CARBOHYDRATE-INDUCED MODULATION OF CELL MEMBRANE IX. BINDING WITH GALECTIN-1 DISRUPTS BACTERIAL MEMBRANE

RAJESH KUMAR GUPTA, KSHITIJ GUPTA, SUMATI HAJELA, KRISHNAN HAJELA

Abstract: Galectins are a family of animal lectins (carbohydrate binding proteins)with an affinity for beta-galactosides.Whether the binding of galectin-1 to bacterial cell surface has any effect on membrane disintegration has never been studied. The release of β-galactosidase enzyme in culture supernatant upon membrane disintegration as a measure of membrane lytic activity has been widely used to study the damage of bacterial and parasites membrane by complement or antimicrobial peptides.This study reports for the first time that the binding of bacterial cells by galectin-1 results in disruption of the bacterial cell membrane as assayed by the release of β-galactosidase enzyme in the supernatant measured by the cleavage of fluorescent substrate 4-methylumbelliferyl β-D-galactopyranoside (MUG).

Keywords: lectin; galectin-1; gram-negative bacteria; lysis; β-galactosidase assay.
Running title: Galectin-1 mediated lysis of bacterial cells.

Introduction: Galectins are animal lectins with an affinity for beta-galactosides widely distributed from lower invertebrates, such as sponges and nematodes, to higher vertebrates [1]. Galectin family of lectins recognizes saccharide ligands on a variety of microbial pathogens, including bacteria, viruses and parasites [2]. By recognizing β-galactoside-related carbohydrates (polygalactose epitopes), commonly present in pathogen-associated glycoconjugates, galectins may contribute to almost any step in host-pathogen interaction and may regulate the magnitude and quality of the immune response following infection [2]. Galectins interact with distinct surface glycans to trigger adhesion, growth regulation or migration/tissue invasion [3, 4]. Galectins secretion follows a non-classical pathway [5-7]; and galectin-3, a chimera-type of galectins, having weak agglutinating activity, can interact directly with membrane phospholipids and cholesterol and spontaneously traverse the lipid bilayer of liposomes in either direction [8]. With emphasis on proto-type galectin-1, a homodimeric lectin, it is known to interact with ganglioside GM_1 on neuroblastoma cells to exert growth control [9-11] and it also harbors a site to interact with hydrophobic tails of oncogenic H-Ras [12]. These studies point toward the capacity of galectins for glycolipid binding and chances of their association with hydrocarbon chains. In a first demonstration of antimicrobial activity for a member of the galectin family of lectins, Kohatsu et al. [13] showed that binding of galectin-3 specifically to *Candida albicans* species, that bear β-1, 2-linked oligomannans on their cell surface, but not to *Saccharomyces cerevisiae* that lacks β-1, 2-linked oligomannans, resulted in death of *Candida* species containing specific β-1, 2-linked oligomannans. More recently, Stowell et al. [14] showed that human galectin-4 and -8 recognize blood group antigen-expressing (BGB^+) *Escherichia coli* (*E. coli)* and directly kill BGB^+ *E. coli.*

Using hemolysis of red blood cells (RBCs) as a model membrane system we have earlier demonstrated that galectin-1 can perturb the lipid bilayer [15]. Agglutination of trypsinized red blood cells with galectin-1 resulted in increase in membrane fluidity, enhancing the cells' osmofragility. Furthermore, agglutination of trypsinized RBC by galectin-1 resulted in their lysis in a concentration and time dependent manner [15]. We hereby report for the first time that binding of galectin-1 to bacterial cells induces disruption of membrane integrity and finally lysis of these cells as assayed by the release of β-galactosidase in the supernatant. β-galactosidase activity was measured by cleavage of fluorescent substrate 4-methylumbelliferyl β-D-galactopyranoside (MUG).

Materials and Methods: Phenylmethyl sulfonyl fluoride (PMSF), aprotinin and Isopropyl-β-D-thiogalactopyranoside (IPTG) were purchased from Sigma, USA. 4-methylumbelliferyl β-D-galactopyranoside (MUG) fluorescent substrate was purchased from Calbiochem, UK. All the bacterial cultures were purchased from Microbial Type Culture Collection (MTCC), Chandigarh, INDIA. Tryptone, Nutrient Broth, and Yeast Extract were purchased from Hi-Media Laboratories, Mumbai, INDIA. All other reagents used were of highest analytical grade available.

Galectin-1 from sheep liver was isolated as described previously and identity of the purified protein with galectin-1 was confirmed by amino acid sequencing of a partial trypsin digest [16]. In order to maintain galectin-1 activity in a non-reducing environment, batches of carboxyamidomethylated galectin-1 (cam-galectin-1) were regularly prepared by reacting the galectin with 25 mM iodoacetamide at 4 °C for 8 h in

Tris/HCl buffer (pH 7.5) containing 150 mM NaCl and 5 mM ⍰-mercaptoethanol in the presence of lactose. The galectin-1-containing solution was extensively dialysed against buffer without ⍰-mercaptoethanol to remove excess iodoacetamide [15].

