGOT and SGPT level in experimental group C.

The 3-carbon compound malonaldehyde (MDA) is a major carbonyl decomposition product of autoxidized, polyunsaturated lipid materials. Spectrophotometric detection of the Malonaldehyde-Thio barbituric acid (TBA) complex has been widely used for measuring lipid oxidation in food and biological tissues. lipid peroxidation gives complex products including hydroperoxides, cleavage products such as aldehydes, and polymeric materials, and that these products exert cytotoxic and genotoxic effect. In present investigation clearly proves that the amount of MDA in ethylene glycol induced group is significantly increased in contrast the level of MDA is significantly reduced with administration of Cystone in experimental rats.

5. CONCLUSION

The pathophysiology and lipid peroxidation of kidney and liver revealed that, herbal medicinal Cystone minimize the stone formation and damage caused by EG in experimental animals. Dose dependent recovery may be possible depending upon administration of CS to preserve normal condition. Results indicate and strengthen antiurolithiactic and phytoremedial activity of CS in animals.

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