Human neutrophils were seperated by a mehod using Histopaqu 1119 and Hiatopaque 1077 as described earlier by English and Anderson [29].Cell viability was checked by Nitrobluetetrazolium (NBT) reduction assay.The phagocytic activity of neutrophils after incubation with sheep hepatic galectin -1 (GHG-1) was assayed by a colony counting assay [30].In an experiment 100 ul of neutrophils ($6.0x10^6$ cells /ml) in HBSS were primed with 100 ul of GHG-1 (0.25mg/ml final conc) in absence of serum and incubated for 30 minutes at 37 °C and were directly used for the phagocytic assay [31].Primed neutrophils (in 200 ul) were then incubated with 400 ul ($7.2 x10^8$) cells of E Coli MTCC 448 for 30 min and voulme made upto 1000ul with HBSS.Simultaneously unprimed neutrophils were incubated directly with Ecoli .As a control 400 ul (($7.2 x10^8$) cells of E Coli MTCC 448 were incubated with 100 ul of GHG-1 (0.25mg/ml) for 30 min and volume was made up to 1000 ul with HBSS. 100 ul from these tubes was plated on LB plates and incubated overnight at 37 °C.The control and test plates were counted with AlphaDigidoc ™ AD-1200 software and results are presented in form of % lysis.Later this plate assay [30] was also used to assay direct bactericidial activity of galectin.Bacterial cells 10 ul ($1.8X10^6$ cells of 0.1 O.D at 600 nm were incubated with 25mg/ml final conc of reduced SHG-1 for 30 minutes and plated on LB agar plates.

A β-galactosidase release assay described essentially by Bachy *et al*., [17] was used to further study the sheep hepatic galectin-1 (GHG-1) induced damage to *E. coli* MTCC1679 cells. Fresh culture of *E. coli* cells were regularly prepared by inoculating 10 ml of LB (Luria-Bertani) media with 40 µl l of saturated culture of *E. coli* and grown for 1 h at 37 ºC with constant shaking. The *E. coli* cells were then induced by the addition of 1.0 mM (final concentration) of IPTG and cells were again grown for 1 h at 37 ºC to get an O.D. of 0.2 at 600 nm. $3.6 x 10^6$ IPTG induced *E. coli* cells in 10 µl of PBS were incubated for 1 h at 37 ºC with reduced GHG-1 (kept in the presence of 5 mM β-mercaptoethanol) or cam-GHG-1 (in the absence of β-mercaptoethanol) in a final volume of 100 µl. Spontaneous and total release were measured by incubating 10 µl of cells with 90 µl of PBS or PBS containing 0.1% Triton-X for 1 h at 37°C, respectively. After incubation, 100 µl of assay buffer (100 mM sodium phosphate buffer pH 7.0, 10 mM KCl, 1 mM $MgSO_4$, and 50 mM µl -mercaptoethanol) was added to all the tubes. Bacterial cells were then pelleted by centrifugation at 2400 g for 10 min at 30°C (Micro Centrifuge Remi model 12C). To quantitate the released µl -galactosidase enzyme in the supernatant, 160 µl of the supernatant was mixed with 40 µl of 0.5 mM MUG in the assay buffer. The tubes were incubated for 15 min at 37°C, followed by addition of 200 µl of ice cold 1M sodium carbonate buffer (stop solution). 0.6 ml of assay buffer was further added to all the tubes to bring the volume to 1 ml and fluorescence was read in a JASCO spectrofluorimeter FP-6300 in a 1 ml cuvette (10 mm path length). The instrument settings were as follows: excitation wavelength 360 nm, emission wavelength 440 nm, and excitation slit width 5 nm, emission slit width 5 nm, sensitivity: high, Response: 1 sec. The percent lysis was calculated according to the formula:

Percent Lysis (% lysis) =

$$\frac{\text{Experimental Release} - \text{Spontaneous Release}}{\text{Total Release} - \text{Spontaneous Release}} \times 100$$

Results and Discussion: Both galectin-1 and galectin-3 have been shown to activate the NADPH oxidase of human neutrophils in a lactose sensitive manner and have shown to induce oxidative burst in neutrophils [31]. The effect of SHG-1 on phagocytosis of bacteria by neutrophils was thus studied. The results are presented in Figure 1. The SHG-1 primed neutrophils shows 96.5% killing of bacteria , which was surprising.Whereas bacterial cells incubated only with neutrophils show 37% killing.Surprisingly bacterial cells incubated with SHG-1 only ,shows 52% killing. This proved that it was not the activation of neutrophils but a direct interaction of SHG-1 with Escherichia coli resulting in its killing. On direct interaction of SHG-1 with bacteria it was found that incubation with SHG-1 killed around 50-55% of the bacteria tested.The results are present in Table I . Figure 2 depicts the killing of E coli MTCC 448 on incubation with SHG-1.Incubation with galectin-1 resulted in binding and agglutination of bacterial cells as observed under the microscope(Figure 3) . It was noted that agglutination by SHG-1 was a prerequisite for lectin induced killing.All the killed bacteria were agglutinated by the lectin .SHG-1 was able to kill gram negative bacteria (Table I) . Among the species tested Bacillius subtilis NCIM 2724 and Baccilus pumilus 2327 were not killed by SHG-1.

The membrane integrity of *E. coli* MTCC 1679 cells in the presence and absence of galectin-1 (at reducing conditions, kept in the presence of β-mercaptoethanol), and cam-galectin-1 (in the absence of β-mercaptoethanol) was studied by measuring the release of β-galactosidase in the supernatant.. In detail, E. coli MTCC 1679 cells were incubated with increasing concentrations of galectin-1 (1.05 to 2.45 µM) in PBS for 1 h at 37°C and membrane disruption

was measured by monitoring the cleavage of fluorescent substrate MUG by assaying for released β-galactosidase in the supernatant (Figure 4). With increase in concentration of both galectin-1 preparations, the percentage lysis of bacterial cells was also enhanced. At 1.05 μM galectin-1 concentration, 3% lysis of E. coli was observed, which increased to 11% at 1.4 μM galectin-1 concentration, 36% at 1.75 μM concentration and 42% at 2.45 μM galectin-1 concentrations (Figure 4). At similar concentrations, cam-GHG-1 exhibited reduced lysis. Only 25% lysis was obtained at 2.45 μM cam-GHG-1 concentration. Our earlier study has shown that the lysis of trypsinized RBC was also reduced when cam-galectin-1 was used [15].

The lysis of galectin-1-bound bacterial cells in isotonic PBS increased with increase in period of incubation (Figure 5). In case of reduced GHG-1, there was a significant increase in lysis from less than 10% lysis in 15 min to 71% lysis in 90 min. Lysis did not increase beyond this even if cells were incubated up to 120 min. Similarly, in time course experiments of galectin-3 mediated death of *Candida albicans*, negligible death was observed after 30 min of galectin-3 binding, dead cells started appearing at 60-90 min and maximal death was observed after 120 min of galectin-3 binding. At longer time points, the effects of galectin-3 were confounded by proliferation of the surviving cells, so authors also limited assays condition for 2h [13].

Since induction of IPTG increases the level of β-galactosidase permease and galactosidase transacetylase permease, they may exert their effect on membrane permeability. To rule out this possibility, *E. coli* MTCC 433, which is positive for β-galactosidase and negative for β-galactoside permease and galactosidase transacetylase permease, was used. *E. coli* MTCC 433 cells agglutinated under similar assay conditions exhibited 27% lysis at 2.45 μM concentration of galectin-1 (Figure 6 bar c), which was 15 % less than that obtained by *E. coli* MTCC1679, which is permease positive (Figure 6 bar b). This indicated that galectin-1 is independently capable of lysing bacterial membrane, although induction of permeases by IPTG had a synergistic effect and enhanced lysis. No fluorescent product was formed when *E. coli* MTCC 82 (β-galactosidase negative) cells were agglutinated with galectin-1 (Figure 6 bar a). When a local isolate of *Klebsiella pneumoniae* cells was agglutinated under similar assay conditions, 71% lysis at 2.45 μM concentrations of galectin-1 was observed (Figure 6 bar d). Thus, our results clearly showed that binding of galectin-1 on bacterial cells result in the damage of bacterial membrane. However, the mechanism of this is not yet clear. We speculate two different mechanisms for increased membrane permeability: (a) local clustering of membrane components upon galectin-1 binding and (b) destabilization of membrane by insertion of galectin-1. Viability of bacteria could not be checked by counting the colony forming units on agar plates as incubation with galectin-1 resulted in their agglutination, thereby drastically reducing the number of colony forming units.

Gram negative bacteria are resistant to a large number of noxious agents as a result of the effective permeability barrier function of their outer membrane (OM). The molecular basis of the integrity of outer membrane lies in its lipopolysaccharides (LPS) [18, 19]. The pulmonary collectins, surfactant proteins A (SP-A) and D (SP-D), have been reported to bind LPS, opsonize microorganisms and enhance the clearance of lung pathogens. SP-A and SP-D are antimicrobial proteins that directly inhibit the proliferation of gram negative bacteria in a macrophage and aggregation independent manner by increasing the permeability of cell membrane [20].

In view of the above, it seems possible that agglutination of *E. coli* by galectin-1 results in its killing, possibly by effecting OM permeability. In agreement with this possibility, agglutination has been shown to be a prerequisite in the lysis of RBCs by galectin-1 [15]. Galectins have been shown to recognize various molecular components of bacteria [21]. With focus on galectin-3, smooth LPS from *Klebsiella pneumoniae,* which has a β-galactoside containing polysaccharide chain, is bound by the C-terminal carbohydrate recognition domain (CRD) of galectin-3 in a lactose inhibitable manner [22]. In addition, the lipid A of LPS from *Salmonella minnesota* R7, which is devoid of beta-galactosides, is recognized by a site within the N-terminal domain of galectin-3. Both the N-terminal domain and C-terminal lectin region of galectin-3 have been shown to interact with LPS from *E. coli* [22, 23]. Furthermore, role of galectin-3 in direct recognition of glycans on the capsular polysaccharide of *Streptococcus pneumoniae* is also proposed [24] and replication of *S. pneumoniae in vitro* is also inhibited by the recombinant galectin-3, suggesting that galectin-3 has a direct bacteriostatic activity and can be involved in reducing the severity of pneumococcal pneumonia [25]. Recent study by Stowell et al. [14] also confirmed that human galectin-4 and -8 have bactericidal activity against (BGB^+) *E. coli*. Both the N- and C-terminal domain of galectin-4 recognizes BGB^+ *E. coli*, whereas only C-terminal domain of galectin-8 does so. Galectin-4 and -8 mediated killing of BGB^+ *E. coli* involves a mechanism that drastically alters membrane integrity of bacteria, and bactericidal activity of both the lectins is mediated via only by their C-terminal domains. Galectin-4 and

-8 were also shown to recognize and kill bacteria expressing the α1-3 Gal epitope (α-Gal *E. coli*), a common glycan moiety found in many mammalian species. The observed Galectin-4 and -8 mediated killing of α-Gal *E. coli* was reduced when compared to BGB^+ *E. coli*, suggesting a reduced binding affinity towards this glycan epitope. Thus, these studies suggest that the galectins may function similar to anti-microbial peptides and play crucial role in innate immunity through this mechanism.

Galectins have been shown to accumulate at membrane structures near to internalized bacteria, and these associations have been shown to depend on both the capacity of internalized bacteria to induce phagosome lysis and host LacNAc-containing glycans which are normally present on the cell surface and in the lumen of endocytic compartments, but exposed to cytosol after pathogen-induced vacuole lysis [21]. Cytosolic galectin-3 has been reported to accumulate at membrane structures in the vicinity of internalized *Shigella flexneri* [26]. During the course of infection, galectin-3 has also been shown to accumulate in *Mycobacterium*-containing phagosomes. This accumulation was found to be very specific for phagosomes containing live *Mycobacterium* and occurred at the cytosolic face of the phagosome membrane. Infection of galectin-3 deficient mice with *Mycobacterium* showed reduced capacity to clear late but not early infection [27]. Similarly, association of galectin-8 and -9 have been found with vesicles containing *Salmonella typhimurium*, *Listeria monocytogenes* and *S. flexneri* [28]. Due to its ability to directly interact with autophagy cargo receptor NDP52, galectin-8 has been shown to target the damaged bacteria-containing vacuoles for autophagosome degradation and hence, it is considered as a danger-receptor which restricts intracellular bacterial proliferation [28].

Our results clearly demonstrate that galectin-1 can agglutinate and directly lyse the bacteria. Because galectin-1 in such a high concentration in a reducing environment may be present only intracellularly, we speculate that this may be a method of killing a intracytoplasmic bacteria.

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References:

1. Kasai, K., and Hirabayashi, J. (1996) Galectins: A family of animal lectins that decipher glycodes, *J. Biochem.*, **119**, 1-8.
2. Rabinovich, G. A., and Gruppi, A. (2005) Galectins as immunoregulators during infectious processes: from microbial invasion to the resolution of the disease, *Parasite Immunology*, **27**, 103-114.
3. Barondes, S. H., Cooper, D. N. W., Gitt, M. A. and Leffler, H. (1994) Galectins, Structure and function of a large family of animal lectins, *J. Biol. Chem.*, **269**, 20807-20810.
4. Gabius, H.-J. (1997) Animal lectins, *Eur. J. Biochem.*, **243**, 543-576.
5. Hughes, R.C. (1999) Secretion of the galectin family of mammalian carbohydrate-binding proteins, *Biochim. Biophys. Acta*, **1473**, 172-185.
6. Nickel, W. (2005) Unconventional secretory routes: direct protein export across the plasma membrane of mammalian cells, *Traffic*, **8**, 607-614.
7. Seelenmeyer, C., Wegehingel, S., Tews, I., Kunzler, M., Aebi, M., and Nickel, W. (2005) Cell surface counter receptors are essential components of the unconventional export machinery of galectin-1, *J. Cell Biol.*, **171**, 373-381.
8. Lukyanov, P., Furtak, V., and Ochieng, J. (2005) Galectin-3 interacts with membrane lipids and penetrates the lipid bilayer, *Biochem. Biophys. Res. Commun.*, **338**, 1031-1036.
9. Kopitz, J., von Reitzenstein, C., Burchert, M., Cantz, M., and Gabius, H-J. (1998) Galectin-1 is a major receptor for ganglioside GM_1, a product of the growth-controlling activity of a cell surface ganglioside sialidase, on human neuroblastoma cells in culture, *J. Biol. Chem.*, **273**, 1205-1211.
10. Kopitz, J., von Reitzenstein, C., André, S., Kaltner, H., Uhl, J., Ehemann, V., Cantz, M., and Gabius, H-J. (2001) Negative regulation of neuroblastoma cell growth by carbohydrate-dependent surface binding of galectin-1 and functional divergence from galectin-3, *J. Biol. Chem.*, **276**, 35917-35923.
11. Siebert, H.-C., André, S., Lu, S.-Y., Frank, M., Kaltner, H., van Kuik, J. A., Korchagina, E.Y., Bovin, N.V., Tajkhorshid, E., Kaptein, R., Vliegenthart, J.F.G., von der Lieth, C.-W., Jiménez-Barbero, J., Kopitz, J., and Gabius, H-J. (2003) Unique conformer selection of human growth-regulatory lectin galectin-1 for ganglioside GM_1 versus bacterial toxins, *Biochemistry*, **42**, 14762-14773.
12. Rotblat, B., Niv, H., Andre, S., Kaltner, H., Gabius, H.-J., and Kloog, Y. (2004) Galectin-1(L11A) predicted from a computed galectin-1 farnesyl-binding pocket selectively inhibits Ras-GTP, *Cancer Res.*, **64**, 3112-3118.
13. Kohatsu, L., Hsu, D. K., Jegalian, A. G., Liu, F. T., and Baum, L.G. (2006) Galectin-3 induces

detah of C*andida* species expressing specific *β*-1, 2-linked mannans, *J. Immunol.*, **177**, 4718-4726.

14. Stowell, S. R., Arthur, C. M., Dias-Baruffi, M., Rodrigues, L. C., Gourdine, J. P., Heimburg-
15. expressing blood group antigen, *Nat Med.*, **16**, 295-301
16. Gupta, R. K., Pande, A. H., Gulla, K.C., Gabius, H.-J., and Hajela, K. (2006) Carbohydrate-induced modulation of cell membrane. VIII. Agglutination with mammalian lectin galectin-1 increases osmofragility and membrane fluidity of trypsinized erythrocytes, *FEBS Lett.*, **580**,1691-1695.
17. Pande, A. H., Gupta, R. K., Sumati, and Hajela, K. (2003) Oxidation of goat hepatic galectin-1 induces change in secondary structure, *Protein Pept. Lett.*, **10**, 265-275.
18. Bachy, M., Bonnin-Rivalland, A., Tilliet, V., and Trannoy, E. (1999) Beta galactosidase release as alternative to chromium release in cytotoxic T-cell assays, *J. Immunol. Methods*, **230**, 37-46.
19. Nikaido, H., and Varra, M. (1985) Molecular basis of bacterial outer membrane permeability, *Microbiol. Rev.*, **49**, 1-32.
20. Varra, M. (1992) Agents that increase the permeability of outer membrane, *Microbiol Rev.*, **56**, 395-411.
21. Wu, H., Kuzmenko, A., Wan, S., Schaffer, L., Weiss, A., Fisher, J. H., Kim, K. S., and McCormack, F. X. (2003) Surfactant protein A and D inhibit growth of Gram-negative bacteria by increasing membrane permeability, *J. Clin. Invest.*, **111**,1589-1602.
22. Chen, H.Y., Weng, I.C., Hong, M.H., and Liu, F.T. (2014) Galectins as bacterial sensors in the host innate response, *Curr Opin Microbiol.*, **17**, 75-81.
23. Mey, A., Leffler, H., Hmama, Z., Normier, G., and Revillard, J. P. (1996) The animal lectin galectin-3 interacts with bacterial lipopolysaccharides via two independent sites, *J. Immunol.*, **156**, 1572-1577.
24. Li, Y., Komai-Koma, M., Gilchrist, D.S., Hsu, D.K., Liu, F.T., Springall, T., and Xu, D. (2008) Galectin-3 is a negative regulator of lipopolysaccharide-mediated inflammation, *J. Immunol.*, **181**, 2781-9.

Molinaro, J., Ju, T., Molinaro R. J., Rivera-Marrero, C., Xia, B., Smith, D. F., and Cummings, R. D. (2010) Innate immune lectins kill bacteria

25. Vasta, G. R. (2009) Roles of galectins in infection, *Nat Rev Microbiol.*, **7**, 424-38.
26. Farnworth, S.L., Henderson, N.C., Mackinnon, A.C., Atkinson, K.M., Wilkinson, T., Dhaliwal, K., Hayashi, K., Simpson, A.J., Rossi, A.G., Haslett, C., and Sethi, T. (2008) Galectin-3 reduces the severity of pneumococcal pneumonia by augmenting neutrophil function, *Am J Pathol.*, **172**, 395-405.
27. Paz, I., Sachse, M., Dupont, N., Mounier, J., Cederfur, C., Enninga, J., Leffler, H., Poirier, F., Prevost, M.C., Lafont, F., and Sansonetti, P. (2010) Galectin-3, a marker for vacuole lysis by invasive pathogens. *Cell Microbiol.*, **12**, 530-44.
28. Beatty, W. L., Rhoades, E. R., Hsu, D. K., Liu, F-T., and Russell, D. G. (2002) Association of a macrophage galactoside-binding protein with *Mycobacterium*-containing phagosomes, *Cell Microbiol.*, **4**, 167-76.
29. Thurston, T.L., Wandel, M.P., von Muhlinen, N., Foeglein, A., and Randow, F. (2012) Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion, *Nature*, **482**, 414-8.
30. English D and Anderson B R (1974) Single step seperation of red blood cells .Granulocytes and mononuclear leukocytes on discontinuous density gradient of ficoll- hypaque. J Immunol. Meth. **5**,249.
31. Leijh P.C.J , Furth V.,and Zwet TLV (1986) in : Chapter 46 ;In vitro determination of phagocytosis and intracellular killing by polymorphonuclear and mononuclear phagocytes .Vol2 , Cellular immunology,Handbook of experimental immunology Eds IV edited by D M weir. Blackwell Scientific Publications , california (USA) pg 46.1-46.21.
32. Almkvist J .,Dahlgren C, Leffler H and Karlsson A (2002) Activation of the neutrophil nicotinamide adenine dinucleotide phosphate oxidase by galectin -1 .J Immunol , **168,**4031-4041.

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School of Life Sciences/ Devi Ahilya Vishwavidyalaya/Khandwa Road Indore/452001/India
Dr. D. Y. Patil Biotechnology & Bioinformatics Institute/
Dr. D. Y. Patil Vidyapeeth (Deemed University)/Tathawade/ Pune, 411 033/ India
Department of Pathology/ School of Dental Medicine/ University of Pennsylvania/
Philadelphia/ Pennsylvania/ USA/Department of Biotechnology/ IPS Academy/
Indore/ 452001/India/ hajelak@gmail.com

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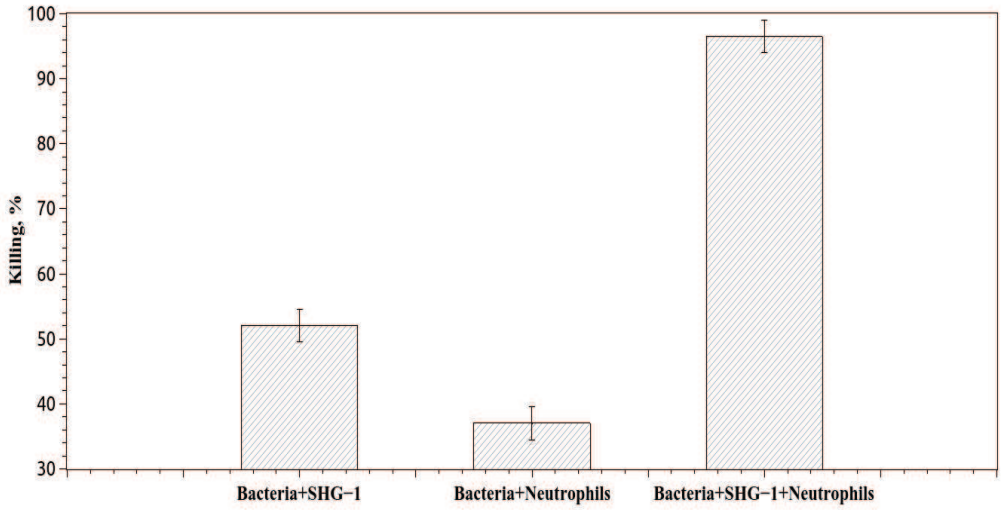

Figure 1. Goat hepatic galectin -1 induced phagocytic activity in primed and unprimed neutrophil.neutrophils were oreincubated for 30 min at 37 °C with or without GHG-1 after which they were incubated with bacteria (E coli MTCC 488) .As control bacteria were incubated directly with GHG-1.

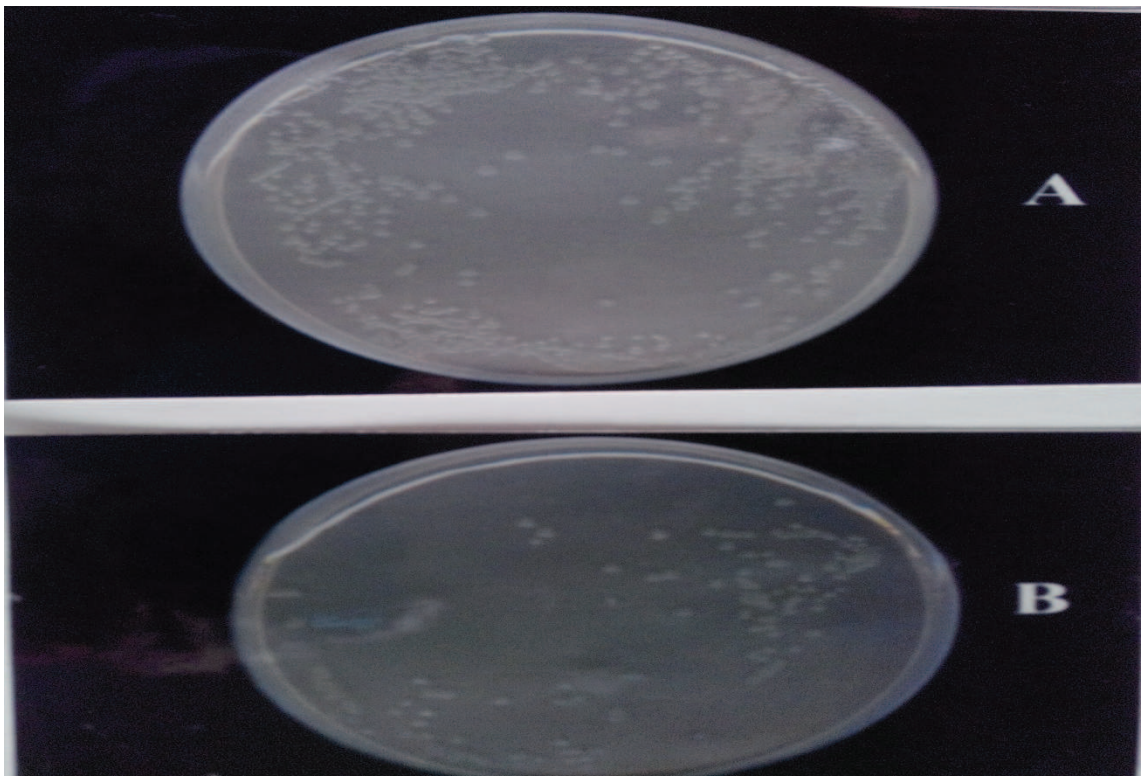

Figure 2. Representative photograph of killing of E coli MTCC 488 on incubation with GHG-1.(A) Bacterial cella incubated without GHG-1 (control) .(B) Bacterial cells incubated with GHG-1 (test).

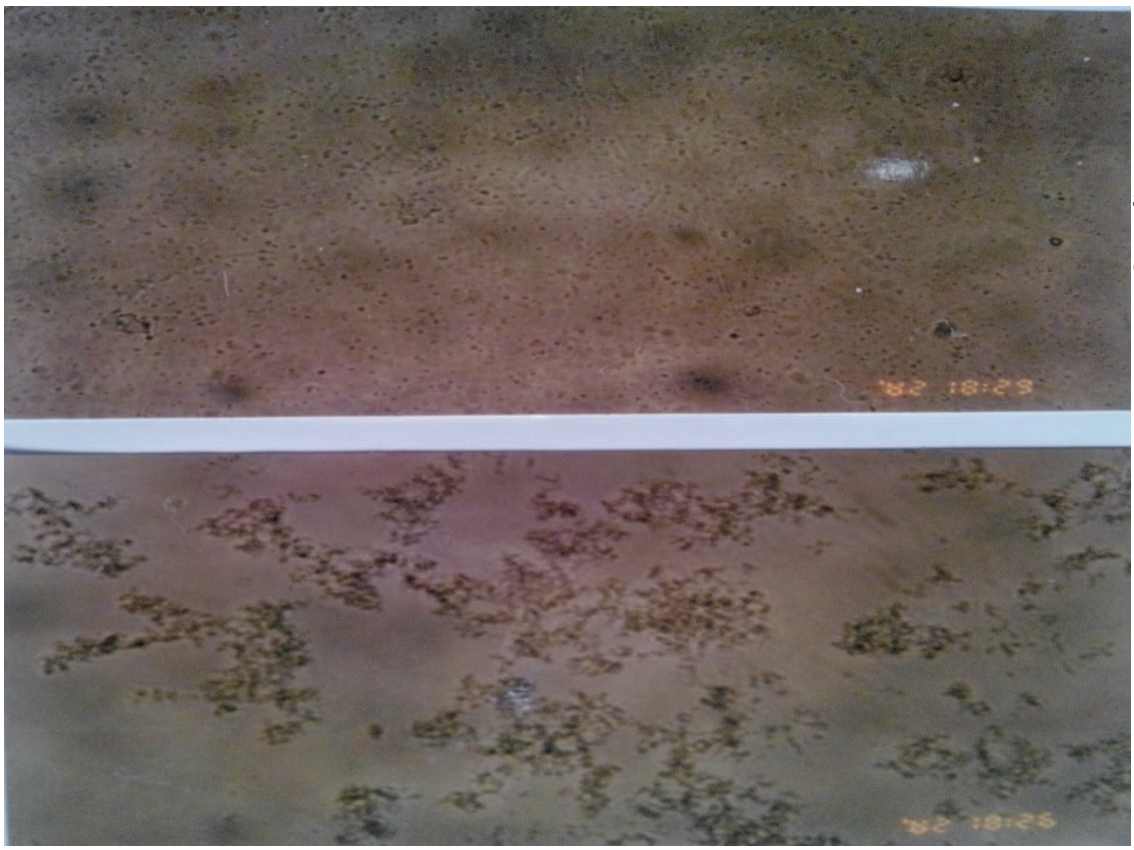

Figure 3. Representative photograph of agglutination of E coli MTCC 488 with GHG-1.(A) in absence of GHG-1 (control) and (B) in presence of GHG-1 (test) .the photographs were taken on Leica Dme microscope equipped with XR 3000 camera under 40X magnification .Enlargment during photoprinting has not been considered.

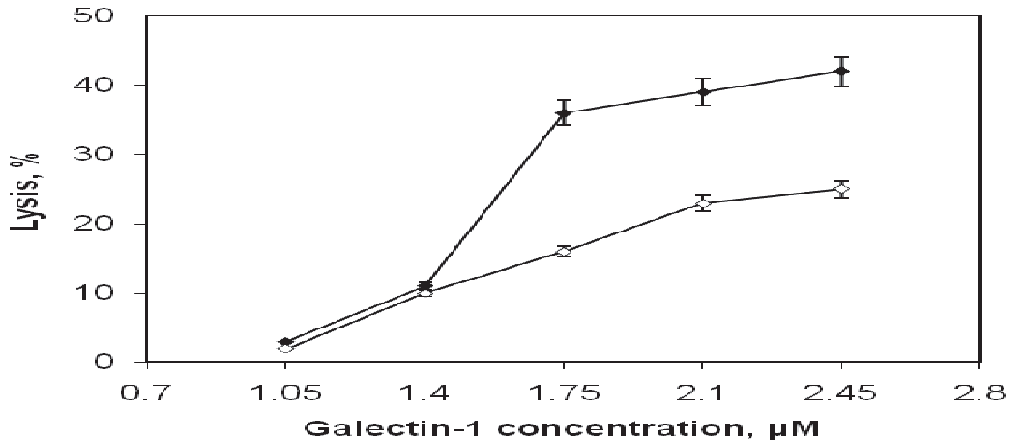

Figure .4. Effect of change of concentration of goat hepatic galectin-1 on lysis of *E. coli* MTCC 1679 cells in isotonic PBS. The incubation was carried out for 1 h at 37 °C. galectin-1 (-●-), cam-galectin-1 (-○-). The results are the means ± SD of three independent experiments.

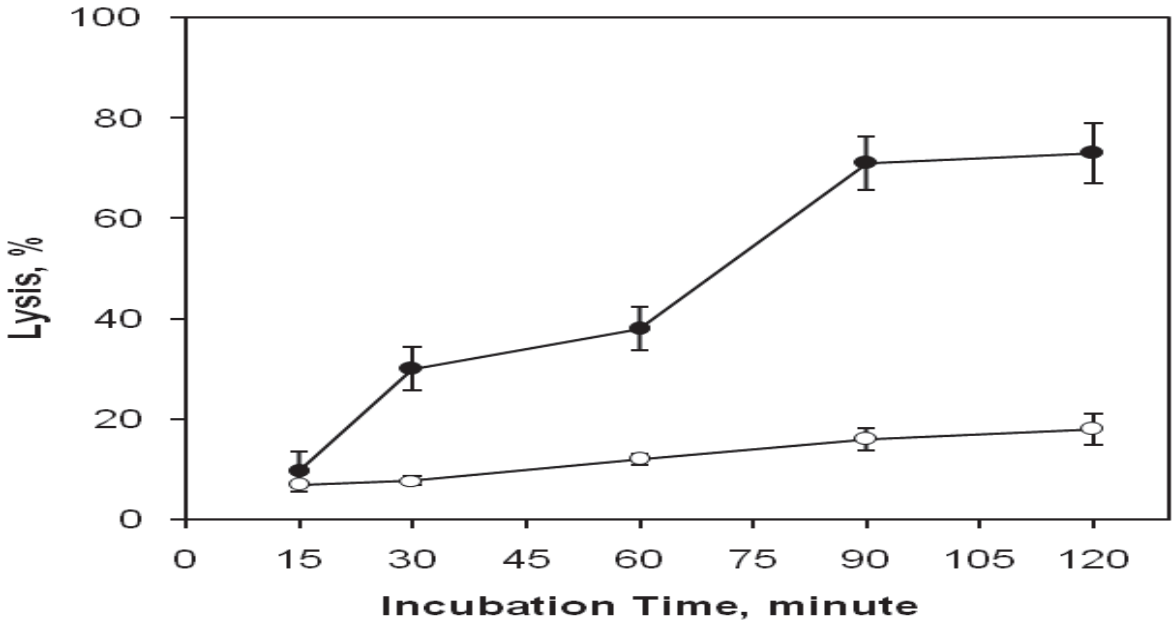

Figure .5. Effect of incubation time on goat hepatic galectin-1-induced lysis of *E. coli* MTCC 1679 in isotonic PBS. The agglutination of E. coli MTCC 1679 cells was carried out at a concentration of 1.75 µM of goat hepatic galectin-1, and agglutinated bacteria were incubated for designated time period at 37 °C. galectin-1 (-●-), cam-galectin-1 (-○-). The results are the means ± SD of three independent experiments.

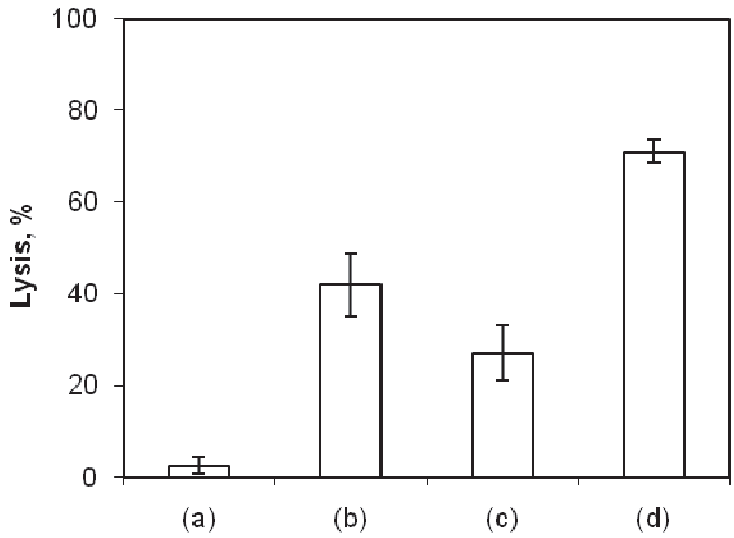

Figure .6. Goat hepatic galectin-1 induced lysis of bacteria: Agglutination was carried out at a concentration of 2.45 µM of goat hepatic galectin-1, and agglutinated bacteria were incubated for 1 h at 37 °C. (a) *E. coli* MTCC 82 (Control *β*-galactosidase negative strain), (b) *E. coli* MTCC 1679 (*β*-galactosidase positive strain), (c) *E. coli* MTCC 433 (*β*-galactosidase positive, negative for β-galactoside permease and galactosidase transacetylase permease) and (d) *Klebsiella pneumoniae* (*β*-galactosidase positive strain, local isolate). The results are the means ± SD of three independent experiments.